UNITED STATES DISTRICT COURT
FOR THE EASTERN DISTRICT OF PENNSYLVANIA

UNITED STATES OF AMERICA, *ex rel.* ROBERT P. BAUCHWITZ, M.D., PH.D. Plaintiff, v. WILLIAM K. HOLLOMAN, Ph.D., CORNELL UNIVERSITY MEDICAL COLLEGE, ERIC B. KMIEC, Ph.D, THOMAS JEFFERSON UNIVERSITY Defendants	CIVIL ACTION No. 04-2892 (TJS) JURY TRIAL DEMANDED

AFFIDAVIT RE EVIDENCE
PRODUCED IN THE CASE
UNITED STATES OF AMERICA V. HOLLOMAN ET. AL.

ROBERT P. BAUCHWITZ, M.D., Ph.D., being duly sworn, deposes and states:

1. I am fully competent to make this affidavit and I have personal knowledge of the facts stated herein. To my knowledge, all of the facts stated in this affidavit are true and correct.[1]

2. The quoted information following is taken from documents available in the docket of the *qui tam*[2] case *U.S. ex rel Bauchwitz v. Holloman et. al.*, in which I was the Plaintiff and Relator[3]. It is also taken from information disclosed to the government, or similar information provided to expert science reviewers, as indicated.

[1] The above caption identifies the case of interest, but this affidavit was not entered into the court record. Rather, this affidavit was produced as part of a formal submission requesting investigation and retraction to the American Society of Microbiology and its journal, Molecular and Cellular Biology, in June, 2012. A pdf version of this file can be found here.

[2] False Claims Act, 31 USC 3729-3733 (1986 version applied throughout unless otherwise noted).

[3] Reference abbreviations: First Declaration of Plaintiff re Motion to Dismiss First Amended Complaint, docket document 63; *First Decl.* Second Declaration of Plaintiff re Motion for Summary Judgment Pursuant to the Statute of Limitations, docket document 110; *Sec. Decl.* Plaintiff's Statement of Additional

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A. Synopsis of New Evidence

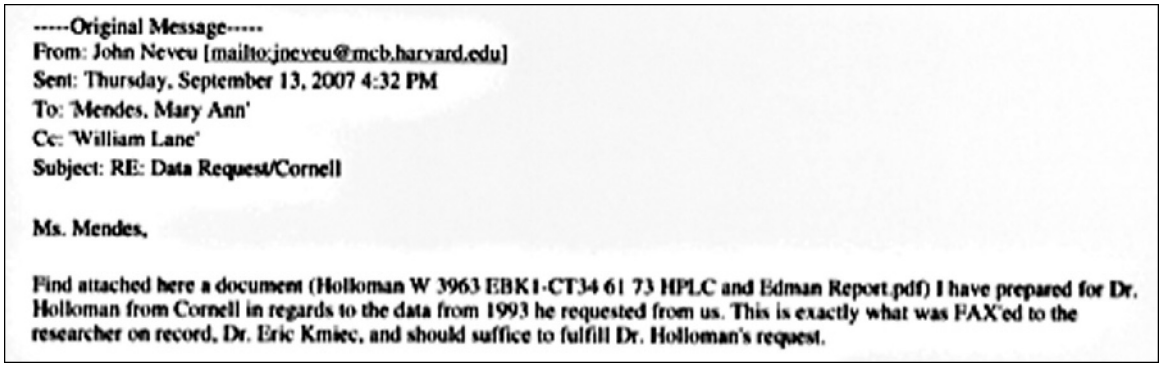
I. Fabrication of Data Showing Purported "Rec1" Strand Exchange Factor Had Amino Acid Sequence of Rec2 Protein

First Allegation:

> "***the Harvard [Microchemistry] laboratory never in fact performed the sequencing work which Holloman and Kmiec represented had been performed by it in Kmiec, et al., 1994***" (*First Decl*., par. 34; emphasis added.)

Internal written statement by external contract laboratory at Harvard University, revealed by subpoena in March 2010:

> "**These sequences are not consistent with the data we provided.**"



-----Original Message-----
From: John Neveu [mailto:jneveu@mcb.harvard.edu]
Sent: Thursday, September 13, 2007 4:32 PM
To: 'Mendes, Mary Ann'
Cc: 'William Lane'
Subject: RE: Data Request/Cornell

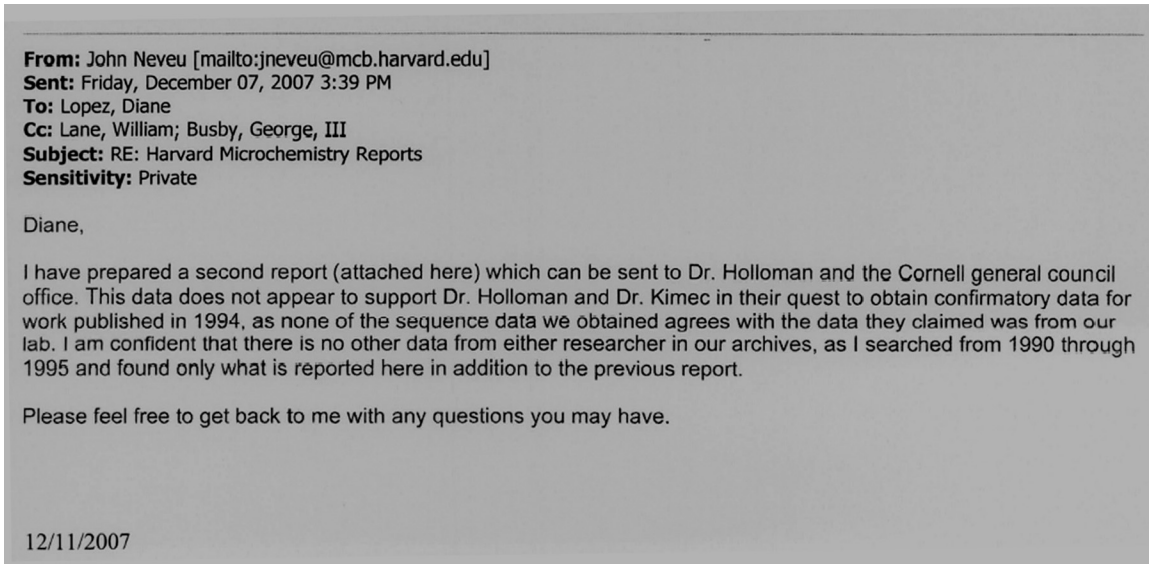
Ms. Mendes,

Find attached here a document (Holloman W 3963 EBK1-CT34 61 73 HPLC and Edman Report.pdf) I have prepared for Dr. Holloman from Cornell in regards to the data from 1993 he requested from us. This is exactly what was FAX'ed to the researcher on record, Dr. Eric Kmiec, and should suffice to fulfill Dr. Holloman's request.

Also included here for your records is a copy of a letter sent to us on July 10, 2007, from Dr. Holloman (Holloman Letter July 10 2007.pdf) in which he requests a copy of the original data from 1993. In this letter, he lists the amino acid sequences that he and Dr. Kmiec published in 1994 (Trp1-5). Note that these sequences are not consistent with the data we provided him, leading me to think that they are barking up the proverbial wrong tree for support in his serious legal situation. Also included was a HPLC chromatogram that was published with the sequence data that has been modified to reflect peaks from which the sequences he claims to have come from us were obtained. Note that in our original data, peaks #'s 34, 61 and 73 were sequenced (the pencil checks are visible) leading to peptide sequences EBK1-CT34, EBK1-CT61 and EBK1-CT73 (which produced no sequence data) as reported to Dr. Kmiec at that time.

The Harvard Microchemistry Laboratory also made it clear that ***there was no potentially relevant missing data***, by writing to the Harvard General Counsel:

> **"...none of the sequence data we obtained agrees with the data they claimed was from our lab."**
>
> **"I am confident that there is no other data".**



From: John Neveu [mailto:jneveu@mcb.harvard.edu]
Sent: Friday, December 07, 2007 3:39 PM
To: Lopez, Diane
Cc: Lane, William; Busby, George, III
Subject: RE: Harvard Microchemistry Reports
Sensitivity: Private

Diane,

I have prepared a second report (attached here) which can be sent to Dr. Holloman and the Cornell general council office. This data does not appear to support Dr. Holloman and Dr. Kimec in their quest to obtain confirmatory data for work published in 1994, as none of the sequence data we obtained agrees with the data they claimed was from our lab. I am confident that there is no other data from either researcher in our archives, as I searched from 1990 through 1995 and found only what is reported here in addition to the previous report.

Please feel free to get back to me with any questions you may have.

12/11/2007

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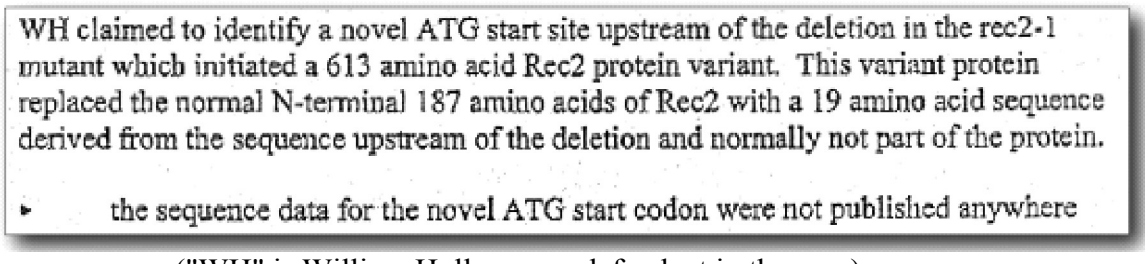
II. Falsification of DNA Sequence for *rec2-1* Mutant Gene Deletion Endpoints

Second Allegation:

> "in Kojic et. al., 2001, and the relevant grant applications and progress reports, Holloman made **specific false statements about the *rec2* mutant gene sequence** in furtherance of his goal of linking the three false claims, *i.e.,* to explain to his colleagues, reviewers, and other scientists ***how it would have been reasonable for a Rec1 protein to emanate from the REC2 gene***, given data to the contrary they had presented in the research articles that he and Kmiec had published previously." (*First Decl*., par. 36; emphasis added.)

The Office of Research Integrity noted in a January 12, 2005 letter to the Department of Justice:



WH claimed to identify a novel ATG start site upstream of the deletion in the rec2-1 mutant which initiated a 613 amino acid Rec2 protein variant. This variant protein replaced the normal N-terminal 187 amino acids of Rec2 with a 19 amino acid sequence derived from the sequence upstream of the deletion and normally not part of the protein.

- the sequence data for the novel ATG start codon were not published anywhere

("WH" is William Holloman, a defendant in the case).

Holloman only made claims in text and schematics for this important "novel ATG start site" for the *rec2-1* mutant allele; as ORI noted, despite alluding otherwise, he never presented actual DNA sequence in a cited paper, or in the federal Genbank.

Moreover, Holloman had apparently removed information specifying the 5' end of the *rec2-1* open reading frame and exact deletion points of the *rec2-1* mutation from a paper initially presenting *REC2* gene sequence (Rubin et. al. 1994). As Rubin, the graduate student who performed the sequencing of *REC*2 and *rec2-1,* noted:

> Rubin: ***You know what is funny is***, that somehow the, I actually did notate that the rec2-1 mutant, that on this figure originally, Figure 1, and uh, in the legend, 5 the description, I am looking at my thesis right now, and it was the same figure, and it is in there. Bauchwitz: Oh, it is? Rubin: ***Bill must have taken it out for some reason***." (*Sec. Decl.*, par. 28.)

According to Holloman's testimony during the case, his graduate student, Brian Rubin, was the only person in his laboratory who had sequenced the *rec2-1* allele, and purportedly was the sole source of the data Holloman relied upon. Rubin, however, stated that he had *not* observed an upstream ATG:

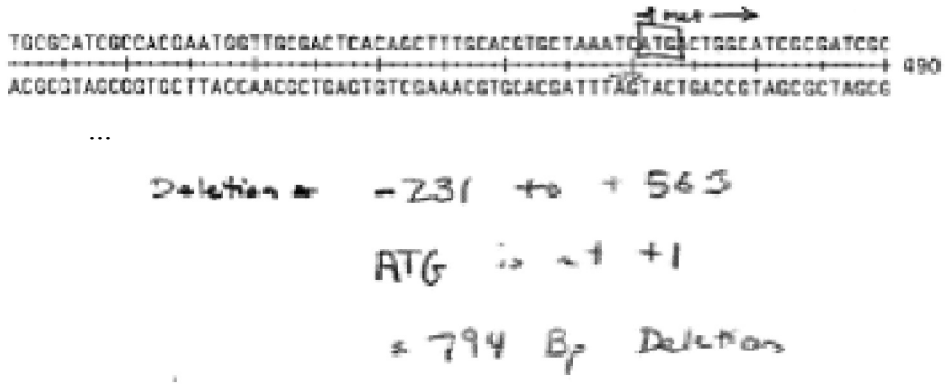
R : But **you sequenced across the rec2-1 breakpoint, right.**
B : **Oh, yeah.**
R : So you know the whole sequence. You could look at it in a second and see if there is an ATG.
B : Upstream?
R : Absolutely.
B : **I did look, actually and there wasn't.**
R : **There wasn't.**
B : **No.**
R : How far did you go sequencing upstream?
B : To that HindIII site. That's to the left of the map.
R : Oh, really?
B : Yeah.
R : **You went all the way up to the HindIII site [] and you saw no ATG.**
B : **Right.**

R: Relator; B: Brian Rubin

As there was no public record, including not in Rubin's thesis, of such sequence, I sequenced the relevant portion of the *rec2-1* allele and published it in the federal Genbank in 1999 (accession number AF027108; docket document 90, Exhibit 7):

```
LOCUS       AF027108                 66 bp    DNA     linear   PLN 31-DEC-1999
DEFINITION  Ustilago maydis recombination-repair protein (REC2) gene, sequence
            across the deletion point of the rec2-1 allele.
ACCESSION   AF027108
      ...
ORIGIN
        1 tgagccaaga ccaagaccac caaccaacac gcagcaagcg tcacacgtca cgcgacccac
       61 gatgtt
```

Only upon court subpoena in 2010 did Holloman release what he purported to be some basis for his claims about the novel, upstream ATG start site for *rec2-1*:



Holloman further claimed that the above numbers, "-231 to +563", which also appeared in Rubin's thesis, would produce the sequence he claimed in Kojic et. al., 2001, using ATG = +1, as noted above. The actual sequence Holloman claimed, however, did not appear in Rubin's thesis.

Open reading frame analysis showed that the above numbers purportedly relied upon, could not have produced the *rec2-1* open reading frame claimed by Holloman. This conclusion was verified by two experts in molecular biology[4]:

> 3. Does the evidence provided by H support "a 613 amino acid [R2] protein variant with a novel 19-residue leader sequence derived from upstream of the deletion" as claimed in JUH2001?
>
> No *ExpRev-1*
> **NO** *ExpRev-2*
>
> 4. Would H's claim to have relied on the sequence numbers "-231 to +563" be consistent with the 613 amino acid open reading frame he published in JUH2001? If so, how?
>
> No *ExpRev-1*
> **NO** *ExpRev-2*
>
> 8. Do you believe that it is more likely than not that H made an innocent error or was acting by incompetence in his claims about finding an ORF for *r2-1* as described in JUH2001? If so, why?
>
> I do not believe that these mistakes were innocent. *ExpRev-1*
>
> **It is more likely that H was deliberately making things up given the collective observations presented. In either case, I would argue against an innocent error.** *ExpRev-2*

[4] The first reviewer is a former director of the Carnegie Institution of Washington, D.C. The second is a Mayo Clinic professor. The reviewers were not given the names of the various parties, nor of the proteins involved. "H" is Holloman, "R" is Relator, "R2" is Rec2, "JUH2001" is Kojic et. al., 2001. Additional evidence obtained and relevant DNA sequence is provided in the main body of the text and in attached documents for analysis by the reader.

9. Based upon the information presented here, do you believe that it is more likely than not that H falsified his *r2-1* data claims in JUH2001?

`Yes` *ExpRev-1*
YES *ExpRev-2*

Furthermore, someone, most likely Rubin, had marked the precise boundaries of the actual *rec2-1* DNA sequence on the documents revealed by Holloman. That sequence exactly matched the *rec2-1* sequence that appears in Genbank AF027108. This supports Rubin's contention that he, too, did not see the upstream ATG claimed by Holloman, but instead had obtained the correct *rec2-1* DNA sequence.

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III. Protein Activity Data Falsification

On December 27, 1994, Holloman's graduate student, Brian Rubin, made the following comments:

> Rubin: "I think of him as just a guy that develops stories in his office and then comes into the lab and says produce the data that fit my stories. And ***in fact in that paper, there are figures straight from my thesis that have totally nothing to do, really, with what was published***."
>
> Bauchwitz: "What do you mean by that?"
>
> Rubin: "Well like the protein, he [Defendant Holloman] used my purification gel and Western showing the anti-Rec2 antisera binds this protein."
>
> Bauchwitz: "Yeah."
>
> Rubin: "And ***then he claims that this protein is active***. And he would ***rationalize it*** by saying 'Well, it's just a better looking gel than Eric's [Defendant Kmiec]' Of course, we are using the same strain, ***but that prep wasn't active***.

As Rubin also stated in that conversation:

> Rubin: "So he [postdoctoral fellow Naoto Arai] came, and he tried to figure out if the Rec2 protein was a strand exchange protein. He worked on it for, well, almost two years. I'd say about two years. **And he got the same results I did. Basically, we could never show this did anything. And we purified numerous helicases from *Ustilago* and *E. coli*, and basically we were looking for a DNA dependent ATPase**. We tried to follow, that was sort of our base assay, was DNA dependent ATPase. We also did extremis. **We tried strand exchange on our purified preps, but we never got it to do anything. …**

Therefore, it was alleged that Holloman falsified Rec2 activity data presented in Kmiec, Cole, Holloman, 1994, according to the information provided by his former graduate student, Rubin.

In addition to the specific issue of data characterization, more generally with respect to the "Rec1 is Rec2" protein activity allegations I argued: "Holloman's reliance on irreproducible data purportedly derived by Kmiec, and ***his disregard of the data produced by researchers in his own laboratory***, which demonstrated no Rec2 activity **at the time of the publication and grant submission**, demonstrate that Holloman acted, at a minimum, with reckless indifference or in deliberate disregard of the truth, given Holloman's awareness that Kmiec had a serious history of reproducibility issues: 1) his Rec1 protein activity work had not been notably reproduced by those inside or outside Holloman's laboratory (excepting subsequently by Kmiec), and 2) Kmiec's work as a postdoctoral fellow with Abraham Worcel had been publicly retracted." (*First Decl.* par. 42, emphasis added.)

In response to the third allegation, and improperly but repeatedly ascribed to all the allegations, Holloman's attorneys used a publication of Holloman's in 2001 that purported to show Rec2 protein purified from *E. coli* as having "Rec1"-like transferase activities (*Bennett and Holloman, 2001*), as a defense against claims that he had acted inappropriately in trusting Kmiec over those in his laboratory.

My response, from the court record:

> "First, I note that defendant Holloman himself actually backed away from the validity of the methodology in the Bennett Paper one year after renewal of a $1.7 million grant for project GM42482, *i.e.* GM42482-12A2. In that competitively renewed, and twice amended grant, Holloman claimed, "We have **only just recently succeeded in being able to produce sufficient amounts** of both Rec2 and Rad51 …" (p. 14) [at RPG 00842]. However, in the next year's Progress Report, it is stated that, ""Isolation of Rec2 protein has **continued to be a formidable problem** … yields of active protein were low and **the method was *not reliable***."" (*Sec. Decl*., par. 42.)

By the following year, Holloman effectively declared that the Bennett procedure had been abandoned:

> "Isolation of Rec2 has continued to be a formidable problem. ... We have continued seeking a better system for expression of Rec2 and have pursued our finding that soluble Rec2 could be obtained when the gene was expressed in yeast ..." (GM42482-14, p.2.)

But even obtaining soluble Rec2 from yeast did not work:

> "... we have not still not [sic] been able to purify Rec2 past one or two fractionation steps before it becomes badly degraded. This disappointing result has led us to **reconsidering our strategy**." (GM42482-14, p.3.)

Notably, under such desperate circumstances in which no experiments were reported as having produced expected data for years, Holloman's reconsideration apparently did not include having Bennett or Kmiec personally produce active Rec2[5].

Therefore, it appears that the Bennett protocol of 2001 was quite the opposite of the "vindication" of the Holloman's Rec2 work with Kmiec, as proclaimed by the defendants. Rather, it seems that the experience of Holloman's personnel in not reproducing the activity claimed by Kmiec was the only thing reproduced with Bennett's method.

As I noted in the court record, "It is my suspicion, for many reasons to be further elaborated, but not germane to the current motions, that the Bennett and Holloman publication of 2001 is likely part of a continuing fraud. Its lack of scientific value in terms of reproducibility are strongly suggested by Defendant Holloman's own statements in GM42482-13." (*Sec. Decl.*, par. 46.)

After the third year of grant funding, Holloman reported that his lab had found that soluble Rec2 could be produced in *E. coli* by fusion to maltose binding protein (MBP). The soluble Rec2 "thorn" had been removed, Holloman declared, but ***no in vitro activity was mentioned as having been observed***.

Indeed, Holloman failed to provide any evidence of active purified Rec2 at any time after funding of the 2002 grant at issue in response to discovery in the legal case, either in 2002 when the his grant first claimed "sufficient" Rec2 "to begin" studies, or up to the point three years later when the original, "fairly manageable" procedure as Holloman portrayed it to NIH, had been abandoned and replaced with one using a different Rec2 (MBP) fusion. To my knowledge, no publication examining *in vitro* activity of such a soluble Rec2 has appeared in the scientific literature.

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IV. False statements to NIH that Holloman's laboratory had never been able to study soluble Rec2

There is probably a good reason why Holloman made no report of soluble Rec2 as having *in vitro* activity - if indeed that was the case in subsequent progress reports to the

[5] Holloman did not reveal any Rec2 purifications by his laboratory from 2002 or thereafter, or any assessment as to why the Bennett and Kmiec protocols were so irreproducible in the hands of others. This situation is very reminiscent of that with the "Rec1" purported strand exchange protein purification, which remained effectively unattainable by others in Holloman's laboratory for more than a decade. Note that when those in Kmiec's postdoctoral laboratory could not reproduce his data, Kmiec was summoned back to that laboratory by its head, Dr. Abraham Worcel. Under the observation of the Worcel laboratory, Kmiec apparently could not reproduce his work either (see Background section, below).

NIH[6]. The latter were not produced by Holloman during discovery despite direct request, so at this time ***adverse inference*** is taken, i.e., had any evidence to support active Rec2 existed, then Holloman would have had strong motive to produce it to the Court. By not doing so, one is entitled by adverse inference to assume that such evidence did not exist.

More than suspicions and adverse inference about the final results from soluble Rec2 are at issue, however.

Holloman made specific claims to the NIH about *not* having previously obtained soluble Rec2 for study. In his 2003 grant GM42482-13 Progress Report he stated:

> "In previous investigations we established that recombinant protein could be highly expressed in bacteria, ***but could not be obtained in a soluble form without the use of denaturing solvents***."

Holloman had also stated in the original grant:

> "Unfortunately, the protein [Rec2] is produced in insoluble form in E. coli",

Examination of Holloman graduate student Brian Rubin's 1994 thesis, however, indicates that soluble Rec2 was the primary form of Rec2 that was first examined (p.71):

> **Purification of Rec2.** Since we did not know the biochemical activity of Rec2, it was decided that only soluble Rec2 should be used in purification. Our main concern was that because the activity of Rec2 could not be assayed, we may not have been able to tell when it was properly renatured.
>
> As a first step in purifying Rec2, the soluble portion of the IPTG-induced BCM464 lysate was precipitated by addition of ammonium sulfate.

Rubin never denatured Rec2 in these experiments, and there is no indication he ever used "denaturing solvents"[7]. (See also *Rubin Thesis*, p.116.) He expressly focused on using soluble Rec2.

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[6] Attorneys involved in the case claimed to have lost the relevant FOIA documents.

[7] Since it was Rubin who subsequently made the Rec2 expression plasmid that did produce insoluble Rec2, we can assume that he used it as well under such denaturing conditions; however, for unknown reasons, none of this work appears in his thesis. (Note that I previously provided in the court record an example of Holloman's negative influence on my own graduate thesis, in which he withheld Rec1 Western blot data.) Nevertheless, it is known that this was the expression construct that Holloman's post-doctoral fellow Arai used, and which Holloman sent to Kmiec for use in the Kmiec et. al., 1994 paper. It would appear that **Holloman may have not wanted any documentation in Rubin's thesis of failures using this Rec2-hexahistidine approach**; indeed, he never mentioned Rubin or Arai's claims that they had performed such work, even when directly questioned about it by interrogatory during court proceedings.

Therefore, it is alleged that Holloman made additional false claims regarding his having obtained and studied soluble Rec2 to the NIH in progress reports associated with his grant GM42482-12A2.

Back to TOC.

B. Background

"I am the relator in this action and make this declaration in opposition to defendants' motions to dismiss the First Amended Complaint on jurisdictional grounds pursuant to Rule 12(b)(1) of the Federal Rules of Civil Procedure. I have personal knowledge of the facts set forth herein, and am willing and able to testify to them under oath." ... (*First Decl.* par. 1; see footnote 2 for reference abbreviations).

"To assist the Court in making the necessary findings regarding the absence of any public disclosure of the relevant allegations or transactions, and particularly to assist the Court in understanding the nature and extent of my role in investigating and developing the allegations in the Original and First Amended Complaints, I believe it is essential for me to provide the Court with some background information about myself, and about the scientific research which was the subject of the grant applications in connection with which the defendants made the false claims at issue in this action." (*First Decl.* par. 3.)

"My educational background is as follows. I received an A.B. degree in Biochemistry from Harvard University in 1982. Thereafter, I completed a joint M.D./Ph.D. program at defendant Cornell University Medical College ("Cornell") and the Sloan-Kettering Institute. I received my Ph.D. degree in Molecular Biology from Cornell in 1990, and my M.D. degree from Cornell in 1991." (*First Decl.* par. 4.)

"From approximately 1987 through 1990, while pursuing my Ph.D. degree, I worked as a graduate student in the laboratory of defendant William K. Holloman, Ph.D. ("Holloman") at defendant Cornell. At the time, most of the research conducted in the Holloman Laboratory was focused on a gene[8] known as *REC1* and, to a lesser extent, on a gene known as *REC2*. During the period that I worked in the Holloman laboratory, I, along with others, was able to successfully isolate the genes for *REC1* and *REC2*. My work as a molecular biologist in the laboratory also included characterizing and obtaining sequence from those genes[9]. In addition, I had extensive interaction with others in the

[8] In scientific notation, the names of proteins (from cells with nuclei as relevant here) are written with only the first letter capitalized, and without italics. Genes, which produce those proteins, are written in italics. The gene name is all capitalized when it is the unmutated form, and in all small case when a mutant form. As there can be various mutant forms of a gene, such notation is often followed by a number or letter. For example, in this action we deal with Rec1 and Rec2 proteins, *REC1*, *REC2*, and *rec2-1* genes, of which the latter is a mutant form.

[9] During the same period, I co-authored with Holloman two articles published in peer-reviewed scientific journals involving the *REC1* and *REC2* genes: (i) Bauchwitz, R. and Holloman, W.K., 1990. Isolation of

laboratory who were attempting to reproduce and extend the Rec1 work of Kmiec and Holloman, including by my attending laboratory meetings in which these issues were discussed. Therefore, my status as an "insider" of the Holloman laboratory gave me extensive knowledge and access to issues regarding Rec1/*REC1* and Rec2/*REC2* research." (*First Decl.* par. 5.)

"From 1991 through 1996, I continued to pursue research related to that performed in the Holloman lab[10] while a Postdoctoral Fellow in the Columbia University Department of Genetics and Development."[11] (*First Decl.* par. 6.)

"The work I performed on the *REC2* gene during the period of my postdoctoral fellowship was related to demonstrating that the *rec2-1* gene was a functional null. Based on observations made during that work, I also obtained evidence concerning the mutator status of *rec2* mutants. Finally, I sequenced upstream regions of the *REC2* and *rec2-1* genes. (These sequences are directly relevant to this case and which establish my position as an original source for this vital information.)" (*First Decl.* par. 7.)

"From 2001 to [October 2007], I [was][12] employed by the St. Luke's-Roosevelt Institute for Health Sciences at Columbia University as Director of the Cognitive Neuroscience Laboratory. From 2000 through 2002, I assisted science journalist Gary Taubes with an article[13] on various phases of concern in the scientific community over the work of the Defendants. The phases of such concern are summarized in the following paragraphs." (*First Decl.* par. 7 [sic - the second of two paragraphs numbered "7"].)

"From the early 1980's through the mid-1990's. Holloman and his graduate student, Kmiec, claimed to have been the first to purify and study a protein[14], Rec1, which they identified as coming from a gene known as *REC1*. This protein had special properties common to a class known as DNA recombinases. However, only Kmiec could

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the *REC2* gene controlling recombination in *Ustilago maydis*. Gene 96: 285-8; and (ii) Tsukuda, T., Bauchwitz, R., and Holloman, W.K. 1989. Isolation of the *REC1* gene controlling recombination in *Ustilago maydis*. Gene 85: 335-41. I also co-authored a book chapter with Holloman (and others) on a related subject: Holloman, W., Bauchwitz, R., Fotheringham, S., and Tsukuda, T., 1988. Molecular Genetic Analysis of Recombination in *Ustilago maydis*. In Intermediates in Genetic Recombination, Amar S. Klar, Ed., Cold Spring Harbor Press, Cold Spring Harbor, NY.

[10] In free time during the evenings and weekends when I was not performing my fellowship research.
[11] [After this, I became a research associate (non-tenure track assistant professor), and then obtained my own lab and grant funding, all in the same department.]
[12] [I was also for several years during this period an adjunct professor in the Department of Natural Sciences of Fordham University.]
[13] Attached here as Exhibit A.
[14] The amino acid order in a protein which specifies its nature, much like the order of letters in a word specify its meaning, are determined by an associated DNA sequence known as a gene. By analogy, if the word to be printed by a computer were "truth", then the computer might assemble the requisite letters from an internal code such as 010 = t, 110 = r, 001 = u, and so on. Therefore, a word generating machine might use the sequence of DNA-like codes (010)(110)(001)(010)(111) to specify the printed letter sequence "truth", which we call a word. A word would have an activity, its meaning; a protein would have an activity, its biochemical function.

produce the purified Rec1 activity, despite prodigious efforts by many others in the Holloman laboratory and outside it. This situation was of such longstanding concern in the scientific community that it became public enough to be described in a Science magazine article published in 2002. I term this the "*Rec1 Phase*" of the controversy and disputes noted by the Defendants." (*First Decl.* par. 8.)

"Upon leaving Holloman's laboratory to perform a postdoctoral fellowship in the laboratory of Dr. Abraham Worcel, Kmiec seemingly replicated his remarkable Rec1 feats by producing data ostensibly showing that he had purified another important eukaryotic enzymatic activity known as a gyrase. The activity Kmiec purified had already been known as TFIIIA. After sustained objections from a second laboratory challenging Kmiec's TFIIIA results, Dr. Worcel had numerous members of his laboratory attempt to replicate Kmiec's results. They could not do so. In stark contrast to Holloman's relentless and self-serving support of his irreproducible Rec1 work with Kmiec, Worcel issued a very public retraction of the work which Kmiec had done on TFIIIA in his laboratory)[15]. I term this the "*TFIIIA Phase*" of the controversy and disputes noted by the Defendants." (*First Decl.*, par. 9.)

"Phases I (Rec1) and II (TFIIIA) were based upon purification of proteins (Rec1 and TFIIIA) and study of their biochemical activities. The outcome of those two phases ended negatively enough that Holloman told me when I was a graduate student in his laboratory that he was "under a cloud", of suspicion by other scientists. The reason was quite apparent. If the TFIIIA work was a near certain fraud by Kmiec which had to be retracted by his employer, and no one had replicated the Kmiec Rec1 work in nearly a decade of attempts, then it would be natural to assume that this work was fraudulent also." (*First Decl.*, par. 10.)

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[15] Kmiec's TFIIIA work became heavily challenged by the laboratory of Dr. Donald Brown of the Carnegie Institution of Washington. When several other members of Worcel's laboratory were unable to reproduce Kmiec's work, Worcel retracted the findings. Dr. Worcel wrote in his retraction of work with Defendant Kmiec, "Our laboratory has previously reported that 5S-specific transcription factor IIIA (TFIIIA) can trigger cooperative DNA gyration of 5S DNA plasmids in Xenopus oocyte extracts (Kmiec and Worcel, Cell 1985; Kmiec et. al., PNAS 1986; Kmiec et. al., Cell 1986; Kmiec and Worcel, J. Cell Biol. 1986)."

Worcel then published the following statement: "After another research group failed to reproduce these results (Wolffe et. al., Cell 49, 1987), we became aware that many TFIIIA preparations would not activate DNA supercoiling, and we began to search for conditions to produce fully active TFIIIA (Worcel, Cell 49, 302-303, 1987). During the last 18 months our laboratory has performed more than twenty TFIIIA purifications by different means. All but two of these TFIIIA preparations were active as assayed by specific 5S DNA binding and transcriptional activation of 5S RNA genes. However, none of the TFIIIA preparations induced 5S DNA supercoiling nor 5S gene-dependent TFIIIA proteolysis as previously reported. We therefore wish to state at this point that we have not been able to reproduce those results and that we cannot confirm the conclusions of these papers. "

Worcel further wrote: "We do not have a satisfactory explanation for the previous results." "What is relevant is that those observations do not represent a reproducible biological phenomenon." "We apologize for any inconvenience that the reports of the TFIIIA effects in the oocyte S-150 may have caused to other workers studying 5S RNA gene expression." Shortly thereafter, Worcel committed suicide.

"Holloman also apparently understood that a defense to views against the veracity of his prior Rec1 publications would be the determination of its gene sequence. As noted above, I and others were able to successfully isolate the gene for the Rec1 protein, i.e. *REC1*. Holloman's hopes for sequence salvation were dashed, however, when it became apparent that the *REC1* DNA sequence would not specify the protein elements common to known recombinases, such as Rec1 was purported to be. However, I and other molecular biologists working for Holloman had also cloned and sequenced a second recombination-related gene known as *REC2*. "As fortune would have it", Holloman noted in one of the grants at issue in this case, the *REC2* gene did have a sequence consistent with a recombinase. However, among other problems, Holloman and Kmiec had in two earlier publications produced data linking the Rec1 protein activity as emanating from the *REC1* gene and explicitly not from *REC2*. Therefore, Holloman was faced with the need to transform the disputed Rec1 protein activity claimed by himself and Kmiec into the Rec2 protein, which undoubtedly did exist and would be predicted to have recombinase activities. It is three specific [alleged] fraudulent actions that the Defendants took to effect this transformation of irreproducible Rec1 into the predictable Rec2 recombinase that are the focus of this case. I term this the "*Rec1-is-Rec2 Phase*" of the controversy and disputes noted by the Defendants." (*First Decl.*, par. 11.)

"Beginning in a 1996 work, and continuing to the present, the Defendants (primarily Kmiec) have published research on a technique related to the earlier phases (the recombination and repair of DNA), but with more direct therapeutic potential. This technique, termed by them "chimeraplasty", has been the subject of substantial public controversy, including as the primary focus of the aforementioned Science magazine article. However, we do not make allegations in this action regarding this [fourth] phase of concern regarding the work of the Defendants, other than to note that it is our understanding that Defendant Thomas Jefferson University was asked to investigate Kmiec's chimeraplasty work while he was employed there, but turned this request down[16]. Nevertheless, Dr. Kmiec was a known issue to the university. (Repeated red flags and warnings to Defendant Cornell about Holloman will be detailed at trial.[17]" (*First Decl.*, par. 12.)

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C. Basis for the Allegations

"... on or about November 24, 1994, in a conversation with a colleague, Hamish Young, a professor at Columbia University who had worked with me [while he had been] on sabbatical in the Holloman lab ... [Dr. Young] informed me that a "Rec1 is Rec2"

[16] "The letters to *Science* did prompt Carlo Croce, Kmiec's department chair at Thomas Jefferson, to suggest to the TJU administration that they form a "committee of investigators" to "review the data reported in [the *Science*] publication". The TJU administration, however, chose not to act on the suggestion". (*Taubes021102draft1ek2[chimeraplasty] SOL discovery*).

[17] Subsequently submitted as *Holloman misconduct allegations to Cornell in resp SOL Discovery 012808*, and within docket document 86, Exhibit B (both on the CD).

paper had been published by defendant Holloman. *See* Bauchwitz Dep. 240:1-245:23. That paper turned out to be the 1994 Paper. Dr. Young also told me that defendant Holloman had been taking "flak", so that the "Rec1 is Rec2" paper represented a "vindication." My notes show that I had considered such a claim quite unexpected. *Id*." (*Sec. Decl.*, par. 13.)

"Upon hearing [] from Dr. Young that the "Rec1 is Rec2" findings had been published, I obtained a copy of the 1994 Paper in or about late November or early December 1994. I immediately noticed that Brian Rubin – my successor in the Holloman lab -- was not listed as an author, which I found to be both unusual and disturbing, given my own prior negative experiences in scientific publishing with defendant Holloman (as summarized in Table 2 of Plaintiff's Statement of Additional Facts, and in Answer to Interrogatory No. 18 of Plaintiff's Answers to Jefferson Interrogatories)[18]. As a result, I contacted Rubin and had two telephone conversations with him about the 2004 Paper – the first on December 27, 1994, the second on February 13, 1995. I tape-recorded both conversations, and later (in February 2004) transcribed and annotated these two recordings myself." (*Sec. Decl.*, par. 14.)

False Claim 1: Rec1 protein sequence fabrication/falsification

"In our conversation on December 27, 1994, Rubin informed me that, despite working on the issue for a period of time, he and another post-doctoral fellow in the Holloman laboratory, Naoto Arai, had been unable to reproduce the results indicated in the 1994 Paper, and, given Kmiec's prior history, did not agree with its conclusions and did not want their names listed on the 1994 Paper. As Rubin stated in our December 27, 1994 conversation:

> Rubin: "So he [postdoctoral fellow Naoto Arai] came, and he tried to figure out if the Rec2 protein was a strand exchange protein. He worked on it for, well, almost two years. I'd say about two years. **And he got the same results I did. Basically, we could never show this did anything. And we purified numerous helicases from Ustilago and E. coli, and basically we were looking for a DNA dependent ATPase**. We tried to follow, that was sort of our base assay, was DNA dependent ATPase. We also did extremis. **We tried strand exchange on our purified preps, but we never got it to do anything. …**
>
> **But, so in the meantime, what Bill did was he sent my overpurification, Ok, so he sent my overexpressing strain to Eric's [Defendant Kmiec's] lab.** Because, in my heart, I kind of believe what happened was with him was, he just really wanted this result. To be a strand exchange protein. And I think what he did was he figured, well, these guys in my lab are not getting this. So I am going to send it to Eric because Eric was starting to get back into Ustilago research. So he sent him our overexpression strain. **And then Eric came up with this incredible result that, he purified Rec1 protein from Ustilago and sent it to Harvard for**

[18] Attached as *Answer to Jefferson Defendants First Interrogatories*.

> **sequencing, and several of the peptides were identical to Rec2 sequence.** I very skeptical about all this."[19]

See Transcription of Recording of December 27, 1994 Telephone Conversation with Brian Rubin, attached as Exhibit N to Defendants' Joint Statement of Undisputed Facts [at RPG 00009-00010]." (*Sec. Decl.*, par. 15.)

"Rubin stated, he was "***very skeptical about all this***". (emphasis added). I too was skeptical about the results reported in the 1994 Paper, but note that elevated ***skepticism*** about the results reported in a published research paper is not the equivalent of knowledge that a fraud had occurred, or even evidence of such." (*Sec. Decl.*, par. 16.)

"As I testified at my deposition (*see* Bauchwitz Dep. 131:21-138:15), if the situation had [] *only* been that Rubin and Arai couldn't get the same result that Kmiec purported to have gotten, I would NOT have even called ORI in 1995, as I would not have expected this to be sufficient basis for them to [] investigate. It was only in the context of their prior pattern of dishonest behavior, which preceded and was separate and distinct in nature from the current information I was hearing for the first time, that I felt an investigation by ORI was warranted in 1995. Clearly, this same consideration of a prior pattern of questionable performance had influenced Rubin, as he noted in the same conversation:

> "**This totally disagrees with my conclusions and, knowing the nature of Eric having had several problems in science, of reproducibility, I said "Just take my name off the paper."**

See Transcription of Recording of December 27, 1994 Telephone Conversation with Brian Rubin, attached as Exhibit N to Defendants' Joint Statement of Undisputed Facts." (*Sec. Decl.*, par. 17.)

"As Rubin stated, "knowing the nature of Eric [defendant Kmiec] having had several problems in science [irreproducibility of Rec1 and TFIIIA protein activities]". The pattern of prior behavior by the Defendants was integrally involved in interpreting the current unlikely scenarios that I was hearing." (*Sec. Decl.*, par. 18.)

"As a result, the information that I received from Rubin in their conversation of 12/27/94 ***did not constitute notice of a likelihood of fraud***." (*Sec. Decl.*, par. 19.)

"Upon being contacted by me in 1995, the ORI could have done an investigation and perhaps have developed such information. To start, it would have been able to

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[19] Additional evidence of related suspicions about Rec1 activity having emanated from Rec2 as Kmiec and Holloman claimed were discussed by Relator and Rubin, as in Rubin's 1995 reply to question as to why Holloman had student Ferguson attempt to produce Rec2 in a new expression system when it supposedly worked so well in *E. coli*: "Rubin: **Exactly. Why would anyone go into vaccinia if you have milligrams of overexpressed protein that is fully active**."

interview Rubin and me, as well as transcribe my recordings if required[20]. However, as it turned out, ORI apparently did not investigate." (*Sec. Decl.*, par. 20.)

"As the transcript of my December 27, 1994 conversation with Rubin makes clear, Rubin did not say anything to me to suggest that any of the defendants had sought or obtained grant funding based on the 1994 Paper. There was no basis for me to be watching for future grant applications containing "false" statements based on the 1994 Paper, since I did not have adequate knowledge that they were false." (*Sec. Decl.*, par. 21.)

What I eventually did, after learning of the later false claim published by defendant Holloman in the 2001 Paper, and deciding to investigate further, was to reconsider and analyze the circumstances of the Rec1 protein sequence in more detail. ***It occurred to me that*** for the Rec1 protein sequence to have been as the Defendants claimed (identical to that expected for Rec2), data might have been available at a laboratory external to theirs which had performed part of the work claimed. In other words, as I explained in detail in my July 26, 2004 FCA Disclosure to the ORI (Exhibit U to Defendants' Joint Statement of Undisputed Facts), and in the First Bauchwitz Declaration, ***it might be the case that purified Rec2 protein made from bacteria and submitted as if Rec1 protein purified from fungi would show evidence of bacterial rather than fungal protein background***. (*Sec. Decl.*, par. 22; emphasis added.)

"To pursue this possibility, on April 30, 2003, I contacted the Harvard Microchemistry laboratory, the outside laboratory defendants claimed to have employed. *See* Bauchwitz Dep. 296:21-298:23. Amazingly, I never even got to the point at which data could be identified for subsequent analysis (bacterial vs. fungal origin). Instead, I received information that ***led me to conclude that there was a very high likelihood that the Defendants had never sent anything at all to that laboratory in the relevant time frame***. Consequently, *at that moment in 2003, a* ***suspicion*** *about whether Rec1 could have been Rec2, which had been reported to authorities in a timely manner in 1995, had upon my investigation* ***led to evidence of a potential fraud - a lie***." (*Sec. Decl.*, par. 23.)

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False Claim 2: falsification of *rec2-1* mutant DNA sequence

"As Rubin and I discussed in our February 13, 1995 telephone conversation:

[20] I made recordings of my conversations with various Holloman lab members in 1994-1995 in order to prevail in any "he-said, she-said" controversy. It was my strong hope that upon my notice to ORI, they would investigate. As part of such investigation, I expected that I would be contacted by investigators from the institutions or ORI itself. I took notes during the conversations, but as a scientist, I was well aware of the utility of the best quality data. Recordings of what actually was said was in my view the most accurate, best quality data that could be presented to investigators, and also the best defense for me to challenges that what I wrote in my notes was not accurate. However, no investigation was forthcoming, so far as I have ever learned.

Bauchwitz: "Another question I had ***out of curiosity***, this is probably very simple but, you show the sequence and the map of the deletions. ***Where is the methionine, the ATG***, where would that be that you could start the truncated message? Because the the Kmiec paper they give the whole story about how this rec2-1 smaller message produces a smaller truncated protein."

Rubin: "Oh, OK. So ***its methionine is gone***. What they would ***postulate*** is that you picked up a methionine that is downstream of the deletion. So it just basically starts at the first methionine."

Bauchwitz: " … there aren't any methionines until after the recA and Walker A homology sites. In fact it is two amino acids after, there is not even AT [or G except] immediately after the [] site."

Rubin: That's interesting. ***I never really thought about that*** but,"

Bauchwitz: "Oh so there wasn't actually a methionine in mind."

Rubin: "Oh no, no, they wouldn't, they ***hand waved***. If you brought that, what you just told me up, I think what they would probably say is "Oh, it must read an upstream methionine then, that is basically to the left of the deletion."

See Transcription of Recording of February 13, 1995 Telephone Conversation with Brian Rubin, attached as Exhibit P to Defendants' Joint Statement of Undisputed Facts [at RPG 00001-00002]." (*Sec. Decl.*, par. 25.)

"This key interchange clearly indicates that ***Rubin had not even considered the relevance of the rec2-1 start site*** as being in a position in which it would have eliminated activity from the protein which was claimed by Kmiec and Holloman, in seeming contradiction to earlier data, to be active (*in vitro*). Rubin's response to me was that "***hand waving***" would have been the likely response of Holloman and Kmiec to the questions I was raising." (*Sec. Decl.*, par. 26.)

"Rubin's characterization of the likely response of Holloman and Kmiec to my questions as "hand waving" did not suggest to me any likelihood of fraud. Clearly, my conversations with Rubin did not suggest or place me on notice of the second alleged fraud (falsification of rec2-1 sequence)." (*Sec. Decl.*, par. 27.)

"What is much more important in the conversation with respect to this second fraud is when I asked Rubin ***where to find his rec2-1 sequence*** (and the comparable region in REC2), as noted in this exchange:

Rubin: ***You know what is funny is***, that somehow the, I actually did notate that the rec2-1 mutant, that on this figure originally, Figure 1, and uh, in the legend, 5 the description, I am looking at my thesis right now, and it was the same figure,

and it is in there. Bauchwitz: Oh, it is? Rubin: ***Bill must have taken it out for some reason***." (*Sec. Decl.*, par. 28.)

"So it seemed to Rubin ***an oddity*** that Holloman had removed the sequence of interest from his own 1994 publication with Rubin (a distinct publication from Kmiec et. al., 1994, from which Rubin had removed his name). In 2005, ORI would note to the Department of Justice that Holloman many times alluded to having published the relevant sequence but did not actually do so (*See* ORI Review of Complaint at 5, Exhibit TTT to Defendants' Joint Statement of Undisputed Facts). Indeed, even the comparable region of REC2 (wild-type gene) was not given in Rubin's thesis. ***These numerous omissions by defendant Holloman made me suspicious enough*** that I endeavored to produce the sequence myself, which I published in the federal Genbank database in 1999. Again, suspicion led to investigation. Investigation led to information that put me in a position to perceive a lie (fraud) when it was eventually published in 2001." (*Sec. Decl.*, par. 29.)

"Why was Holloman seemingly hiding the relevant rec2-1 sequence? Neither Rubin nor I knew in 1995 that ***in 2001 Holloman would publish an explicit falsified statement about that sequence*** as part of a cover-up of the Rec1 is Rec2 situation. *See* Bauchwitz First Declaration, ¶ 26." (*Sec. Decl.*, par. 30.)

"In fact, if I had not produced and by 1999 published my own relevant *rec2-1* sequence, ***in 2002 I still would only have had suspicions and concerns about what Defendant Holloman had written in 2001***, since I would not have had access to any sequence from Rubin (1994 Paper or 1995 sequence) or Holloman (Genbank database – no actual entry; *e.g. see* Exhibit 7 to Plaintiff's Statement of Additional Facts [Doc. 90]). Therefore, it was my own investigation of the rec2-1 sequence which gave me the specialized knowledge that enabled me to detect and understand the patent lie published in the 2001 Paper. It was at that moment that this *qui tam* case began. *See* Bauchwitz First Declaration, ¶¶ 26-28." (*Sec. Decl.*, par. 31.)

"The only question with respect to alleged frauds 1 and 2 would seem to be ***whether I had any duty in 1995*** - long before I even knew what a *qui tam* action was, or had even considered whether defendants might seek *future* federal grant funding based on the False Claims 1 and 2 – to act as a perpetual investigator and monitor of future grant applications by the defendants, based solely on my ***skepticism*** about the results reported in the 1994 Paper. I respectfully submit that that this cannot be the case. To the contrary, I believe that in 1995 I went above and beyond the call of duty by notifying ORI of continuing suspicious circumstances that warranted investigation, and subsequently developing my own rec2-1 sequence, when nothing by Rubin could be found." (*Sec. Decl.*, par. 32.)

"Once I developed solid evidence of lies -- of frauds -- as I understood them, in the spring of 2002, I took ***very timely*** action to obtain relevant grants by the end of 2002. My continued investigation led to additional findings of fraud in 2003 and 2004, resulting in the filing of my original Complaint on June 30, 2004, ***less than two and a half years***

after I first saw what I knew to be a published false statement by one of the defendants (in the 2001 Paper)." (*Sec. Decl.*, par. 33.)

"In 1995, I more than discharged any duty on my part to act by alerting ORI to ***suspicious circumstances***. I did not give them specific allegations of fraud ***because I myself did not have them until 2002***, as detailed above. ORI has clearly stated the same, i.e. that I never gave them specific allegations of fraud until our meeting in July 2004 (*see* ORI Time Line, Exhibit 10 to Exhibits to Plaintiff's Statement of Disputed Facts and Plaintiff's Statement of Additional Facts [Docs. 89 and 90])." (*Sec. Decl.*, par. 34.)

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False Claim 3: falsification of Rec2 protein activity

"In our December 27, 1994 telephone conversation, Rubin also made the following comments:

> Rubin: "I think of him as just a guy that develops stories in his office and then comes into the lab and says produce the data that fit my stories. And ***in fact in that paper, there are figures straight from my thesis that have totally nothing to do, really, with what was published***."
>
> Bauchwitz: "What do you mean by that?"
>
> Rubin: "Well like the protein, he [Defendant Holloman] used my purification gel and Western showing the anti-Rec2 antisera binds this protein."
>
> Bauchwitz: "Yeah."
>
> Rubin: "And ***then he claims that this protein is active***. And he would ***rationalize it*** by saying 'Well, it's just a better looking gel than Eric's [Defendant Kmiec]' Of course, we are using the same strain, ***but that prep wasn't active***.
>
> Bauchwitz: "But the gel is of yours, then it really isn't of the same prep that he used to show activity."
>
> Rubin: "Exactly."
>
> Bauchwitz: "He was accused of that, ***I am not sure if that is the right word***, but Toyoko[21] said that he did that repeatedly with Rec1. There would be some crude fraction of unknown meaning that had some activity that he was ascribing to some purified band."
>
> Rubin: "Exactly."

[21] "Toyoko" was a senior graduate student in the Holloman lab while I worked there.

Bauchwitz: “A decade ago.”

Rubin: “This is more of the same.”

See Transcription of Recording of December 27, 1994 Telephone Conversation with Brian Rubin, attached as Exhibit N to Defendants’ Joint Statement of Undisputed Facts [at RPG 00011-00012]." (*Sec. Decl.*, par. 35.)

"The third fraud claim alleges falsification of a data image in the 1994 publication. The key issue as to whether I was put on notice of this claim from my December 1994 telephone conversation with Rubin is what I derived (or a reasonable person in my position would have derived) from the conversation. My language in the call indicates that Rubin’s passing comments about his gels did not trigger any immediate suspicion of fraud. Neither Rubin nor I mentioned the word “fraud”. Instead, both Rubin and I concluded that the behavior was “***more of the same***”, i.e. consistent with behavior which had generally been associated with Defendant Holloman by those who had worked for him. We then returned to the topic of the call, namely how Rec1 could be Rec2 and why Rubin (and Arai) had removed themselves as authors from this claim. While I do not recall mentioning any of this information about the misuse of Rubin’s gels in my call to ORI in early 1995, I believe that I more than discharged any duty to generally bring information of the suspicious circumstances surrounding the 1994 Paper to the attention of authorities (ORI)." (*Sec. Decl.*, par. 36.)

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D. Timeline and Handling of the Case by the Office of Research Integrity

During the summer of 2000, a journalist, Gary Taubes, contacted me regarding an investigative article he was writing about the (future) defendants, for which Taubes sought assistance. Upon completing his work (*Taubes2002Chimeraplasty Exhibit*, in the Case Documents folder on the CD), the journalist suggested to the federal government’s Office of Research Integrity (ORI) that they contact me to consider a misconduct investigation.

I was leery of working with the ORI based on my prior experiences in reporting concerns about possible misconduct to them and their predecessor, OSI. Those contacts were summarized in tables in the case document, *Additional Facts* (also in the Case Documents CD folder):

Table 2. STATEMENTS TO OSI 1990

Statement		Type	Research Topic	
1	Harm to students by Defendant Holloman, apparently because they could not adequately reproduce potentially	Serious concern	Rec1 protein purification and activity (“Rec1”)	

	irreproducible work by an earlier graduate student (Defendant Kmiec) who had his subsequent postdoctoral work retracted			
2	Misconduct in research publishing by Defendant Holloman	Allegation	REC1 cloning, REC2 cloning	
3	Were negative data omitted in claims made in new NIH grants?	Suspicion	rec2-1 homologous integration issue	

Table 3. STATEMENTS TO ORI 1995

Statement		Type	Research Topic	
1	What became of the concerns and suggestion for investigation made to OSI in 1990?	Follow up	1990 1-3 (see Table 2)	
2	Defendants have now published a paper under highly suspicious circumstances, based on results purportedly obtained by Defendant Kmiec but which two members of Defendant Holloman lab were unable to obtain. (Reminiscent of the Rec1 situation reported to OSI in 1990.) Prior scientific findings are not consistent with the published claims and suggest the new claims are highly unlikely to be true.	Suspicion	Regarding research publication: Kmiec et. al., 1994 (MCB) ("Rec1 is Rec2")	

"Regarding the events which specifically led to this legal action, I provide the ***following timeline***: On February 9, 2002, science journalist Taubes brought to my attention a new article published by Defendant Holloman (Kojic et. al., MCB, 2001, hereinafter, Kojic et. al. 2001) which pertained to a mutant DNA sequence of interest in this case ("*rec2-1*"). After eventually obtaining and reading Kojic et. al., 2001, I saw what I knew to be a clearly false statement made by Defendant Holloman regarding the mutant sequence:

> "Inspection of the *rec2-1* DNA sequence on both sides of the deletion indicated that a novel ORF could be generated through conjunction of the flanking sequences. This ORF would be predicted to encode a 613 amino acid Rec2 protein variant with a novel 19-residue leader sequence derived from upstream of the wild-type Rec2 protein."

I also realized that this false statement was meant to provide cover for the earlier incredible claims made in Kmiec et. al., 1994, (namely that the Rec1 protein activity had actually emanated from the *REC2* gene). Specifically, the motivation for this false statement was to explain explicitly how the Rec1 protein Defendant Holloman had originally published with Defendant Kmiec could have been derived from a different gene (the *REC2* gene), despite genetic and other evidence to the contrary." (*First Decl.* par. 26.)

"Furthermore, according to information elicited by me from Rubin, Holloman knew of the true state of the mutant gene in question prior to his false representations in 2001. ... " (*First Decl.* par. 38.)

"... On April 30, 2003, I called the Harvard University Microchemistry Facility. I spoke with intake specialist Liam McCallum. McCallum told me that the last record for Kmiec was a user id on December 31, 1992. With respect to the other two authors with Defendant Kmiec, there were no records for Allyson Cole, and the first record for Defendant Holloman was not until 1995 (after the date of acceptance for publication of Kmiec et. al. MCB 1994 on July 28, 1994). McCallum noted that the Kmiec record had "**no activity on it**". He said that it remained their practice to give anyone who contacts them a user id. Contemporaneous records from other researchers did indicate activity. He noted that ***it was possible that further records, if any, (such as invoices sent for services rendered) existed at an "off-site storage area"***. I felt that this information was obviously consistent with the amino acid data having been fabricated[22]. (*First Decl.* par. 27.)

As it turned out during discovery, Harvard had indeed retained complete paper records in its archive facility. Their records indicated that protein microsequencing *had* been performed at Harvard for Kmiec during the period in question. The records also indicated that the reason the intake specialist did not see relevant entries was that the electronic database had been changed shortly after that time.

Nevertheless, and most importantly, the documents released by Harvard also clearly indicated that the **Harvard Microchemistry laboratory had not produced the data claimed by Kmiec and Holloman in their paper** KCH1994, and in associated federal grants. [See New Evidence, Section E., below.]

The Court has argued that I had a duty to call Harvard's Microchemistry Laboratory within a specified time of being put on notice of fraud. As noted above, it is arguable as to what conclusions about fraud were reasonable at the time of the reading of the 1994 publication and speaking with Rubin. However, it is also **important to note** that when I ultimately did contact Harvard's Microchemistry Laboratory, ***its director did not release any records nor even confirm that his facility had produced the findings claimed***. Therefore, **contrary to the Court's implication that such information would be readily available** for those who contacted the third party, ***in reality the use of subpoenas was ultimately vital*** to resolving the nature of what data had or had not been obtained.

With respect to the third allegation, "After many months of attempting to have commercial firms provide the transcriptions, I myself completed them on January 27, 2004." (*Sec. Decl.*, par. 14.)

[22] In my Disclosure to the government, I noted that had any protein Defendants Kmiec and Holloman might have sent to the facility actually been produced from the bacterium *E. coli* rather than the fungus *U. maydis* - the latter as claimed in Kmiec et. al., 1994, then evidence of such data falsification could have also been observed in subpoenaed records.

Ultimately, ORI agreed to proceed on the basis of a *qui tam* suit. The case *United States ex rel Bauchwitz v. Holloman et. al.,* No. 04-2892 (E.D. Pa.) was filed under seal on June 30, 2004 in federal district court in the Eastern District of Pennsylvania (the location recommended by the ORI). The ORI was to produce a report for the Department of Justice on the science involved.

Back to TOC.

ORI Statements and Responses

ORI produced a report for use by the Department of Justice (DOJ), which was received by me via DOJ on January 12, 2005. In that report, the first of what collectively have come to be called the "**ORI documents**", ORI concluded that each of the Plaintiff's allegations had merit.

However, ORI also noted what they believed could be two primary issues in pursuing the case: 1) ORI thought there might be no additional evidence available beyond that which the Plaintiff had brought to the government, and 2) ORI claimed that it might not be possible to prove intent for one of the false claims alleged. (The complete ORI documents are available at court docket Doc. 90 attachment 9 and Doc. 90 attachment 10 of 4/16/08 and on the enclosed CD).

The following are excerpts from the conclusion of ORI's first memorandum of November 23, 2004 [with emphasis added]:

> i. "***Each [of the Relator's claims] has some merit***, but all lack definitive proof of being deliberate falsifications, and ORI does not believe that evidence is available to provide such proof."
>
> ii. "Dr. Bauchwitz' complaint identifies three false claims, as identified above. ***ORI notes that these false claims deal with only a very small portion of the much larger scope of possible misconduct issues that have been linked to Drs. Kmiec and Holloman*** (see footnote 8). The reason for this is that Dr. Bauchwitz has limited his claims to issues that he has direct knowledge of. He has made a solid case that the 'story' on *Ustilago maydis* recombination genes, their associated proteins and their enzymatic properties has shifted dramatically over the past 20 years. Many scientists working in this area appear to have believed that erroneous claims have been consistently published by Drs. Holloman and Kmiec."
>
> iii. "Even if it could be shown that some of the grant applications unequivocally contain the false statements described in the complaint, ORI believes that the evidence is inadequate and generally unobtainable to prove that the questioned statements are intentionally false".

With respect to ORI's purported concern about not finding additional evidence, my attorney responded that ***ORI had made no attempt to obtain such evidence***. There was then correspondence back and forth as to the general lack of investigation and what should have been done to investigate. Excerpts of communications and reviews related to the ORI's handling of the case and general failure to investigate, are provided in the endnotes (i).

Ultimately, contrary to the ORI's stated concerns, additional, important evidence *was* obtained during the limited discovery that was performed in the case. That evidence and its assessment are presented in the following sections.

Back to TOC.

E. New Information Obtained During General Discovery

I. Amino Acid Sequence Data Fabrication/Falsification - First Allegation

Holloman has claimed repeatedly that he has identified the Rec1 strand exchange activity [KSSEP][23] as being produced by the Rec2 protein. The claim is based on peptide sequencing he and Kmiec published in Kmiec, Cole, and Holloman, 1994 ("KCH1994"):

> **Amino acid sequence analysis has established that the homologous pairing protein of *Ustilago maydis*, known previously in the literature as rec1, is encoded by *REC2*, a gene essential for recombinational repair and meiosis with regional homology to *Escherichia coli* RecA. The 70-kDa rec1 protein is most likely a proteolytic**

Selected "Rec1 is Rec2" Grant Claims making similar claims (as anonymized for expert reviewers)[24]:

> "From one, **peptides were isolated after digestion with trypsin and amino acid sequencing was performed on five isolated peptides**. From the second, N-terminal sequencing was carried out. All the amino acid sequences obtained were found to be contained within the R2 open reading frame (**K et. al., 1994, see Appendix**)".
> GM042482-08 1996 – **2001** $3, 487, 424

> "We obtained **amino acid sequence of peptides** derived from the 70 kDa protein purified from fungus XX. As fortune would have it the sequence information obtained corresponded precisely to R2, a gene which had also been cloned and was under study in the laboratory (R and H, 1990; B et. al., 1994; **K et. al. MCB 1994; Appendix**)"
> GM53732-01 1996 – **1999** $1,127,533

[23] "KSSEP" refers here to an apparently "Kmiec-specific strand exchange protein" activity, which was originally purported to derive from the *REC1* gene (hence called "Rec1"), but subsequently from the *REC2* gene.
[24] The GM42482 and GM53732 grants are available as pdf files on the CD. The AR grant is available in the court docket online through PACER (document 86, Exhibit S).

"However, despite the name and the absence of detectable activity in extracts from the r1 mutant, the R1 protein is encoded by the r2 gene (25)."
AR44092-01 1996 – **2000** $432, 657

The amino acid sequences claimed in Kmiec, Cole, and Holloman, 1994 are shown in a figure from that publication:

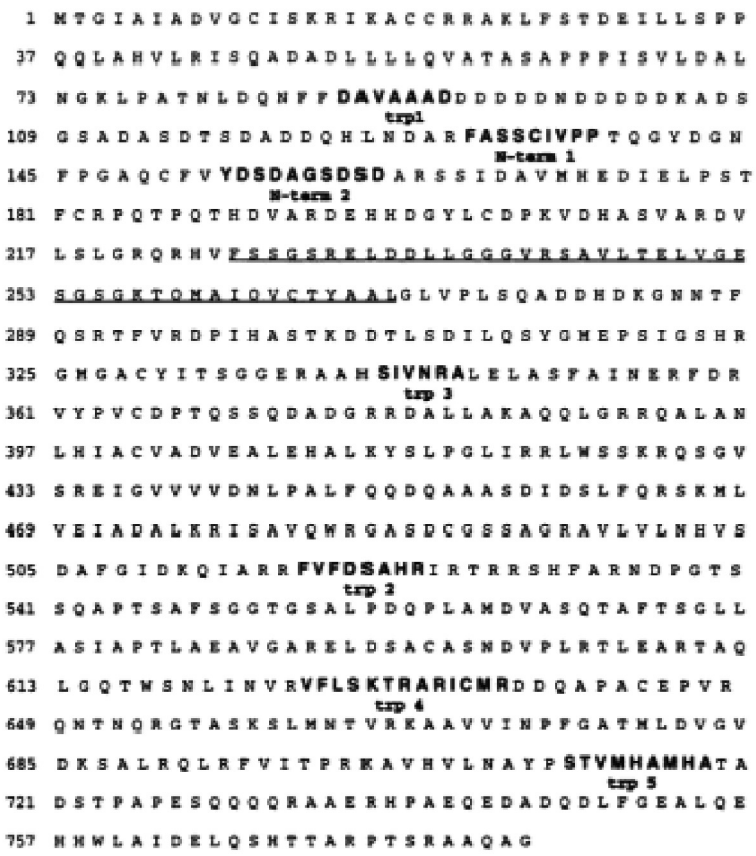
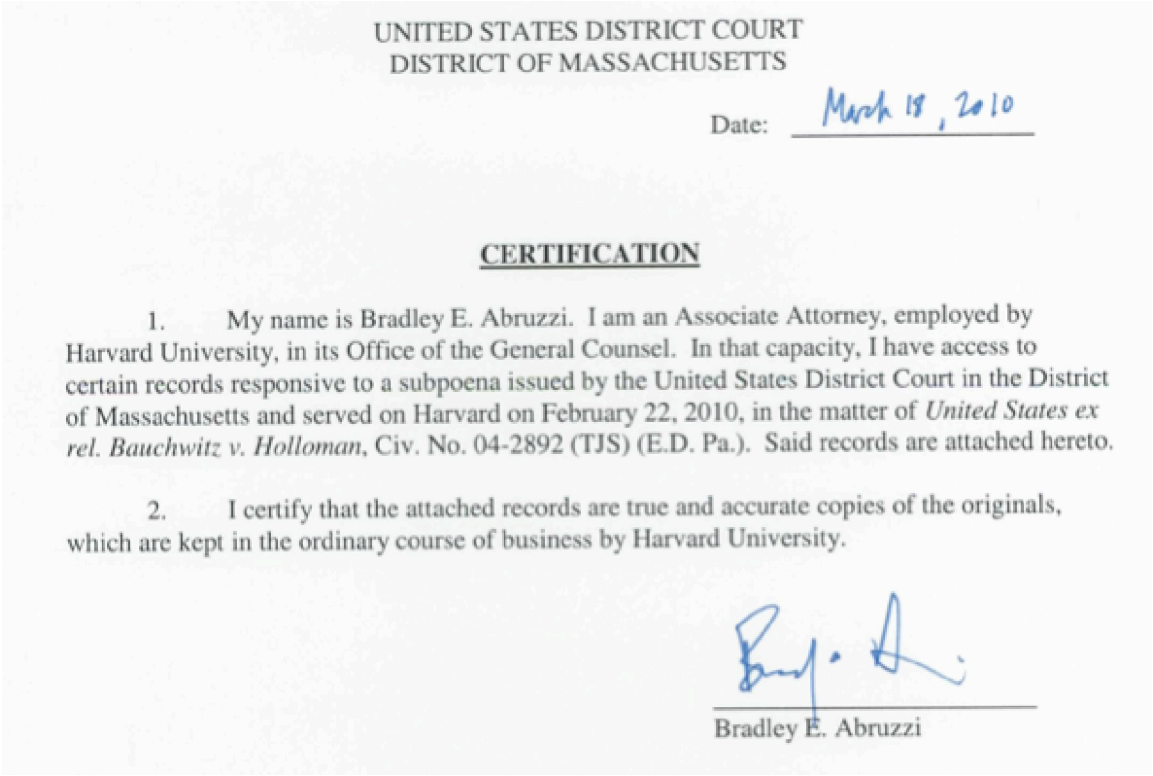


FIG. 1. Amino acid sequence alignments of peptides from the homologous pairing protein and the REC2 gene product. Trypsin digestion, peptide separation, and amino acid sequence determination were performed as described in Materials and Methods. The corresponding sequences in the *REC2* gene are indicated in boldface. Sequences designated trp were from tryptic peptides. Those designated N-term were from N-terminal sequencing of the doublet members migrating at an M_r of 70,000 after SDS-gel electrophoresis. Also presented for orientation (underlined) is the sequence with strong homology to a sequence in RecA protein which spans the ATP binding loop motif (residues 251 to 258).

As reviewed in the Basis for the Allegations section, above, I considered the "Rec1 is Rec2" claim highly suspect for several reasons. Among the most important reasons was that "Rec1" [KSSEP] activity was found in rec2-1 mutant cells, but not rec1 mutant cells. Yet rec2-1 mutants appeared to be functional nulls when I had worked with Holloman. My sequencing of the *rec2-1* allele after I had left Holloman's lab had strongly indicated that any protein fragment produced by it would not have strand exchange activity. (See also Section E.II., below.)

Therefore, records of the peptide sequencing of Rec1 strand exchange protein claimed in KCH1994 were sought during general discovery in the *qui tam* science fraud case.

The Harvard Microchemistry Laboratory had been cited for peptide sequencing in KCH1994, as had Thomas Jefferson's Cancer Center. Only Harvard's Microchemistry facility produced relevant research records. Kmiec had been employed by Thomas Jefferson University.



UNITED STATES DISTRICT COURT
DISTRICT OF MASSACHUSETTS

Date: March 18, 2010

CERTIFICATION

1. My name is Bradley E. Abruzzi. I am an Associate Attorney, employed by Harvard University, in its Office of the General Counsel. In that capacity, I have access to certain records responsive to a subpoena issued by the United States District Court in the District of Massachusetts and served on Harvard on February 22, 2010, in the matter of *United States ex rel. Bauchwitz v. Holloman*, Civ. No. 04-2892 (TJS) (E.D. Pa.). Said records are attached hereto.

2. I certify that the attached records are true and accurate copies of the originals, which are kept in the ordinary course of business by Harvard University.

Bradley E. Abruzzi

In a letter from Holloman to William S. Lane, Director of the Harvard Microchemistry Facility on July 10, 2007, Holloman identified the date on which he and Kmiec claim that Harvard performed the microsequencing presented in KCH1994.

Is there any possibility that you might have some record of tryptic peptide sequencing results that were performed on a sample (EBK1-CT.D) on or about 10 September 1993. I have attached an old fax copy of an HPLC profile of tryptic peptides of that sample that was performed by your facility which was sent to my collaborator, Dr. Eric B. Kmiec, who was on the faculty at Thomas Jefferson University Medical School at that time. According to his and my records the five indicated peptides were sequenced on an ABI 477A sequencer and the following sequences were obtained.

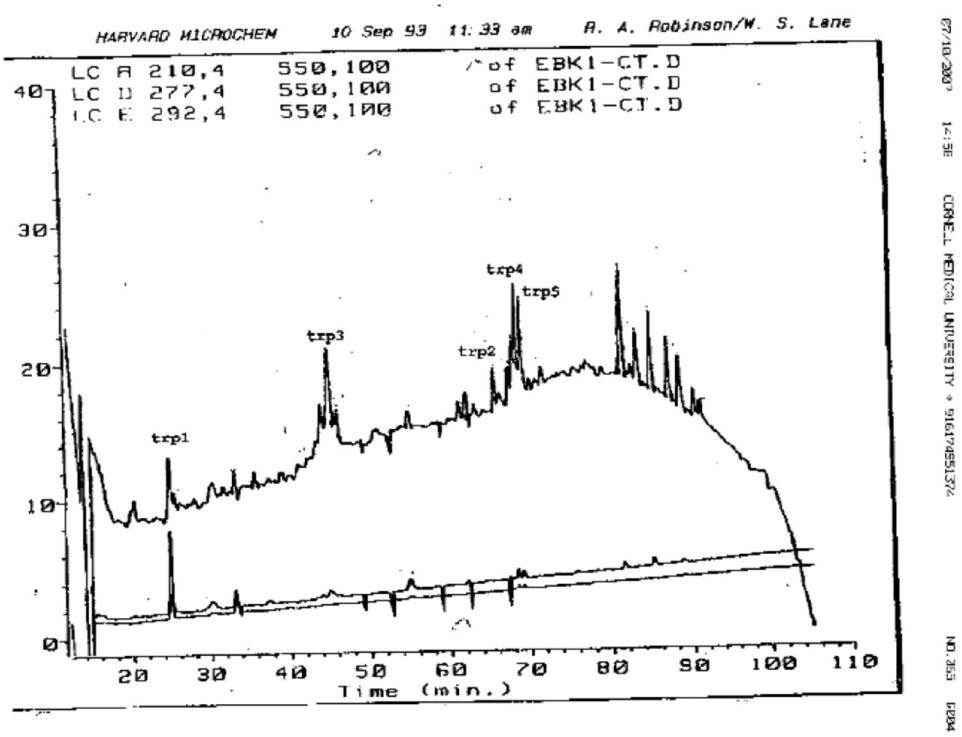
Trp1 DAVAAAD
Trp2 SI(V/M)N/DA
Trp3 FVFD(S/A)A(H/G)R
Trp4 (V/G)(F/Y)LSKTR(A/T)RIC(M/G)R
Trp5 S(?/T)(V/M)MH(A/D)MHA

Kmiec and I published this in Mol. Cell. Biol. 14: 7163-7172 (1994). Unfortunately, we do not have the original fax transmission of the sequences that you sent to Kmiec

Holloman also provided to Lane an HPLC profile of tryptic peptides[25], labeled with “trp” designations, which Holloman claimed his records indicated that Harvard had produced for him and Kmiec on September 10, 1993.

The profile of the peptides sent to Lane by Holloman is shown below. It is important to note that someone[26] had labeled five of the peaks "trp1" through "trp5". Those designations correspond to the peptides listed in Holloman's letter to Lane, and to what Holloman and Kmiec claimed in their 1994 paper that Harvard had produced for them.

[25] HPLC stands for high performance liquid chromatography. Chromatography is a method of separating molecules. In this case, the molecules being separated were fragments of protein, called peptides. The peptides had been produced by digestion of protein samples by an enzyme called trypsin. The protein supplied to Harvard came from Kmiec and was purportedly from a purification of "Rec1" (KSSEP).
[26] Almost certainly Holloman or Kmiec, but not the Harvard laboratory, as they state below.



However, documents obtained from Harvard University during discovery showed that ***only three peptides had been sequenced, not five as claimed by Holloman***:

To: William Holloman, Ron Sarachan
Email: wkhollo@med.cornell.edu; sarachan@ballardspahr.com
Fax: 212-746-587, 215-864-8333
Voice: 212-746-6510
Date: 9/13/07

From: John Neveu
Voice: 617-495-4043
Fax: 617-495-1374

Dr. Holloman, Mr. Sarachan,

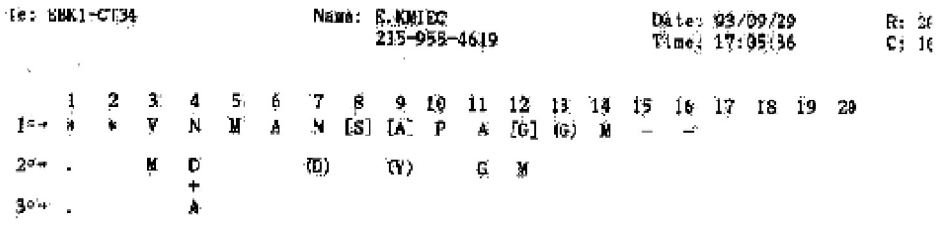
Find here copies of the EBK1-CT sample HPLC chromatogram and Edman sequencing reports which I retrieved from our off-site storage area. The data sent started with the HPLC chromatogram which was FAX'ed to Dr. Eric Kmiec on 9/10/93. From this chromatogram, three peaks were sequenced, numbers 34, 61 and 73. The report for EBK1-CT73 (peak #73) was sent by FAX to Dr. Kmiec on 9/28/93, EBK1-CT34 was sent on 10/5/93, and EBK1-CT61 on 10/21/93.

Please review these results and then feel free to call with any questions you may have regarding them.

Sincerely,

John Neveu

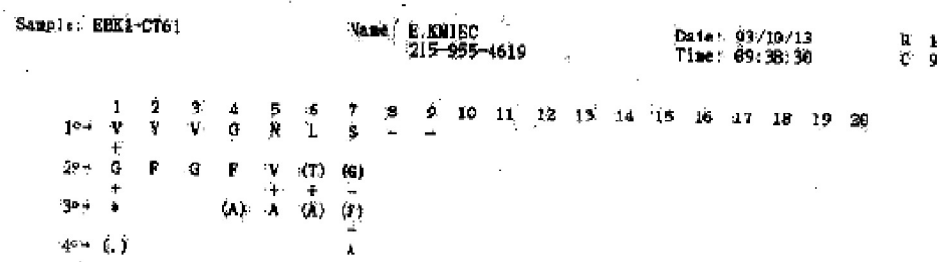
The actual data produced by the Harvard Microchemistry Laboratory is shown below. It does not show the five printed "trp" labels Holloman had on his version of the data, but rather has check marks over three of the peaks (numbers 34, 61, and 73).



```
le: EBK1-CT34                    Name: E.KNIEC                    Date: 93/09/29        R: 2
                                       215-955-4619               Time: 17:05:36        C: 1

     1   2   3   4   5   6   7   8   9  10  11  12  13  14  15  16  17  18  19  20
1=>  N   *   V   N   M   A   N  [S] [A]  P   A  [G] (G)  M   -   -
2=>  .       M   D           (D)      (Y)      G   M
                 +
3=>  .           A
```

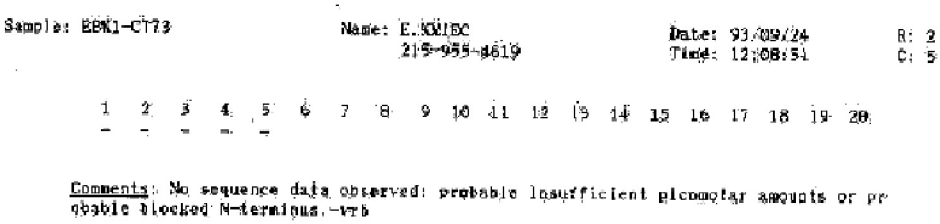
EBK1-CT34 amino acid data from Harvard Microchemistry



```
Sample: EBK1-CT61                Name: E.KNIEC                    Date: 93/10/13        R: 1
                                       215-955-4619               Time: 09:38:30        C: 9

     1   2   3   4   5   6   7   8   9  10  11  12  13  14  15  16  17  18  19  20
1=>  V   V   V   G   R   L   S   -   -
     +
2=>  G   P   G   F   V  (T) (G)
     +                  +   -
3=>  *               (A)  A  (A) (P)
                             -
4=>  (.)                     A
```

EBK1-CT61 amino acid data from Harvard Microchemistry

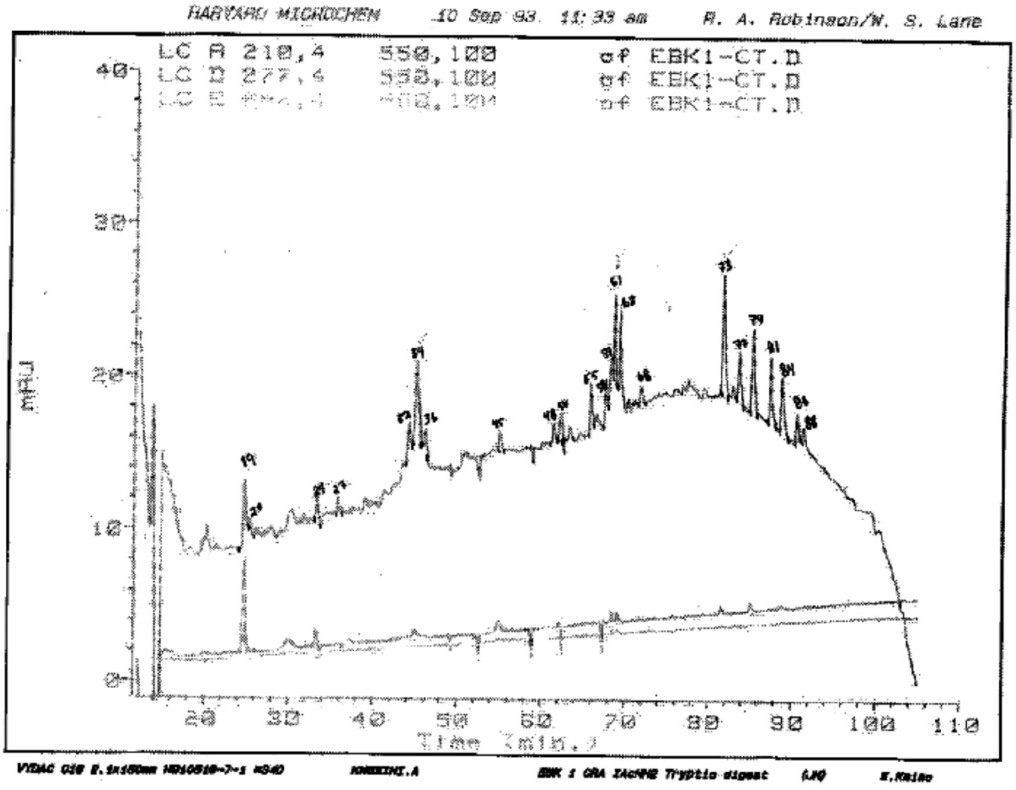


```
Sample: EBK1-CT73                Name: E.KNIEC                    Date: 93/09/24        R: 2
                                       215-955-4619               Time: 12:08:51        C: 5

     1   2   3   4   5   6   7   8   9  10  11  12  13  14  15  16  17  18  19  20
     -   -   -   -   -

Comments: No sequence data observed: probable insufficient picomolar amounts or pr
obable blocked N-terminus.-vtb
```

EBK1-CT73 - no amino acid data observed - from Harvard Microchemistry



In comparing the profile sent by Holloman to Lane with the profile Harvard claims it actually produced, it can be seen that:

1) peaks 34 and 61 correspond to those labeled by Holloman and Kmiec as “trp3” and “trp4”,

2) peak 73 was not listed by Holloman as having been sequenced, and,

3) Harvard does not note having sequenced peaks labeled “trp1”, “trp2”, or “trp5” by Holloman.

Of even greater importance is that ***Harvard also produced copies of the actual sequence data that they had provided to Holloman and Kmiec in 1993***. They gave this information to Holloman in 2007 in response to his inquires (e,g, the letter noted above), and to the court handling the False Claims Act action in 2010.

Copies of the sequence data for the three peaks is provided in the file, "*Harvard Amino Acid Sequences*" (on the CD). Also shown in those documents is a data form indicating that Harvard had tried to directly sequence the (presumably Rec1/KSSEP) protein as received from Kmiec. However, Harvard obtained no sequence, which is what

led to their digesting and purifying fragments from the protein Kmiec had sent to them. (All of the samples have an "EK" or "EBK" designation.) Thus, tryptic peptides from sample EK1 were subsequently HPLC purified to produce peaks EBK1-34, -61, and -73.

The following list summarizes the amino acid sequences claimed by Holloman in his letter to Harvard, those that were actually produced by Harvard upon subpoena, and those published in Figure 1 of Kmiec, Cole, Holloman 1994[27]:

trp1	DAVAAAD
trp2	SI(V/M)N/DA
trp3	FVFD(S/A)A(H/G)R
trp4	(V/G)(F/Y)LSKTR(A/T)RIC(M/G)R
trp5	S(?/T)(V/M)MH(A/D)MHA

EBK1-CT34	(V/M)(N/D/A)MA(N/D)S(A/Y)P(A/G)(G/M)(G)M[28]
EBK1-CT61	(V/G)(Y/F)(V/G)(G/F/A)(N/V/A)(L/T/A)(S/G/?)
EBK1-CT73	"No sequence observed" was the result sent by Harvard to the defendants.

trp1	DAVAAAD
trp2	FVFDSAHR
trp3	SIVNRA
trp4	VFLSKTRAR
trp5	STVMHAMHA

The **key conclusion** is that the sequences claimed to have been sequenced do not match those that Harvard says it actually produced for Holloman and Kmiec.

Indeed, Harvard actually stated this explicitly in their 2007 review of the work they had performed for Kmiec:

"**These sequences are not consistent with the data we provided.**"

[27] trp3 comes before trp2 in the actual Rec2 sequence, which is what was used to provide the more exact sequences in the paper.
[28] The amino acid sequences underlined in peaks 34 and 61 correspond to a single predicted *U. maydis* peptide, but it is not Rec2; see expert analysis below.

-----Original Message-----
From: John Neveu [mailto:jneveu@mcb.harvard.edu]
Sent: Thursday, September 13, 2007 4:32 PM
To: 'Mendes, Mary Ann'
Cc: 'William Lane'
Subject: RE: Data Request/Cornell

Ms. Mendes,

Find attached here a document (Holloman W 3963 EBK1-CT34 61 73 HPLC and Edman Report.pdf) I have prepared for Dr. Holloman from Cornell in regards to the data from 1993 he requested from us. This is exactly what was FAX'ed to the researcher on record, Dr. Eric Kmiec, and should suffice to fulfill Dr. Holloman's request.

Also included here for your records is a copy of a letter sent to us on July 10, 2007, from Dr. Holloman (Holloman Letter July 10 2007.pdf) in which he requests a copy of the original data from 1993. In this letter, he lists the amino acid sequences that he and Dr. Kmiec published in 1994 (Trp1-5). Note that these sequences are not consistent with the data we provided him, leading me to think that they are barking up the proverbial wrong tree for support in his serious legal situation. Also included was a HPLC chromatogram that was published with the sequence data that has been modified to reflect peaks from which the sequences he claims to have come from us were obtained. Note that in our original data, peaks #'s 34, 61 and 73 were sequenced (the pencil checks are visible) leading to peptide sequences EBK1-CT34, EBK1-CT61 and EBK1-CT73 (which produced no sequence data) as reported to Dr. Kmiec at that time.

The complete email is presented in the *Harvard Amino Acid Sequences* document.

The Harvard Microchemistry Laboratory also made it clear that ***there was no potentially relevant missing data***, by writing to the Harvard General Counsel:

> **"...none of the sequence data we obtained agrees with the data they claimed was from our lab."**
>
> **"I am confident that there is no other data".**

From: John Neveu [mailto:jneveu@mcb.harvard.edu]
Sent: Friday, December 07, 2007 3:39 PM
To: Lopez, Diane
Cc: Lane, William; Busby, George, III
Subject: RE: Harvard Microchemistry Reports
Sensitivity: Private

Diane,

I have prepared a second report (attached here) which can be sent to Dr. Holloman and the Cornell general council office. This data does not appear to support Dr. Holloman and Dr. Kimec in their quest to obtain confirmatory data for work published in 1994, as none of the sequence data we obtained agrees with the data they claimed was from our lab. I am confident that there is no other data from either researcher in our archives, as I searched from 1990 through 1995 and found only what is reported here in addition to the previous report.

Please feel free to get back to me with any questions you may have.

12/11/2007

Therefore, the five peptides Holloman and Kmiec claim in their paper, KCH194, and in subsequent grants as having been sequenced at Harvard either were not those actually isolated (trp 1, 2, and 5) or did not have the sequences claimed (trp 3, 4).

Back to TOC.

Expert Assessments of the Harvard Amino Acid Sequence Situation

Three expert assessments were made of the data obtained from Harvard. The principal issue was whether any of the amino acid sequences obtained by Kmiec and Holloman from Harvard were from the Rec2 protein. Secondary issues dealt with individual responsibility and the potential for penalties and correction of the literature.

In the primary assessment, it was determined that **Harvard was correct** in claiming that there was no connection between the sequence data they had produced and what was claimed by Holloman and Kmiec. The protein specified by the amino acid sequence produced by Harvard was most likely from *Ustilago maydis*, but it was not Rec2:

Midwest Bio Services <info@midwestbioservices.com> **Tue, Feb 9, 2010 at 1:21 PM**
To: Fraud Investigations <sciinvest@gmail.com>

Dear Robert,
The research documents appear to be from the experiments that are **not** related to the data that is presented in the paper.

It looks like the Edman degradation was performed on peptides from two samples: recB and EK1.
From sample EK1 two different peptides were sequenced that appear to belong to the same protein from Ustilago maydis:

1 maak**vyvgnl swnttddsla** hafstygqlt dyivmkdret grsrgfgfvt fatqaeadaa
61 iaalneqeld grrir**vnman srpaggm**ggg yggvtgqyga naygaqggyg gyggqpggfq
121 qpggfqqqgg ypqqggyggy qqpgfqpqqg gygapqqgyg apqqggyggy gaqqgfqqpa
181 qqnsgsyngq gy
http://www.ncbi.nlm.nih.gov/protein/71013156

One peptide was sequenced from sample recB. This 35 amino acid long peptide is from unknown protein, there are no similarities to the proteins that are currently in public databases. It could mean that the protein comes from the organism that is not sequenced yet, or that the sequence is not deposited in the public databases or that it is a synthetic peptide.

As specified in the expert's letter, the likely identity of protein from which amino acids were obtained by Harvard is given at http://www.ncbi.nlm.nih.gov/protein/71013156. That protein is not Rec2, but rather:

LOCUS XP_758559 192 aa linear PLN 25-APR-2006
DEFINITION hypothetical protein UM02412.1 [Ustilago maydis 521].
ACCESSION XP_758559 XP_400027

By comparison, Rec2 sequence is found at:

LOCUS AAA64741 781 aa linear PLN 04-APR-1995
DEFINITION REC2 protein [Ustilago maydis]. ACCESSION AAA64741

(Kmiec had also had a second protein sample, labeled "RecB" sequenced at Harvard during the period of interest. Harvard produced this data during discovery as well. The first expert was unable to find related sequence from any organism in the databases she searched; however, it was determined that the RecB amino acid sequences did not emanate from *U. maydis* Rec2.)

The Thomas Jefferson University (TJU) protein facility, part of Kmiec's former employer and a co-defendant of Kmiec's, did not provide any evidence regarding whether they had performed any amino acid sequencing as claimed by Kmiec and Holloman in KCH1994.

However, as the claimed N-terminal sites purportedly sequenced at Thomas Jefferson University no longer exist in the *rec2-1* deletion mutant's genome (see below), but the purported purified protein and activity appear unchanged[29], I have argued that there was no relationship between whatever might have been sequenced at TJU and any Rec2 protein purified from its host fungus.

More importantly, I have argued that examiners are entitled to draw an ***adverse inference***[30]**,** which in this case means that, as TJU and Holloman and Kmiec had good reason to produce any evidence that "Rec1" (KSSEP) *did* have Rec2 amino acid sequences if they had indeed found such at TJU, but did not do so despite substantial opportunity during the investigation of this case, then ***it is reasonable to infer that no such evidence exists***.

Therefore, not only have Holloman and Kmiec failed to provide any evidence that data existed to support their claims that the amino acid sequence of the purported "Rec1" strand exchange activity (KSSEP) was that of Rec2, but the evidence that was obtained from Harvard University indicates that with a very high likelihood the results claimed were based on falsified or fabricated data. (Whether the misconduct is called falsification or fabrication might come down to the degree of relation between the data obtained and the results claimed. In this case, **as there appears to be no relationship to the actual amino acid sequences**, it is suggested that the data was ***fabricated***, i.e. essentially made up from whole cloth to meet the needs of the researchers.)

Assessing Responsibility For Falsified Data - Allegation 1

Given that the amino acid sequence data appears with reasonable certainty to have been, at a minimum, **falsified**, the next question addressed by two of the experts was that of responsibility.

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[29] see also "*1 Background.pdf*" and "*3 DNA Sequence Fabrication-Falsification Discovery.pdf*" files in the "Reviewer Documents" folder on the CD.

[30] A legal principle based in common law regarding evidence which has been destroyed by a party, which a party refuses to produce, or which a party has under his control but which is not produced. (*Wikipedia*). This assumes there was no innocent reason for loss of the evidence; in the court case discussed here, no response or explanation was given, nor was this purported data even mentioned in defense. Therefore, it is my strong suspicion that the N-terminal amino acid sequences represent a further data fabrication.

Kmiec received the primary data from Harvard, and from what Rubin has stated, produced the original manuscript which would become Kmiec et. al. 1994 ("KCH1194"). It seems clear from Rubin's statements after having read the Kmiec manuscript (see above) that a major point made by Kmiec was that amino acid sequence of the Rec1 protein activity had purportedly been shown to emanate from Rec2. Therefore, it does not seem likely that Holloman changed truthfully reported amino acids sequences in the paper (i.e. that Kmiec reported correctly), nor made major new claims about Rec1's identity with Rec2. Thus, Kmiec would appear to have been a principal actor in falsifying/fabricating this data.

It remained, however, to assess the responsibility of Holloman, who stated in writing during court proceedings that he had "relied upon the sequencing information Kmiec provided". But was this reliance reasonable?

As noted in the Background and Basis for Allegations sections, Holloman knew several issues had arisen with respect to Kmiec's work in science, including much that had been performed with Holloman himself:

1) Kmiec's Rec1 (KSSEP) work with Holloman from the 1980's had never been replicated in any meaningful way by anyone else (other than Kmiec) in Holloman's or any other laboratory[31];

2) Holloman also knew that a serious controversy and retraction of Kmiec's work as a post-doctoral fellow had occurred. As Holloman had written to Kmiec's post-doctoral advisor, Abraham Worcel, in the late 1980's regarding the TFIIIA issue:

"**There has been enough talk in the scientific community so that Eric's results are looked at with skepticism**".

> Holloman then went to Kmiec, who was so forthright, wrote Holloman to Worcel on May 2, 1988, that he began to think some "incredible miscommunication" had been to blame for the problems. "There has been enough talk in the scientific community so that Eric's results are looked at with skepticism," Holloman wrote.

(from information obtained by G. Taubes).

3) Holloman also knew that on the very same KCH1994 paper in which he and Kmiec claimed that "Rec1" (KSSEP) strand exchange activity had the amino acid sequence of Rec2, two members of his laboratory, an M.D./Ph.D. student ("B" is Rubin) and a post-doctoral fellow, insisted on removing their names as authors

[31] According to statements made by those who had talked to me and to journalist Gary Taubes (see Background section).

because they did not find activity for Rec2 protein as claimed by Kmiec. As noted above, the student told me:

> **B: This totally disagrees with my conclusions and, knowing the nature of Eric having had several problems in science, of reproducibility, I said, "Just take my name off the paper."**

4) Holloman also knew that he had published two papers which showed genetic and biochemical data indicating that "Rec1" (KSSEP) strand exchange activity was present in rec2 mutant cells but not rec1 mutants[32]. Indeed, Holloman referred to this situation as "paradoxical" in KCH1994 itself:

> **The apparently paradoxical observations reported earlier indicating the presence of homologous pairing activity in extracts from the *rec2* mutant but the absence in extracts from the *rec1* mutant remain unexplained (15). Given the identity of *REC2* as the structural gene encoding the homologous pairing activity and the identity of the *REC1* gene as encoding an exonuclease (33), the earlier published results appear virtually opposite to expectations. We have not yet resolved these**[33]

return to DNA sequence falsification, below

5) It is further alleged here that Holloman knew that the rec2-1 mutant had a large 5' deletion in its *rec2-1* gene that would have removed its active site. (See the evidence obtained in the next section on DNA Sequence Falsification).

Nevertheless, Holloman accepted Kmiec's claims for "Rec1" (KSSEP) amino acid sequence without verification. Indeed, it had been my experience that Holloman had consistently argued against any of Kmiec's work with him as being incorrect despite the failure of numerous others in his laboratories to replicate it. Rather, Holloman claimed to others that inability to reproduce Kmiec's results was the result of the inferior abilities of those trying to replicate, not any possible wrong-doing. As noted by Taubes:

---

[32] Cell 29: 367-374, 1982; Cold Spring Harbor Symposia on Quantitative Biology, Vol. XLIX, pp. 669-673, 1984. The latter publication, whose first author was one of Holloman's postdoctoral mentors, Robin Holliday, made the following statement: "We also present new evidence that the rec1+ locus is the structural gene for the Rec1 protein." As presented in the court record, "Replication and extension of the rec1 mutant data published in Cell, 1982, strongly argues that this cannot now be ascribed to simple "error". This is especially the case since Holliday found a revertible allele of *rec1*, *rec1-5*, which he used in the paper to show that Rec1 activity is restored." (Bauchwitz analysis of November 10, 2000, at the request of Gary Taubes, (document 90, Exhibit 9).

[33] This affidavit can be considered the explanation.

While *Cell* published Kmiec's Rec1 papers year after year to much acclaim, the reviews from Holloman's own lab were mixed. Kmiec's success generated what Holloman later described as "hostility" among his other researchers. Only Kmiec could successfully purify the Rec1 protein, which Holloman ascribed to Kmiec's "excellent laboratory skills." When his other students failed or questioned Kmiec's veracity, Holloman attributed the former to incompetence and the latter to jealousy.

Above and beyond the aforementioned red flags, Holloman also had a strong motive for wanting Rec1 (KSSEP) strand exchange activity to derive from the *REC2* gene:

1) Holloman was aware that there was serious skepticism about his work with Kmiec on the "Rec1" (KSSEP) protein, as he noted in an NIH grant application:

Since the *U. maydis* activity stood apart from the rest in terms of key biochemical properties, skepticism was raised about the value and veracity of our work.

2) The sequence of the *REC1* gene had not shown any motifs known to be conserved among DNA recombinases. Therefore, it seemed unlikely that the *REC1* gene had encoded "Rec1" (KSSEP) strand exchange activity, (if in fact "Rec1" (KSSEP) strand exchange activity had ever existed).[34]

3) Holloman also knew at the time he published KCH1994, that the *REC2* gene sequence *did* have motifs that were consistent with being a DNA recombinase. Ultimately, Holloman would be the principal beneficiary of large amounts of NIH grant funding if he could justify and continue the "Rec1" (KSSEP) work as associated with the real *REC2* gene.

Indeed, it is important to note that ***it was Holloman who went to Kmiec*** and provided him with the Rec2 expression construct when Holloman's own laboratory members could not obtain activity which would connect what he and Kmiec had purported to be "Rec1" strand exchange activity with Rec2 protein. As Holloman's graduate student Rubin stated:

"So he [postdoctoral fellow Naoto Arai] came, and he tried to figure out if the Rec2 protein was a strand exchange protein. He worked on it for, well, almost two years. I'd say about two years. **And he got the same results I did. Basically, we could never show this did anything.** ... **what Bill did was he sent my overpurification, Ok, so he sent my overexpressing strain to Eric's [Defendant Kmiec's] lab.** Because, in my heart, I kind of believe what happened was with him was, ***he just really wanted this result. To be a strand exchange***

[34] See the work of Holden et. al., in the Background section.

protein. And I think what he did was he figured, well, these guys in my lab are not getting this. So I am going to send it to Eric".

In addition to wanting Rec2 to have strand exchange/transferase activity, Holloman also had a major motive that Rec2 be the source of the strand exchange activity he and Kmiec had ascribed to Rec1, i.e. especially once Holden et. al. had published the *REC1* gene sequence and comments that it did not seem to be a strand exchange protein (see above).

Kmiec, as Holloman's collaborator on the Rec1 claims, which had also proven to be irreproducible by others, especially in Holloman's own lab, shared the same motives and goals. It remains remarkable that Holloman not once but twice apparently ignored the negative findings of multiple of his own laboratory members in favor of those from Kmiec, whose reputation after the TFIIIA retractions would have raised at least some concern about his production of remarkable results. As noted above, Holloman explicitly had written just this himself: "There has been enough talk in the scientific community, so that Eric's results are looked at with skepticism."

Therefore, **Holloman's thinking about the perception of Kmiec was rational**. Yet he himself refused to apply any skepticism at all; he stated in the *qui tam* case that he simply "relied upon" what Kmiec provided to him. An obvious reason for doing so was that Holloman got just the results he wanted. Hence in the legal case we alleged that the relationship between Kmiec and Holloman could be seen as a conspiracy.[35]

The standard by which one is determined to have had "knowledge" of a fraud, as defined by the applicable U.S. federal law (31 U.S.C. 3729-3733), and consistent with the legal principles of scienter, is the following[36]:

[35] It was argued in the legal case that Holloman easily fit within the legal definition of having had knowledge that something was likely wrong, namely that he was acting, at a minimum, in deliberate ignorance of or in reckless disregard for the truth (see below). However, it is also entirely possible, as suggested in the complaint, that Holloman was in a much more active and long-term conspiracy with Kmiec.

[36] Scienter is Latin for knowingly. A detailed legal analysis of deliberate ignorance and reckless disregard can be found at § 4:47 of The False Claims Act: Fraud Against the Government, by Claire M. Sylvia, Thompson-West, 2004. In brief, deliberate ignorance is willful, while reckless disregard is acting with "gross negligence".

(b) **Knowing and Knowingly Defined**. –

For purposes of this section, the terms "knowing" and "knowingly" mean that a person, with respect to information –

(1) has **actual knowledge** of the information;

(2) acts in **deliberate ignorance** of the truth or falsity of the information; or

(3) acts in **reckless disregard** of the truth or falsity of the information,
and **no proof of specific intent to defraud is required**.

Therefore, the question arose as to whether it was more likely than not (greater than 50% likelihood), that with respect to accepting Kmiec's claims that "Rec1" (KSSEP) had the amino acid sequence of Rec2, Holloman was acting with reckless disregard for the truth or in deliberate ignorance.

Subsequently, two additional molecular biologists reviewed the information obtained from discovery.[37] With respect to the issue as to whether misconduct had likely occurred, they commented as follows:

> 1. Do you believe that K acted intentionally in reporting that Harvard Microchemistry had obtained R2 amino acid sequence when [they] did not do so?
>
> "Yes." *ExpRev-1*
>
> **"Yes. In both my experience as a primary collector of data and now more recently as a lab chief, this type of error is not possible. The process has too many checks and balances, preventing an unintended mistake."** *ExpRev-2.*
>
> 2. Given what H knew of K's history of irreproducible and retracted results, and the "unexplained" "paradox" (in H's words) that R1 strand exchange activity should derive from the R2 gene, was it reasonable for H to have "relied upon" K, for such a vital result (putative R1 strand exchange activity had the amino acid sequence of R2)?

[37] Note again that the reviewers received anonymized information; therefore Holloman appears as "H" and Kmiec as "K". Rec1 was "R1" and Rec2 was "R2".

"Clearly unreasonable." *ExpRev-1*

"**It was not reasonable. This in many ways is a critical issue. There is absolutely no reason in my opinion to have an expectation of certainty regarding K's work given the other available information."** *ExpRev-2.*

4. Do you believe, given all the evidence, that it was more likely than not that H was acting in "deliberate ignorance" or with "reckless disregard for the truth" by accepting K's claims about R2 sequence from putative R1 strand exchange activity?

"Yes. More likely than not H knew the truth, but can you prove this?"[38] *ExpRev-1*

"I can't help but believe from my perspective as the Principal Investigator of a laboratory that it was deliberate ignorance. He knew full well that things were not appropriate." *ExpRev-2*

5. Knowing now that Harvard Microchemistry does not support claims made about work attributed to it in the paper KCH1994, now 16 years old, what do you believe should be done?

a) Should the journal ***retract*** the paper?
b) Should K be subject to ***any penalty***? Should H? If so, what?
c) Should the **NIH** take such information into account in considering ***future***
funding for these researchers?

"I believe that the journals should retract this data despite how long ago it was published. In fact they should provide space for a detailed account of the story. K should be tried for falsifying data. H should lose his funding. I do not know if there is any mechanism to recover past misused MIH funds."[39] *ExpRev-1*

a) "**YES, setting the record straight is necessary and appropriate even given the length of the intervening years."**

[38] ORI raised a similar concern, but it should be noted that the standard for "proof" in a U.S. civil case of this nature is "more likely than not", rather than with certainty. Each reader can assess for himself what level of likelihood they would assess to the behavior of Holloman as acting with deliberate ignorance or in reckless disregard for the truth with respect to relying upon Kmiec.
[39] "MIH" should read "NIH", for the National Institutes of Health of the United States. With respect to recovering grant funds, the *qui tam* case from which this information was obtained is a primary mechanism.

b) "YES. Both K and H have engaged in misconduct. H is far more at fault as the supervisor and the person ultimately in charge. ..."[40]

c) " They should be barred from receiving NIH funding in the future. ..."

Back to TOC.

II. DNA Sequence Data Falsification - Second Allegation

It was Holden et. al., in a paper in Current Genetics (20:145-150) in 1991, who first exposed a problem that had arisen from sequencing the DNA of *U. maydis*' recombination genes. As they stated:

> "We have also cloned the gene in order to determine if indeed *REC1* encodes the transferase protein [KSSEP] or, for example, a protein which regulates expression of the transferase and perhaps other genes involved in recombination and repair.
>
> "We have found that the amino acid sequence deduced for the Rec1 protein is ***not compatible*** with the molecular size of the transferase [KSSEP] (Kmiec and Holloman, 1982). It also ***does not contain any of the conserved ATP- or DNA-binding motifs***, although a nuclear localization signal may be present (Holden et. al., 1989 b)". (Emphasis added.)

Therefore, nucleotide sequence of the *REC1* gene indicated that, contrary to Holloman's hopes, the *REC1* gene did not have a sequence comparable to other known recombinases. If it had, he would have claimed justification of his earlier KSSEP results with Kmiec.

Instead, the difference put Holloman in a precarious position. Any new grants could be attacked on the basis that his claimed "Rec1" strand exchange activity (KSSEP) would have been unlikely to have had the size and activities originally claimed by him and Kmiec.

A much more obvious explanation, given Kmiec's subsequent history of retracted experiments and failure of Holloman's own laboratory personnel to extend the Rec1 strand exchange work, would be that the original Rec1 (KSSEP) claims were also false. Therefore, Holloman faced the likelihood that any critics among his federal reviewers would again reject his grant applications.

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[40] It is important to note that not only has Cornell known of potential misconduct related to Holloman for many years (see Background, above), apparently without investigating, according to their response in the *qui tam* case, but also since 2007 they have specifically had information from Harvard, cited above, which strongly suggests serious data fabrication. Nevertheless, there is no sign that any correction of the literature has been made. Therefore, the same scienter considerations that apply to Holloman also apply to his university supervisors.

Motivation and Importance of Rec1 becoming Rec2

But, "as fortune would have it", as Holloman stated in a federal grant application, the sequence of the *REC2* gene did have a sequence compatible with activities they had described for "Rec1" strand exchange activity (KSSEP).

The associated document "***REC2* and *rec2-1* technical information**" includes diagrams of the various forms of *REC2* gene and Rec2 protein discussed in this section.

In addition to the "paradoxical" biochemistry and genetics from "earlier published results [being] virtually opposite to expectations", i.e. with Rec1 having emanated from the *REC2* gene, as noted by Holloman, there were some additional issues related to the sizes of the expected protein products.

The most important size issue, to me, was that the purified "Rec1" strand exchange activity (KSSEP) was always obtained as a pair[41] of approximately 70 kd peptides (see Background above). However, not only was KSSEP activity found in the rec2-1 mutants, but so were the 70 kd peptides (see Western blot for an example in the "*REC2* and *rec2-1* technical information" document).

The problem with this is that it was already known by the time KCH1994 was written that *rec2-1* had been formed by a large deletion of the 5' end of the *REC2* gene, e.g. as shown by Southern blots and subsequently DNA sequence published by Holloman in the Rubin et. al., 1994 paper.[42] However, as noted in the Basis for Allegations section above, Holloman had apparently removed information specifying the 5' end (of the open reading frame) and exact deletion points of the *rec2-1* deletion from the Rubin et. al. 1994 paper.

> Rubin: ***You know what is funny is***, that somehow the, I actually did notate that the rec2-1 mutant, that on this figure originally, Figure 1, and uh, in the legend, 5 the description, I am looking at my thesis right now, and it was the same figure, and it is in there. Bauchwitz: Oh, it is? Rubin: ***Bill must have taken it out for some reason***." (*Sec. Decl.*, par. 28.)

The proteolytic digestion sites claimed in KCH1994, which supposedly reduce the larger Rec2 protein to 70 kd peptides, fall within the *rec2-1* genomic deletion. Therefore, it would have been a remarkable coincidence that the products of proteolysis would produce a peptide pair of effectively identical size as a deletion product of the same gene - particularly when the digestion sites had been removed by that deletion![43]

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[41] Or even triplet.

[42] Those with expertise in molecular biology can see also the diagram and list of expected protein product sizes in the "*REC2* and *rec2-1* technical information" document.

[43] Assessment of the exact level of my "skepticism" of this and other issues, and any duty it imposed upon me to act, were discussed at length in various court documents, including those excerpted in the "Basis for Allegations" section.

Important evidence from the rec2-1 gene sequence

Even if a presumptive rec2-1 protein product were expected to be much smaller in size (56.5 kD predicted) than the 70 kD pair observed in the rec2-1 cells, it would remain unknown whether there was any sequence upstream of *rec2-1* which would extend the open reading frame to make a longer composite protein.[44]

Of even greater importance than peptide sizes, if the *rec2-1* mutant allele were not able to produce an active protein, then normal levels of such activity could not have been isolated from rec2-*1* cells by Kmiec and Holloman, contrary to their claims in two prior publications (Cell 29: 367-374, 1982; Cold Spring Harbor Symposia on Quantitative Biology, Vol. XLIX, pp. 669-673, 1984).

Examination of the *rec2-1* DNA sequence upstream of and across its deletion point should have allowed an assessment of possible size and especially activity issues, since the active site of the protein had been determined. Although Holloman's graduate student, Rubin, had told me that he had sequenced the *rec2-1* allele, I could not find any specification of the relevant upstream sequence, not only in his 1994 paper (as he had noted in surprise, see above), but also not in his thesis, nor in the federal Genbank.

Therefore, in 1997 I produced my own DNA sequence of the *rec2-1* deletion breakpoint and I also extended the upstream sequence of *REC2*. I deposited those sequences in Genbank and made them available to the public in 1999. (Some of the raw data I obtained is shown in the "*REC2* and *rec2-1* technical information" document.)

```
LOCUS       AF027108                 66 bp    DNA     linear   PLN 31-DEC-1999
DEFINITION  Ustilago maydis recombination-repair protein (REC2) gene, sequence
            across the deletion point of the rec2-1 allele.
ACCESSION   AF027108
       ...
ORIGIN
        1 tgagccaaga ccaagaccac caaccaacac gcagcaagcg tcacacgtca cgcgacccac
       61 gatgtt
```

The DNA sequence results I obtained were consistent with what Rubin had told me he had found while he had worked in Holloman's laboratory. These results appeared to be a significant piece of evidence against Holloman's claim that Rec1 had actually emanated from the *REC2* gene, since the results were now not only paradoxical and opposite of expectations, but incompatible - there should not have been any Rec1 activity found in rec2-1 cells if those cells were not capable of producing any active recombinase.

Just how definitive my *rec2-1* DNA sequence was with respect to being put on notice of fraud was discussed at length by the Court in this case (*Memorandum Opinion re Summary Judgment*, doc. 116). It should be noted that U.S. *qui tam* law (31 U.S.C. 3729 - 3733) requires that an actual false statement be made with respect to funding from

[44] No splicing was found in the region: "The boundaries of this RNA as determined by S1 nuclease protection place the termini approximately at position -150 and +2500 with respect to the ORF, with no indication of splicing" Rubin et. al., MCB 1994.

the federal government. In other words, just publishing a false statement is not enough to trigger the laws under which this case was pursued. The ***false statements must be made as part of a false claim for payment or certification*** such that the taxpayers are defrauded of their funds. Holloman never made an explicit statement about the *rec2-1* DNA sequence, at least not one that I knew of, until his 2001 publication with Kojic et. al. (see detailed in the following section). The grant relevant to the *rec2-1* false statements was first funded in 2002.

Nevertheless the Court stated, " Bauchwitz knew the facts material to this second category of fraud as early as February of 1995, when he spoke with Rubin for a second time, and later, in 1999, when he published the results of his own *rec2-1* sequencing."

Leaving aside that there are many significant inaccuracies in the judicial opinion as to the facts that had been presented[45], the question is whether I was really in any position to file a lawsuit based on the inconsistency of the *rec2-1* DNA sequence result with the Hollomans' claims that Rec1 had Rec2 amino acid sequence. Was that information really enough that the clock began to tick on my ability to take legal action to recover federal funds related to the first allegation of fraud? What I did early on was suggest to the relevant federal agency, ORI, that they investigate. They apparently did not do so.[46] As was stated in the court record on this point:

> "The only question with respect to alleged frauds 1 and 2 would seem to be ***whether I had any duty in 1995*** - long before I even knew what a *qui tam* action was, or had even considered whether defendants might seek *future* federal grant funding based on the False Claims 1 and 2 – to act as a perpetual investigator and monitor of future grant applications by the defendants, based solely on my ***skepticism*** about the results reported in the 1994 Paper. I respectfully submit that that this cannot be the case. To the contrary, I believe that in 1995 I went above and beyond the call of duty by notifying ORI of continuing suspicious circumstances that warranted investigation, and subsequently developing my own rec2-1 sequence, when nothing by Rubin could be found." *Sec Decl. par. 32.*

I do not agree that such inconsistent results are as definitive with respect to knowledge of fraud as the Court seems to portray them. As I argued (see Basis for the Allegations section, above), ***had I not known of the prior history of concerns about the work of these researchers***, I might have assumed that **technical errors** had occurred to

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[45] For example, in the same part of the opinion, the judge claimed, " Rubin told him that he had observed no ATG start codons for methionine upstream of the *rec2-1* deletion, and that *the rec2-1* mutation truncated on its own." Rubin never made the latter statements. In fact, as presented in the court record, our conversation actually indicated that Rubin had never thought of the truncation of rec2-1: Bauchwitz: " … there aren't any methionines until after the recA and Walker A homology sites. In fact it is two amino acids after, there is not even AT [or G except] immediately after the [] site." Rubin: That's interesting. ***I never really thought about that ...".*** *Sec. Decl. p. 10.*

[46] It was, by ORI's own policy, their responsibility to investigate, not mine.

produce the inconsistent results. Indeed, this is precisely what was stated in the court record[47]:

> "Rubin stated, he was "***very skeptical about all this***". (emphasis added). I too was skeptical about the results reported in the 1994 Paper, but note that elevated ***skepticism*** about the results reported in a published research paper is not the equivalent of knowledge that a fraud had occurred, or even evidence of such. ... It was only in the context of their prior pattern of dishonest behavior, which preceded and was separate and distinct in nature from the current information I was hearing for the first time, that I felt an investigation by ORI was warranted in 1995." *Sec Decl. par.s 16 and 17.*

Regardless of just how much notice my *rec2-1* DNA gave me, the Court clearly considered this sequence and related information (e.g. Holloman not presenting his own) **highly material** to understanding the case; the same has been true for scientific reviewers to date.

Holloman did eventually publish a specific claim about the *rec2-1* DNA sequence, at which point, in conjunction with the presumptively false statement appearing in relation to funding from the U.S. government, it is without doubt that the statute of limitations would begin, at least for the second alleged fraud.

Publication of rec2-1 sequence claim by Holloman

While I was assisting journalist Gary Taubes, he brought to my attention an article published by Holloman in 2001 in which Holloman claimed that the *rec2-1* sequence *did* have an upstream ATG which would have allowed a rec2-1 peptide to retain the recombinase active site:

> **"** Inspection of the *rec2-1* DNA sequence on both sides of the deletion indicated that a novel ORF could be generated through conjunction of the flanking sequences. This ORF would be predicted to encode a 613 amino acid [Rec2] protein variant with a novel 19-residue leader sequence derived from upstream of the deletion in lieu of the N-terminal 187-residue sequence of the wild-type [Rec2] protein." **(Kojic et. al., 2001.)**

Holloman was claiming that an ATG DNA sequence exists upstream of the *rec2-1* deletion site in a position which would make it reasonable to assume that a protein could be produced from *rec2-1*.

This despite testimony that data Holloman received from his graduate student, Brian Rubin, did not indicate such a sequence, and despite public data (GenBank AF027108 of 1999) also to the contrary.

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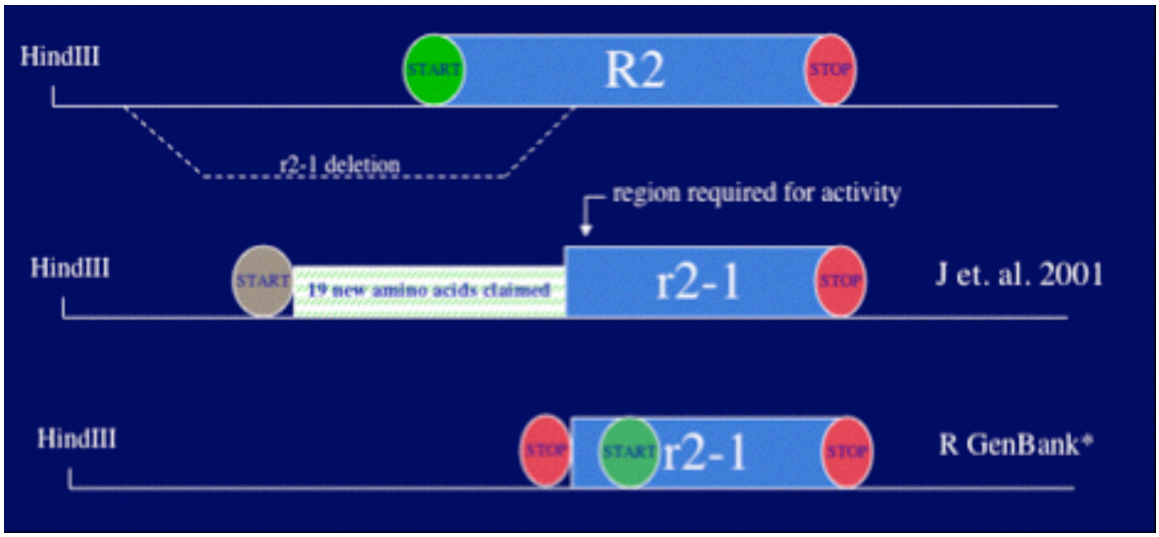
[47] See also my 1997 comments in a letter to Dr. Walter Gilbert, as excerpted in the final endnote of the *Case History,* and present in its entirety on the CD as *Gilbert Letter Exhibit* in the Case Documents folder.

Of particular note, Holloman never explicitly showed the relevant sequence or published it in the Genbank. He only made statements and showed schematics in the paper.

This naturally raised the question as to whether Holloman and his new authors actually had *rec2-1* DNA sequence to support his claims.

I summarized the situation in a disclosure to the U.S. government (ORI and Department of Justice) as follows:



(*) *rec2-1* should not have been able to produce the "Rec1" (KSSEP) protein activity. [48]

To summarize the motivations for the 2001 *rec2-1* DNA sequence claim, if there were no ATG start codon, and hence no methionine which almost all proteins have as the first amino acid, the likelihood of a protein from *rec2-1* would be so remote that without further evidence it would not be proposed as a reasonable explanation of activity from the mutant gene *rec2-1*. Therefore, claiming that ATG was present made Holloman's explicit and implied claims of potential activity from *rec2-1* seem to have been plausible.

In other words, while it would be possible to use "hand waving" to explain why rec1 mutants did not show expected KSSEP protein activity (a negative result), it would be much more difficult to explain why purified KSSEP activity would be present in rec2 mutant cells (a positive, affirmative result) if those cells did not have a *rec2* gene capable of producing an active Rec2 protein.

Back to TOC.

New Information about the rec2-1 DNA sequence

[48] "J" was a name code for the first author, Kojic", of the 2001 Holloman *rec2-1* DNA sequence paper.

What follows is new information that was obtained by subpoena during discovery in 2010 regarding the *rec2-1* sequencing issue. Holloman was required to respond to written questions under oath and provide relevant documents. However, Holloman's graduate student, Rubin, could not be questioned; all that is available are his statements to me in 1994 and 1995.

There was no other *rec2-1* breakpoint sequence produced by Holloman other than by his graduate student, Rubin

Holloman, in his sworn response claimed that he relied upon the *rec2-1* DNA sequence numbering in his graduate student Rubin's thesis for his claims in his 2001 paper.

When asked for the identity of anyone else involved in the *rec2-1* sequence at issue, Holloman did not specify anyone else, but again referred only to the sequence produced by his graduate student, Rubin.

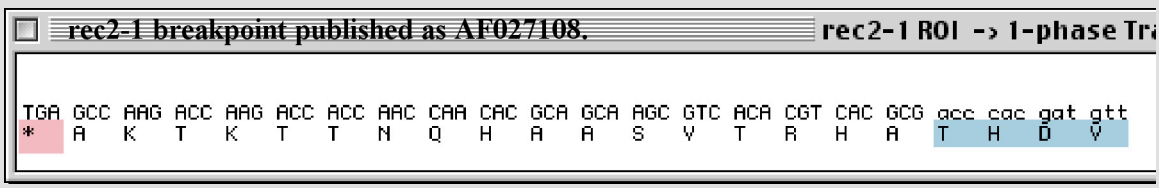
When asked, Holloman also specified no other source for the *rec2-1* sequence he claimed in his 2001 paper other than Rubin's.

Therefore, there appears to have been no rec2-1 DNA sequence performed by Holloman's laboratory after Rubin's work, e.g. nothing by Kojic or Thompson, the other authors of 2001 paper[49].

The DNA sequence position numbers claimed to have been relied upon by Holloman could not have produced the results he published.

The following analysis of the relevant new evidence is technical in nature. For those who do not have experience in assessing DNA open reading frames, this material (shaded in gray) can be skipped. For those who have some experience in molecular biology, the original analysis submitted to the Court (attached, Reviewer Documents, 3a, on the CD) has been supplemented here with some explanatory material, which should make it more accessible to those who are less experienced. A summary and reviews by experts in molecular biology are provided in the subsequent section.

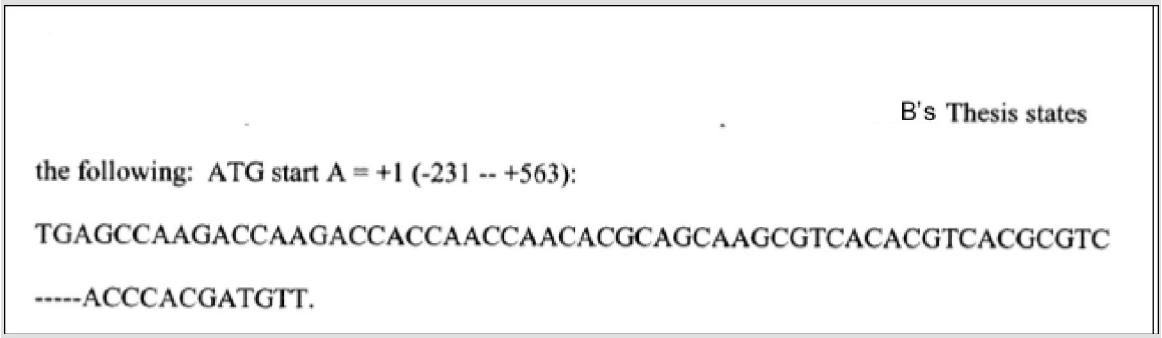
The specific sequences at issue



[49] coded as "JUH2001" in the anonymized expert review documents. Kojic ("J") was a Holloman post-doctoral fellow while Thompson ("U") was a graduate student.

Notes: The extra "TC" at the breakpoint claimed by H is shown at the green box in second figure, above. These two nucleotides originate from just beyond the end of 5' side of the deletion breakpoint.

In a written response to questions, Holloman claimed that he had relied upon a DNA sequence from Rubin's thesis (here the "B" stands for "Rubin"):



However, Rubin's thesis does not show the sequence stated by Holloman, above, nor does it even imply the "TC" at the end of the 5' deletion breakpoint. (See the "*REC2* and *rec2-1* technical information" document for details on the numbering scheme used in Fig. 1.3 of Rubin's Thesis).

Upon production of written documents, it was revealed that the information that Holloman cited was present as **marginalia** on a *REC2* DNA sequence summary document.

On that sequence document, dated June 17, 1991, which was found among other sequencing records apparently produced by Holloman's graduate student Rubin, has been drawn in a very heavy black line a demarcation of what ***exactly*** corresponds to the *rec2-1* deletion breakpoint sequence as it appears in the Genbank entry AF027108, produced by me. (See the "*REC2 and rec2-1 technical information*" file for relevant excerpts of the documents produced; the complete set of relevant documents can be found on the CD in Reviewer Documents/3c.)

This strongly indicated that Rubin, the only other person known from this case to have sequenced the *rec2-1* deletion breakpoint, had obtained the same sequencing results as I had.

The next question was whether there was in fact some dispute as to the presence of an ATG start codon upstream of the *rec2-1* deletion breakpoint. Such a start codon would have been required to retain the Rec2 active site in any rec2-1 protein[50].

[50] Without an upstream start codon, the next ATG would code for a methione at the 260th amino acid (M260) within Rec2, which was three amino acids beyond the (K257) that had been shown by Rubin to be

Open reading frame (ORF) **analysis** is used to predict peptides which might be produced by a segment of DNA. In essence, the process looks for known start and stop signals which control where protein synthesis machinery begins to read the sequence (via its RNA intermediate form) and when it stops synthesis. As with the ATG start codon in DNA, stop codons are also present. This part of the analysis is relatively simple - look for known start and stop triplets of DNA in the gene sequence of interest.

An extra level of complexity is introduced because there are three potential reading frames in each direction of a double-stranded DNA. For example, we know that the amino acid sequence THDV (shown in the rec2-1 breakpoint sequence figure in the *REC2 and rec2 technical information* technical file, found the CD) must be present beyond the 3' deletion breakpoint in any Rec2 or rec2-1 protein. Those amino acids are coded by the DNA sequence triplets: acc cac gat gtt. Therefore, those amino acid and associated DNA sequences anchor the reading frame that must be used.

Then, one can work towards the upstream, DNA triplet by DNA triplet, to look for an ATG start codon. To be in-frame means that the ATG corresponds to the position expected for a triplet, rather than being out-of-frame by overlapping two triplets. For example, ATG CCC acc cac gat gtt would put the ATG "in-frame" with the amino acids encoded by acc, while ATG CC acc cac gat gtt would have it out-of-frame; the actual result would be xAT GCC acc cac gat gtt, for which x represented the next DNA nucleotide upstream of the AT. Performing this ORF analysis is aided by the use of computer software.

The final point to be considered with the discovered evidence is that Holloman never actually presented Rubin's actual *rec2-1* open reading frame as a singular, unbroken string, despite subpoenas for the information[51]. He only showed it within the context of where it existed within the wild-type *REC2* gene sequence.

Nevertheless, it is relatively easy to put the two pieces of the Holloman sequence that correspond to *rec2-1* together. The result shows precisely what was published in the Genbank as AF027108, and as shown in the top portion of the preceding "Specific sequences at issue" figure. This sequence is not what was claimed in the 2001 Holloman paper (Kojic et. al., 2001) nor by Holloman during the court proceedings (see preceding figure, "B's thesis states").[52]

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required for Rec2 activity *in vivo*, and which was within the highly conserved ATPase site found in recombinases.

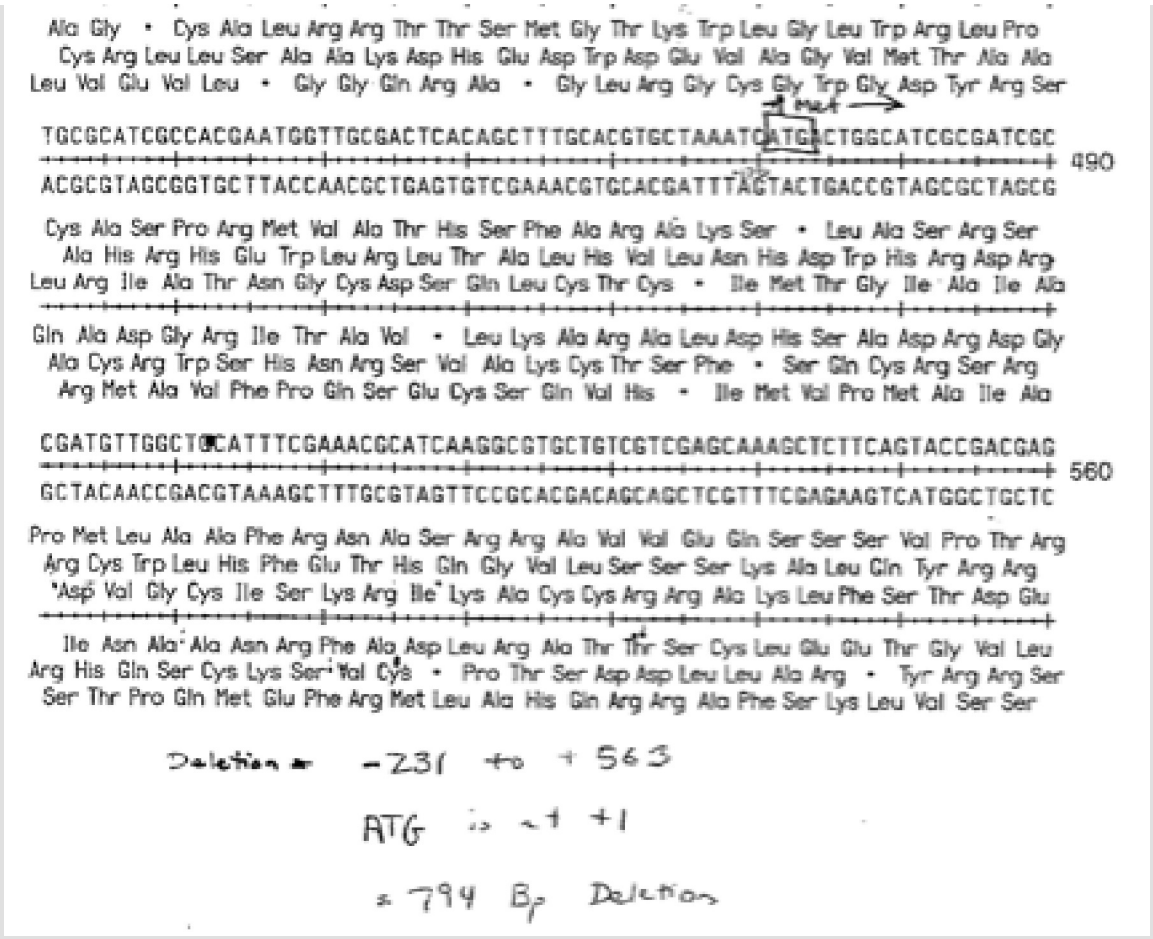
[51] Holloman never provided any open reading frame, including none to support his own claims for the rec2-1 DNA sequence. Yet he presumably MUST have done so himself if he relied upon the numbers purported produced by Rubin to obtain the results he published. Rubin clearly stated he did not obtain the results Holloman claimed. Furthermore, there is good reason why Holloman presented nothing - the numbers actually do not support the ORF claimed - see below.

[52] Nevertheless, Holloman's attorneys persisted in claiming that this was a mere difference in scientific results and therefore a frivolous dispute.

While it is essentially certain that no one would make a specific sequence claim about an open reading frame without actually assembling one as a single, continuous sequence, i.e. in a manner sufficiently specific that the sequence would be appropriate to enter into a database such as Genbank, it might be possible to use numeric shorthand based on relative DNA sequence positions to describe such an open reading frame in a way that would satisfy some research journal reviewers or editors. The basis for so doing would be that they would *trust* the author to have carefully checked his numbers. But responsible reviewers would not have accepted that an author would have produced a claimed open reading frame *without actually assembling it*. In other words, pretending to rely on someone else's numbered sequence positions would not be appropriate.

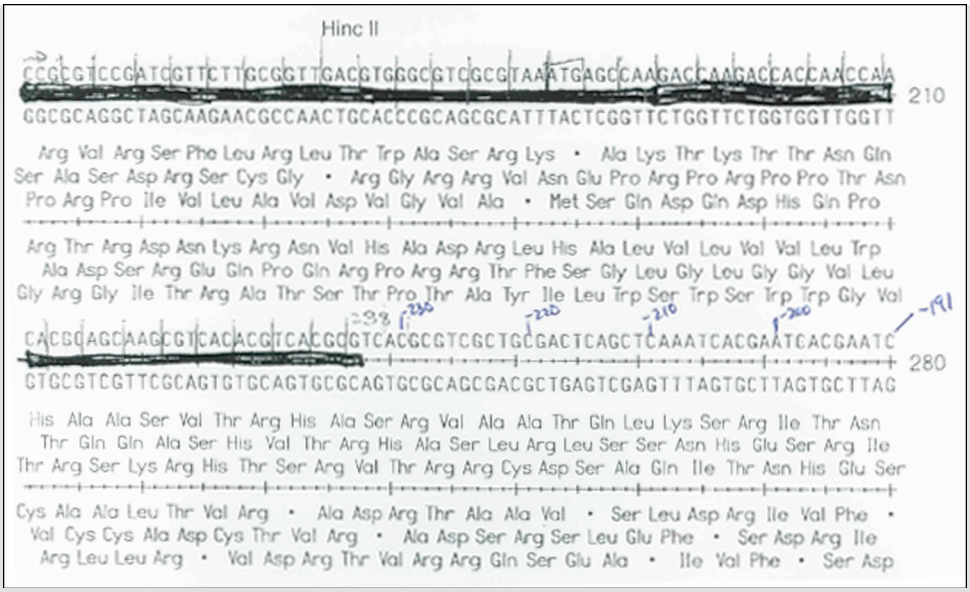
Holloman presented such sequence numbers in two instances in this case. First, as shown above, he wrote: "A = +1 (-231-+563)". Second, in the DNA sequence that Holloman released are numbers written in hand among the sequences and in particular, in the margins ("marginalia").

"A = +1" is a way of saying that the first nucleotide of the wild-type *REC2* DNA sequence, which starts with the usual ATG, will be numbered "+1". This is standard numbering for such sequences. Indeed, it is also shown on the corresponding DNA sequence document he released, in which the *REC2* ATG start codon has a handwritten box around it and a "+1 Met" with a rightward pointing arrow above it:



Furthermore, the "(-231-+563)" numbering also appears again in the margin of the DNA sequence document as a handwritten: "Deletion = -231 to +563". We were not told who the author of those marginalia was, nor when they were made.

The -231 to +563 numbering represents the position of the deletion endpoints relative to the A being +1. Establishing these numbers can be done by a relatively straightforward counting of the DNA nucloetides between the two ends of the deletion, for example as follows:



The figure immediately above shows that the DNA sequence software itself applies numbers to the nucleotide bases based on the first base in the file being +1. Thus, the numbers "210" and "280" can be seen in the right hand margin; these correspond to nucleotides at the end of each line.

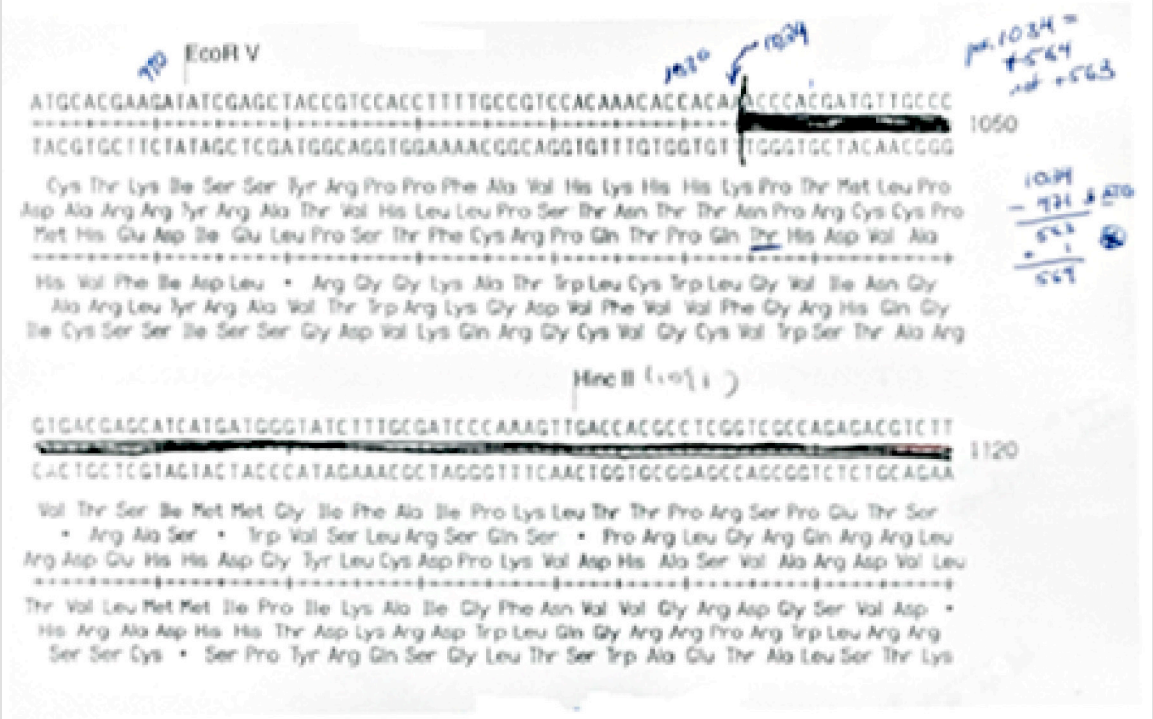
Much of the following discussion was presented in the case document (*Plaintiff's Third Interrogatory Response of March 25, 2010*, question 8, which was presented to expert reviewers, with one added figure, as found on the CD as file 3a_r2-1_seq_new_data_analysis_alt_2010.pdf).

Importantly, above the “T” immediately following the end of the heavy black line corresponding to the 5’ end of the *rec2-1* DNA sequence, has been written the number “238”. This number correctly identifies the position within the sequence file of the “T” above which it is present. This indicates that whoever wrote "238" could count correctly either up from 211 at the start of the line, or back from 280 at the end of the line.

Similarly, if one reexamines the previous figure that shows the boxed "ATG" with "A met" above it, it is apparent that there is a "490" at the end of the same line. Furthermore, in light handwriting a “470” has been written in by hand under the “C” which precedes the ATG. The “A” defined as “+1”, is clearly at position 471 of the sequence file, so again, whoever wrote the "470" could perform this simple counting.[53]

Finally, the discovery document showing the 3' end of the *rec2-1* deletion (see following), indicates that the *rec2-1* deletion ends at position 1034 of the DNA sequence file and also correctly shows the indicated HincII marker as being at position 1091. Once again, these numbers are easily generated by counting from the end of each line.

[53] The ATG is within the deletion, so there is no heavy black line indicating *r2-1* sequence. This, too, is correct.



Therefore, all of the aforementioned numbers drawn within the DNA sequence are correct and demonstrate that whoever wrote them could count using the numbering provided by the DNA sequence program. (Note that numbers in blue are not part of the original documents but were added to facilitate counting by reviewers.)

Notable Discrepancies

The marginalia that claim "Deletion = -213 to +563", however, appear to be inconsistent with the data presented and the aforementioned markings.

First, if the ATG is at position 471 and the 5' deletion begins at 238, as indicated in the documents, then the deletion would begin at -233, not -231.

Furthermore, if the 3' end of the deletion is at 1034, then the deletion would end at +564, not +563.

The most direct way to establish these facts would be to count every nucleotide on the DNA sequence documents, e.g. as they appear in their complete form (CD file 3b in folder Reviewer Documents).[54]

Molecular biologists, and those with comparable skills, could use specialized software that could adjust the numbering scheme, e.g. to set the A = +1, or otherwise to produce a count of the nucleotides between two points.

[54] For example, a dot could be placed over each nucleotide in the deletion as it was counted. This would also establish that, contrary to the claim in the marginalia that the deletion is "794 bp", it is actually 797 bp.

A third method would be to use some simple math involving the endpoint numbers, i.e. subtraction.

If the person who wrote the marginalia thought that it was appropriate to simply subtract the sequence file positions to obtain the deletion point positions, then they would have obtained:

For the deletion end point,
1034 – 471 = 563, as stated, but,

For the deletion start point,
471 – 238 = 233, contrary to the marginalia claiming 231.

Above and beyond the aforementioned problem, the number "+563" is not a correct determination of the actual sequence shown.

When one subtracts, one is determining a difference, e.g. the number of steps between two points. For example, if a person wanted to count the 5 fingers on his hand, then he would not subtract 5 – 1, as this produces 4, which is not the number of fingers. To go from 1 to 5, a person would need to take four steps, but ***it is necessary to add back "1" to get the actual number of fingers (or nucleotides) separating two points***.

The problem does not occur when going backwards in such a sequence file because it contains a "-1" which gets included in the difference, i.e. it obviates the need to add back a 1. So the negative numbers would be correct.[55]

The net result is that +563 is NOT the end of the deleted sequence and ***could not have been relied upon*** to produce the *rec2-1* sequence claimed by Holloman in the answer he made on March 8, 2010 (see preceding).

Clearly, had Holloman relied upon the number +563, with "A" of ATG = +1 as he claimed, he would have found from any *REC2* sequence that an additional "A" would have been present at the start of the 3' *rec2-1* breakpoint sequence.

Therefore, I concluded that Holloman did NOT rely upon this "erroneous" number +563 to produce the sequence he claimed, because if he had done so, an open reading frame analysis would have shown only six amino acids possible following the 3' end of the deletion breakpoint, i.e. following the 19 novel amino acids claimed from the 5' end of the *rec2-1* sequence in Kojic et. al., 2001:

[55] Historically, DNA sequence numbering schemes had the form -3, -2, -1, +1, +2, +3, but they might also use a "0", e.g. as in -3, -2, -1, 0, +1, +2, +3. This scheme appears in one of the figures in Rubin's thesis, but it is not germane to the claims made by Holloman.

```
      M  S  Q  D  Q  D  H  Q  P  T  R  S  K  R  H  T  S  R  V  N
1     atgagccaagaccaagaccaccaaccaacacgcagcaagcgtcacacgtcacgcgtcaac  60
      P  R  C  C  P  *  R  A  S  *  W  V  S  L  R  S  Q  S  *  P
61    ccacgatgttgcccgtgacgagcatcatgatgggtatctttgcgatcccaaagttgacca  120
```

The red asterisks here are in-frame stop codons. The blue "M" is the start of the rec2-1 amino acid sequence claimed in Kojic et. al., 2001.

Thus, there is no connection between the erroneous numbers supposedly relied upon and any *rec2-1* DNA sequence claimed by Holloman, either in Kojic et. al., 2001, or in his interrogatory response.[56]

Summary of the immediately preceding reviewed evidence for the allegation of DNA sequence falsification

In short, the above reviewed evidence shows that Holloman could not have used the numbers "ATG start A = +1 (-231 -- +563)" to produce the *rec2-1* sequence as he claimed in his interrogatory answer number three of March 8, 2010 and as he showed in his publication Kojic, Thompson, and Holloman, 2001. Whoever produced the number +563 apparently misunderstood that the process of subtraction could not be used without an adjustment (adding one).

It is notable, and we make ***adverse inference*** from, the fact that despite the request made in writing during court discovery, Holloman failed to produce the actual open reading frame sequence he purportedly must have produced if he had in fact relied on the numbers he provided, as he stated under penalty of perjury. Holloman never presented an actual *rec2-1* ORF that was produced by him or any author of Kojic et. al., 2001.

Instead, the evidence indicates that Holloman simply chose the sequence he wished to obtain and then presented it without any attempt to determine if it was consistent with what he knew was the true *REC2* sequence, much less with an open reading frame assessment of the *rec2-1* mutation.

Back to TOC.

Many Missed Opportunities to Present rec2-1 ATG Claims or Actual DNA Sequence

Despite Holloman's claim for the *rec2-1* sequence in Kojic et. al., 2001 ("JUH2001"), in at least one half dozen instances in grants, papers, theses, and patents in discussing this issue up to 2001, Holloman did not ever make a claim for the existence of the upstream ATG, despite his clear recognition by his written statements of its relevance and importance.

It was not until after the publication of the Genbank *rec2-1* sequence in 1999 that Holloman claimed a contrary finding in the Kojic et. al., 2001 paper.

[56] The relevant DNA sequences are present in the files (3b and 3c) in the Reviewer Documents folder on the CD, for those who wish to assess the above conclusions themselves.

However, even there, he only did so through textual statements and schematics. Holloman did not present an actual *rec2-1* DNA sequence nor, apparently, to this day has he placed such in the Genbank or any other comparable public database.

Examples of "missed opportunities" to disclose the vital upstream ATG

The importance of an alternative *rec2-1* translation start site was already noted in the Discussion of Kmiec, Cole, Holloman, 1994:

> **but if an alternative translational start site were utilized, a truncated REC2 polypeptide with certain biochemical activities might be produced.**

If Holloman had believed that an alternative start site for translation existed upstream of critical amino acids in the *rec2-1* allele such that "certain biochemical activities", e.g. as associated with a DNA strand exchange recombinase, could have been produced, he most definitely would have been expected to mention it here.

Yet he did not, even though he had the *rec2-1* sequence produced by his graduate student, Rubin.

The first mention of such ATG was not until publication of Kojic, Thompson, and Holloman in 2001, and according to court testimony by Holloman, it was not based on any new *rec2-1* DNA sequence.

Even Holloman's graduate student, Bennett, who followed Rubin in the laboratory, did not mention such an ATG in his 2001 thesis when reviewing the *rec2-1* allele:

> "The rec2-1 mutant is the result of a deletion extending 600 bp into the ORF from the ATG codon and including 200 bp of upstream sequence (Rubin et. al., 1994). However, a truncated mRNA is observed in northern blot hybridization using REC2 sequence as a probe for detection." (*Bennett Thesis*, p.38.)

Bennett's mention of mRNA was apparently intended to hint that protein might be produced. Yet only the Rubin *rec2-1* sequence is cited and no upstream ATG mentioned.

The Bennett thesis was dated May 2001, and like Rubin's thesis, apparently signed by Holloman. It seems apparent there was no general knowledge or discussion in the Holloman laboratory about an upstream ATG, even at this late date, contemporaneous with the Kojic et. al., 2001 paper which proclaimed the upstream ATG.

The Office of Research Integrity made similar note of this situation, e.g. in a January 12, 2005 letter to the Department of Justice:

WH claimed to identify a novel ATG start site upstream of the deletion in the rec2-1 mutant which initiated a 613 amino acid Rec2 protein variant. This variant protein replaced the normal N-terminal 187 amino acids of Rec2 with a 19 amino acid sequence derived from the sequence upstream of the deletion and normally not part of the protein.

- the sequence data for the novel ATG start codon were not published anywhere

and no mention of an upstream ATG in a relevant grant:

- from **GM 42482-04, p.26**:

"The REC2 gene was isolated . . . DNA sequence determination revealed a 2273 bp uninterrupted ORF and RNA blot hybridization indicated the REC2 message to be a 2.7 kb damage-inducible RNA . . . The rec2-1 allele contains a deletion of 800 bp that extends 200 bp upstream from the presumed ATG start of the ORF and 600 bp downstream into the ORF. Blot hybridization of RNA from the rec2-1 mutant reveals a 2.2 kb RNA that is at least 10 fold less abundant than the 2.7 kb REC2 message and that is not damage inducible. We have sequenced the rec2-1 allele and know the boundaries of the deletion. Examination of the remnant sequence has revealed the presence of a heptad repeat element that we think is likely to function as part of the constitutive promoter element."

Back to TOC.

Expert reviews of the rec2-1 DNA sequence evidence

The claims I made above regarding the *rec2-1* DNA sequence were assessed by the two molecular biologists who reviewed the material obtained during discovery in the case. Their complete reviews are attached. The reviewers were not given the actual identities of the defendants or others involved; the name codes used were the same as provided to the federal government. In particular, I am "R", Holloman is "H", and Kmiec is "K". The following are relevant excerpts of their conclusions:

> Does the sequence data presented by R support his contention that the first ATG initiation codon (following an in-frame stop codon) in the *r2-1* DNA sequence is located downstream of the *r2-1* deletion breakpoint?
>
> `Yes.` *ExpRev-1*
> **YES** *ExpRev-2*
>
> Does the evidence indicate that B's *r2-1* breakpoint sequence agrees with that published by R in Genbank?
>
> `Yes` *ExpRev-1*

YES *ExpRev-2*

Does the evidence provided by H support "a 613 amino acid [R2] protein variant with a novel 19-residue leader sequence derived from upstream of the deletion" as claimed in JUH2001?

No *ExpRev-1*
NO *ExpRev-2*

Would H's claim to have relied on the sequence numbers "-231 to +563" be consistent with the 613 amino acid open reading frame he published in JUH2001? If so, how?

No *ExpRev-1*
NO *ExpRev-2*

Do you believe it is more likely that not that H was acting in "deliberate ignorance" or "with reckless disregard for the truth", as defined by law (see Introductory Note, above), in the manner in which he claims to have relied upon sequence numbers for *r2-1* findings stated in JUH2001?

H has tried to construct a logical progression that can account for his and K's original bad data. I do believe that data was fabricated by H and K. *ExpRev-1*

This is a tough call on this issue but nonetheless it is one of these two conclusions. That is, taken as a whole I would have said "deliberate ignorance". However, on this specific issue the evidence would also be consistent "with reckless disregard for the truth". *ExpRev-2*

Do you believe that it is more likely than not that H made an innocent error or was acting by incompetence in his claims about finding an ORF for *r2-1* as described in JUH2001? If so, why?

I do not believe that these mistakes were innocent. *ExpRev-1*

It is more likely that H was deliberately making things up given the collective observations presented. In either case, I would argue against an innocent error. *ExpRev-2*

Based upon the information presented here, do you believe that it is more likely than not that H falsified his *r2-1* data claims in JUH2001?

Yes *ExpRev-1*
YES *ExpRev-2*

If you believe it was more likely than not that H was *not* acting by incompetence or innocent "error" in making sequence claims for *r2-1* in JUH2001:

a) Should the journal ***retract*** the paper?

Yes *ExpRev-1*
YES *ExpRev-2*

b) Should H be subject to ***any penalty***? If so, what?

At least his grants should be revoked. *ExpRev-1*
YES. H again appears to have engaged in misconduct. That is, in my mind he is criminally at fault[57] - there is no excuse other than self-interested, self-motivated, self-indulgent behavior. *ExpRev-2*

c) Should the **NIH** take such information into account in considering ***future funding*** for this researcher?

Absolutely. *ExpRev-1*
He should be barred from receiving NIH funding in the future.[58] *ExpRev-2*

If you were a member of an NIH grant review committee and learned there of these facts concerning *r2-1* sequence claims, regardless of judgment of whether deliberate, would this have had a negative impact on your scoring of the grant?

Yes. *ExpRev-1*
YES *ExpRev-2*

Back to TOC.

III. Protein Activity Data Falsification - Third Allegation

Due to restrictions placed on the time for which we were allowed to perform discovery (see document, "*Case History*"), the only new evidence that I was able to obtain relating to the third allegation of fraud came from Freedom of Information Act (FOIA) requests for grant documents. Prior to filing the case, I had also obtained grants through the FOIA, as well as publicly available graduate student theses. As the new

[57] This qui tam case did not involve criminal charges.
[58] Note that these questions were asked after consideration of the first claim.

information obtained by FOIA did not require a technical analysis, it was not reviewed by experts.

Nevertheless, the FOIA grant documents, as well as the theses of two of Holloman's graduate students, were very revealing with respect to the third allegation in particular, and the entire alleged Rec1 is Rec2 fraud in general. Therefore, information obtained related to the question of what was known about purified Rec2 activity, both in 1994 as relevant to this case, and throughout the period relevant to the grant documents (through 2007), is presented below.

To review from the Basis for Allegations section, Holloman's graduate student, Brian Rubin, had indicated to me that he was of the belief that data produced by him had been used by Holloman in the 1994 Kmiec, Cole, Holloman paper to imply Rec2 activity, when in fact not only was his data from an inactive preparation, but ***all preparations of such protein produced in the Holloman laboratory by him and postdoctoral fellow Naoto Arai had been inactive***.

On December 27, 1994, Holloman's graduate student, Brian Rubin, made the following comments:

> Rubin: "I think of him as just a guy that develops stories in his office and then comes into the lab and says produce the data that fit my stories. And ***in fact in that paper, there are figures straight from my thesis that have totally nothing to do, really, with what was published***."
>
> Bauchwitz: "What do you mean by that?"
>
> Rubin: "Well like the protein, he [Defendant Holloman] used my purification gel and Western showing the anti-Rec2 antisera binds this protein."
>
> Bauchwitz: "Yeah."
>
> Rubin: "And ***then he claims that this protein is active***. And he would ***rationalize it*** by saying 'Well, it's just a better looking gel than Eric's [Defendant Kmiec]' Of course, we are using the same strain, ***but that prep wasn't active***.

As Rubin also stated in that conversation:

> Rubin: "So he [postdoctoral fellow Naoto Arai] came, and he tried to figure out if the Rec2 protein was a strand exchange protein. He worked on it for, well, almost two years. I'd say about two years. **And he got the same results I did. Basically, we could never show this did anything. And we purified numerous helicases from Ustilago and E. coli, and basically we were looking for a DNA dependent ATPase**. We tried to follow, that was sort of our base assay, was DNA dependent ATPase. We also did extremis. **We tried strand exchange on our purified preps, but we never got it to do anything. …**

Therefore, it was alleged that Holloman falsified Rec2 activity data presented in Kmiec, Cole, Holloman, 1994, according to the information provided by his former graduate student, Rubin.

In addition to the specific issue of data characterization, more generally with respect to the "Rec1 is Rec2" protein activity allegations I argued: "Holloman's reliance on irreproducible data purportedly derived by Kmiec, and ***his disregard of the data produced by researchers in his own laboratory***, which demonstrated no Rec2 activity **at the time of the publication and grant submission**, demonstrate that Holloman acted, at a minimum, with reckless indifference or in deliberate disregard of the truth, given Holloman's awareness that Kmiec had a serious history of reproducibility issues: 1) his Rec1 protein activity work had not been notably reproduced by those inside or outside Holloman's laboratory (excepting subsequently by Kmiec), and 2) Kmiec's work as a postdoctoral fellow with Abraham Worcel had been publicly retracted." (*First Decl.* par. 42, emphasis added.)

In response to the third allegation, and improperly but repeatedly ascribed to all the allegations, Holloman's attorneys used a publication of Holloman's in 2001 that purported to show Rec2 protein purified from *E. coli* as having "Rec1"-like transferase activities (Bennett and Holloman, 2001), as a defense against claims that he had acted inappropriately in trusting Kmiec over those in his laboratory. I termed this the "***Bennett Defense***". The following are excerpts from a discussion of this issue in the *Sec Decl*.

"The Bennett publication of 2001 has no direct bearing on any of the allegations in this case[59]. Indeed, it **in no way addresses claims one (Rec1 amino acid sequence fabrication) or two (rec2-1 DNA sequence falsification) at all**. It is *topically related* to claim three, which involves what we allege were false statements related to image(s) presented in a paper produced by the defendants in 1994, and used repeatedly as of foundational importance to several subsequent grants, including through last year (2007)." (*Sec. Decl*. par. 40.)

"Counsel for the Cornell Defendants have mischaracterized my deposition testimony as suggesting that I believe that there are no false statements within the Bennett and Holloman 2001 paper. This is not the case." (*Sec. Decl*., par. 41.)

"First, I note that defendant Holloman himself actually backed away from the validity of the methodology in the Bennett Paper one year after renewal of a $1.7 million grant for project GM42482, *i.e.* GM42482-12A2. In that competitively renewed, and twice amended grant, Holloman claimed, "We have **only just recently succeeded in being able to produce sufficient amounts** of both Rec2 and Rad51 …" (p. 14) [at RPG 00842]. However, in the next year's Progress Report, it is stated that, ""Isolation of Rec2

[59] Leaving aside the obvious issue that there is nothing independent, or even credible, about information in a publication from defendant Holloman.

protein has **continued to be a formidable problem** … yields of active protein were low and **the method was *not reliable***.”" (*Sec. Decl*., par. 42.)

By the following year, Holloman effectively declared that the Bennett procedure had been abandoned:

> "Isolation of Rec2 has continued to be a formidable problem. ... We have continued seeking a better system for expression of Rec2 and have pursued our finding that soluble Rec2 could be obtained when the gene was expressed in yeast ..." (GM42482-14, p.2.)

It is of significant note that, despite having been served with court interrogatories and requests for production of documents, **Holloman failed to produce any data on Rec2 activity** from 2002 or thereafter to support his contention to the NIH in grant application GM42482-12A2 that he had "sufficient" active Rec2 to even "begin" the studies for which he was funded[60]. In other words, <u>there should have been some data produced after the grant was funded from the sufficient Rec2 portrayed as already being on hand</u> (or some explanation given for why such data was not produced).

With respect to concerns about the irreproducibility of the Bennett protocol, I further noted:

"It is my suspicion, for many reasons to be further elaborated, but not germane to the current motions, that the Bennett and Holloman publication of 2001 is likely part of a continuing fraud. Its lack of scientific value in terms of reproducibility are strongly suggested by Defendant Holloman’s own statements in GM42482-13." (*Sec. Decl*., par. 46.)

I obtained Bennett's thesis from public sources. The description of his thesis work suggested to me one possible explanation was that his results may have been an artifact of contamination by the bacterial (*E. coli*) enzyme, helicase II. For reasons not explained, Bennett did not continue precautions to use bacteria that lacked this activity, even though in his predecessor's, Rubin's, thesis, a significant warning regarding this issue had been given.

What follows are three statements Rubin made in his thesis with respect to the experience he had with a bacterial contaminant activity while trying to study the activity of purified Rec2:

> "A mock preparation was also set up in parallel to show that the helicase that had been purified was not present in the strain that had been used to produced Rec2. A culture of BCM465 which carried the pET3b plasmid only (no *REC2* insert) was used to produce a soluble protein lysate as described recently. ***A major shock came*** when the fractions from the heparin agarose column were assayed from

[60] "We have only just recently succeeded in being able to produce **sufficient amounts** of both Rec2 and Rad51 *to begin* comparative studies ..." (GM42482-12A2, p.14.)

DNA-dependent ATPase activity and the activity profile was exactly the same as with the lysate prepared from the Rec2 overproducing strain." (*Rubin Thesis*, p.77; emphasis added.)

"To sidestep the need to purify helicase II away from Rec2, we decided to construct an *E. coli* expression strain with a *uvrD::Tn5* mutation[61] which encodes helicase II. We also took the opportunity to make the strain *endA1* which results in lack of endonuclease I activity. It was desirable to remove endonuclease I from the background biochemical activities because many of the assays that we had in mind for Rec2 involved DNA substrates which would be targets for endonuclease I degradation." (*Rubin Thesis*, p.79; emphasis added.)

"The pitfalls of attempting to assign a biochemical function to a cloned gene are many. In the case of "Rec2", generic biochemical activities were probed for. These included: DNA-dependent ATPase, helicase, ATP binding, and $ATP\gamma S$ binding. ***Failure to do proper controls, coupled with such generic assays*** led to the incorrect assignment of a helicase activity to Rec2. This illustrates an important limitation of this approach. If one looks for generic activities such as DNA-dependent ATPases in *E. coli*, they will be found. ***Only through controls and attention to meticulous biochemistry*** can the assignment of this type of common activity be assigned to an overexpressed protein of unknown activity." (*Rubin Thesis*, p.82; emphasis added.)

Bennett acknowledged the same risks in his thesis:

Detection of contaminating activities Since Rec2 protein is thought to posses RecA-like enzyme activities it was important to ensure that no contaminating enzymatic activities were present in our protein preparations. The *E. coli* strain used for overexpression of Rec2 protein was a *recA* null mutant ensuring that *E. coli* RecA protein would not contaminate the Rec2 protein preparations. In addition, contaminating nuclease or helicase activities could give rise to complications in pairing and strand exchange assays and possibly even lead to false conclusions. The purified Rec2 fractions were tested for endonuclease

Nevertheless, rather than follow Rubin's approach to use bacteria with as few active contaminants as possible, Bennett apparently used an *Eschericia* bacterial strain[62] which retained the same helicase activity.

[61] This mutation was intended to inactivate helicase II.
[62] HMS 174: F^- *recA1 hsdR*(r_{K12}^- m_{K12}^+) (Rif^R)

Furthermore, Bennett relied upon the same sorts of generic assays during purification that Rubin had warned against[63].

Bennett did claim to perform assays intended to find such contaminants, but one has to wonder why, given the risks and Rubin's experience, Bennett did not at least attempt to use Rubin's helicase-disrupted strains.

However, the most troubling lack of experimental control was that Bennett did not employ in his purifications the one which revealed to Rubin that trouble was afoot - ***an expression plasmid lacking the REC2 gene***. It was use of this negative control that led Rubin to his "major shock". Clearly it was available in the laboratory for Bennett's use, and had been shown to be of great value[64].

The problems in producing active Rec2 that had supposedly already been in hand when the NIH first received the grant, continued even after the Bennett procedure had apparently failed. Holloman stated in progress reports to NIH that he would try obtain active Rec2 using yeast expression constructs; however, that, too, did not work:

> "... we have not still not [sic] been able to purify Rec2 past one or two fractionation steps before it becomes badly degraded. This disappointing result has led us to **reconsidering our strategy**." (GM42482-14, p.3.)

Notably, under such desperate circumstances in which no experiments were reported having produced expected data for years, Holloman's reconsideration apparently did not include having Bennett or Kmiec personally produce active Rec2.

As noted above, Holloman did not reveal during discovery in the legal case any Rec2 purifications by his laboratory from 2002 or thereafter, or any assessment as to why Bennett and Kmiec's protocols were so irreproducible in the hands of others. This situation was reminiscent of that with the "Rec1" purported strand exchange protein purification, which remained unattainable by others in Holloman's laboratory for more than a decade (beginning in the 1980's). When those in Kmiec's postdoctoral laboratory could not reproduce his data, Kmiec was summoned back to that laboratory by its head, Dr. Abraham Worcel. Under the observation of the Worcel laboratory, Kmiec apparently could not reproduce his work either.

Therefore, it appears that the Bennett protocol was quite the opposite of the "vindication" of the Holloman's 1994 Rec2 work with Kmiec. Rather, it seems that the

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[63] It is interesting to note that Bennett's helicase assay used the same substrates as the DNA strand exchange assay, although Bennett reports having never observed helicase activity in the Rec2 purified fractions. (*Bennett Thesis* p.58).

[64] It might be that Bennett obtained activity so sporadically (his thesis stated 3 of 11 preparations using his protocol were active; no data were found in discovery to show that he independently obtained activity using Kmiec's protocol) that a negative control would have been at risk of being deemed a false negative. Nevertheless, only a single positive from such a control would have revealed that something was amiss, so there is inherent value to it, if sufficiently repeated.

experience of Holloman's personnel in not reproducing the activity claimed by Kmiec was the only thing reproduced with Bennett's method.

Back to TOC.

IV. New, fourth allegation of false statements by Holloman to NIH that he had never been able to study soluble Rec2

After the third year of grant funding, Holloman reported that his lab had found that soluble Rec2 could be produced in *E. coli* by fusion to maltose binding protein (MBP). The soluble Rec2 "thorn" had been removed, Holloman declared, but ***no in vitro activity was mentioned as having been observed***.

Indeed, Holloman failed to provide any evidence of active Rec2 at any time after funding of this grant in response to discovery in the legal case, either in 2002 when the his grant first claimed "**sufficient**" Rec2 "to begin" studies, or up to the point three years later when the original, "**fairly manageable**" procedure had been abandoned and purportedly replaced with one using a different Rec2 (MBP) fusion. To my knowledge, no publication examining *in vitro* activity of such a soluble Rec2 has appeared either[65].

There is probably a good reason why Holloman made no report of soluble Rec2 having *in vitro* activity - if that was the case in subsequent progress reports to the NIH. The latter were not produced by Holloman during discovery, so at this time ***adverse inference*** is taken, i.e., **had any evidence to support active Rec2 existed, then Holloman would have had strong motive to produce it to the Court**. one is entitled by adverse inference to assume that such evidence did not exist.

More than suspicions about the final results from soluble Rec2 are at issue.

Holloman made specific claims to the NIH about *not* having previously obtained soluble Rec2 for study. In his 2003 grant GM42482-13 Progress Report he stated:

> "In previous investigations we established that recombinant protein could be highly expressed in bacteria, ***but could not be obtained in a soluble form without the use of denaturing solvents***."

Holloman had also stated in the original grant:

> "Unfortunately, the protein [Rec2] is produced in insoluble form in E. coli",

Examination of Holloman graduate student Brian Rubin's 1994 thesis, however, indicates that soluble Rec2 was the primary form of Rec2 that was first examined (p.71):

[65] As of January, 2012.

Purification of Rec2. Since we did not know the biochemical activity of Rec2, it was decided that only soluble Rec2 should be used in purification. Our main concern was that because the activity of Rec2 could not be assayed, we may not have been able to tell when it was properly renatured.

As a first step in purifying Rec2, the soluble portion of the IPTG-induced BCM464 lysate was precipitated by addition of ammonium sulfate.

Rubin never denatured Rec2 in these experiments, and there is no indication he ever used "denaturing solvents"[66]. (See also *Rubin Thesis*, p.116.)

Therefore, it is alleged that Holloman made additional false claims regarding his having obtained and studied soluble Rec2 to the NIH in progress reports associated with his grant GM42482-12A2.

Unfortunately, due to severe constraints placed by the Court on the time to perform discovery, and the failure of my attorneys to act in an expeditious manner to adhere to the limited time available for discovery, no further evidence on either the third allegation of falsification of protein activity, or the soluble Rec2 issue, was obtained.[67]

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[66] Since it was Rubin who subsequently made the Rec2 expression plasmid that did produce insoluble Rec2, we can assume that he used it as well under such denaturing conditions; however for unknown reasons none of this work appears in his thesis. Nevertheless, it is known that Rubin's was the expression construct that Holloman post-doctoral fellow Arai used and which Holloman sent to Kmiec for use in the Kmiec et. al., 1994 paper. It would appear that Holloman may have not wanted any documentation of failures using this Rec2-hexahistidine approach to appear in Rubin's thesis; indeed, he never mentioned Rubin or Arai's claims that they had performed such work even when directly questioned directly about it during court proceedings (interrogatory).

[67] Typographical corrections and parenthetical information in footnote 64 were added in MS dark red font August 31, 2012. "Kmiec" refers to Eric B. Kmiec, Ph.D., throughout the document. "Holloman" refers to William K. Holloman, Ph.D.
October 17, 2012: "Aria" was corrected to "Arai" in three places; discussion of RNA "5' end" implications excerpted in new footnote; complete references to some of the relevant scientific literature is presented here:

1) Kmiec EB, Cole A, Holloman WK. The REC2 gene encodes the homologous pairing protein of Ustilago maydis. Mol Cell Biol. 1994 Nov;14(11):7163-72.

2) Kojic M, Thompson CW, Holloman WK. Disruptions of the Ustilago maydis REC2 gene identify a protein domain important in directing recombinational repair of DNA. Mol Microbiol. 2001 Jun;40(6):1415-26.

3) Bennett RL, Holloman WK. A RecA homologue in Ustilago maydis that is distinct and evolutionarily distant from Rad51 actively promotes DNA pairing reactions in the absence of auxiliary factors. Biochemistry. 2001 Mar 6;40(9):2942-53.

4) Rubin BP, Ferguson DO, Holloman WK. Structure of REC2, a recombinational repair gene of Ustilago maydis, and its function in homologous recombination between plasmid and chromosomal sequences. Mol Cell Biol. 1994 Sep;14(9):6287-96.

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I, Robert P. Bauchwitz, signed this affidavit on _____ at Hershey, PA.

_____

Robert P. Bauchwitz

SUBSCRIBED AND SWORN TO BERFORE ME on _____ at Hershey, PA.

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March 3, 2013: “transcription” typographical error corrected, FN68; other typographical corrections, noted by MS dark red font, as possible; images centered for html viewing; for move from web root at healthsci.org; full case name provided in the caption; second allegation noted in Synopsis of Evidence. February 20, 2014: Resolutions restored to images damaged in March 2013 update; all images converted from png to jpeg. “With” added to “I was leery of working with the ORI”. Additional Genbank information provided for the likely identity of protein from which amino acids were obtained by Harvard.

ENDNOTES

(i) More detail on the ORI handling of the case.

1. The ORI's general failure to investigate

i. Plaintiff's attorney Poserina email to Assistant U.S. Attorneys David Hoffman and Gerald Sullivan, **May 26, 2005**:

> "I am presuming that we are being granted additional time to clear up the issues presented in the ORI letter. I am also concerned, however, that the only review and investigation given in this case was a scientific paper review of the complaint and disclosure; but ***there does not appear to have been any true investigation of the merits of the claims***. For instance, there has been no investigation to determine what, if anything, did occur at the Harvard lab; ***just a statement that this information may be old***, etc. As far as I can see, there has been no attempt to interview Dr. Rubin, who may provide the bulk of the information to corroborate the complaint[1]. There were, however, comments about whether or not Dr. Rubin said these things or just suggested them; and without an interview of Dr. Rubin, even telephonically, this is just mere discussion. ***This is not an investigation, but just conjecture***."

ii. Attorney Poserina email to Relator of the same date:

> "they [DOJ and ORI] are making a decision without any investigation; with only a paper discussion of the facts. This is far short of the investigation required under the FCA".

iii. Attorney Poserina email to Relator of **July 5, 2005**:

> "Robert: either call me or let me know when and where I can call you; got a letter today from [ORI's] Dahlberg that was not good; **basically they do not feel they could ever get sufficient evidence to proceed**."

2. The ORI's apparent misinterpretation of intent standards

7. With respect to the concern about proving intent, I noted in my first response to the ORI that even if no further evidence were forthcoming, the standard for considering intent was more reasonably and broadly specified by the False Claims Act of 1986, under which this action was being taken. **Intent Standards of the False Claims Act of 1986** (from the first ORI response memo of 2005; see ORI documents on the CD):

"What Congress said was "It is intended that ***persons who ignore 'red flags'*** that the information may not be accurate or those persons who deliberately choose to remain ignorant … should be held liable under the Act. This definition, therefore, enables the government not only to effectively prosecute those persons who have actual knowledge, but also those who play 'ostrich'". (Helmer, JB, False Claims Act: Whistleblower Litigation, Third Edition, Lexis-Nexis 2002). Therefore, we do not believe that the "ORI" standard for proof of "intentionally false" is relevant in this case.

More specifically, the False Claims Act of 1986 states:

> (b) Knowing and Knowingly Defined. - For purposes of this section, the terms "knowing'' and "knowingly" mean that a person, with respect to information - (1) has actual knowledge of the information; (2) acts in ***deliberate ignorance*** of the truth or falsity of the information; or (3) acts ***in reckless disregard of the truth*** or falsity of the information, and ***no proof of specific intent to defraud is required***."

3. The ORI's error of biology

The specific example ORI provided for their intent concern, affecting only one of the three allegations of false statements, was based upon an error of biology on their part. I brought this to their attention in a written response which is part of the ORI documents (docket document 90, Exhibit 10; also present on the CD as two pdf files).

Nevertheless, the ORI persisted in not responding to Department of Justice (DOJ) requests for clarification as to whether they had indeed made a error of biology (with their suggestion that protein translation could be expected to begin at the 5' end of a eukaryotic RNA. It would not.) (See also my letter to ORI's John Dahlberg, present in the ORI documents, doc 90, Exhibit 10)[68].

[68] Excerpted here: The ORI wrote in its first memorandum: "The sequence data for the novel ATG start codon were not published anywhere by WH; the upstream ATG *was implied* because the rec2-1 mutant was shown to produce the 2.1 kb mRNA *that could generate* a Rec2 protein variant".
The Relator replied to DOJ and ORI as follows: "It is incorrect to claim that an RNA *implied* an upstream ATG. **Figure 1** (in [a separate] pdf file) is a figure from a biology textbook used by Dr. Bauchwitz in teaching college students. It illustrates that production of an mRNA from a gene need not lead to production of a protein; these are two separately controlled processes in eukaryotes (nucleated, non-bacterial organisms). **Figure 2** (attached) is from a graduate level text by Benjamin Lewin; text box annotations are by Dr. Bauchwitz. The figure illustrates in more detail that the start site of RNA transcription is separate from that for protein translation. The consequences of losing an AUG start site are illustrated in **Figure 3**, which is reprinted from the Disclosure Statement. Holloman's motivation to claim a

To present to the Court expert opinions on the issue as to whether ORI may have been in error, and to further assess the merit of the case based on the evidence initially presented to the government, ***reviews by two science experts who had assessed the information disclosed to the government, as well as the ORI report, were submitted***. One of the reviewers was a member of the National Academy of Science and a longtime director of a prominent biomedical research institution, The Carnegie Institution of Washington D.C., while the other was a professor at the Mayo Clinic. As their complete reports have been produced during subsequent discovery, a summary of their comments provided to the Court on January 17, 2006 are reiterated:

i. The two scientists noted the following concerning the ORI "science" issue:

> Reviewer 1: "The [ORI] argument is ***embarrassingly flawed***. The conclusion that "regardless of who is correct on the sequence data, an initiation codon upstream of the published sequence does seem to exist" [quote from ORI memo 1] is ***not supported by the available data*** and it is both surprising and disturbing that anyone would come to such a conclusion based on the available data".
>
> Reviewer 2 "There is ***no evidence*** that [Defendant] H or anyone in H's lab identified an ATG that begins an open reading frame that would produce a protein ... there are many RNAs made by cells that do not make any protein. Without the sequence of the RNA no conclusion can be made about that RNAs function. ***Why would one [the ORI] conclude that this RNA is 'initiated through a novel and abnormal mechanism'?***"

ii. Regarding their overall view of the case, as requested by the following question: "***Do you feel that the preponderance of the evidence presented indicates that scientific fraud has occurred in this case***?"

> Reviewer 1: "**Yes**. Data, facts, and descriptions thereof appear to have been manipulated. Results were willfully ignored and presented with the intent of misleading grant reviewers as well as others in the scientific community."
>
> Reviewer 2 "**Yes**. Evidence suggests that on more than one occasion experiments were never even done. These scientists have

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novel upstream AUG derives from the knowledge that the next AUG in the protein lies downstream of a critically important, conserved protein sequence required for activity of similar proteins. The ORI author further makes the claim that the purported RNA *could generate* a Rec2 protein variant. There is no data whatsoever anywhere indicating that the purported 2.1 kb RNA even points in the same direction as the REC2 gene, much less can generate a protein product."

been the subject of refutation not once, not twice, but three times." [emphasis added]

Therefore, although these science experts seriously disagreed with ORI regarding the question of biology, they agreed with ORI that the allegations had merit.

Even had I not challenged the ORI's error, in reality what was at issue were lies made by the Defendants about the actual data they had in their possession, and their use of such false statements to obtain federal funding. Alternatives by which desired proteins might have been produced were irrelevant to consideration of the fraud or underlying scientific misconduct. Subsequent discovery strongly supported my contention about the availability of evidence as well as the scientific misconduct involved.

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