IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Cabilly et al	)	
	)	Interference No. 102.572
v.	)	
	)	Examiner-in-Chief
Boss et al	)	Mary F. Downey

DECLARATION OF WILLIAM HOLMES

1. I, William Holmes, declare and state that I am a citizen of the United States residing in Pacifica, California. My Curriculum Vitae is attached as Cabilly Exhibit No. 10. I am a co-inventor of United States Patent 4,816,561 entitled "Recombinant Immunoglobulin Preparations".

2. I have been employed at Genentech, Inc., which is located at 460 Pt. San Bruno Blvd., South San Francisco, California 94080 from August 2, 1979 to January 4, 1985 and from June 1, 1987 to the present. My present position is that of Scientist.

3. I was approached by Herb Heynecker to participate in a project, to express antibodies directed against human carcinoembryonic antigen (CEA) in E.coli. I started working on this project on July 12, 1982 (Cabilly Exhibit No. 11, Bates No. 00941).

4. I received a sample of polyA mRNA from The City of Hope which had been isolated from the hybridoma cell line CEA.66-E3. This cell line produces an anti-CEA antibody. From this mRNA sample I prepared a cDNA which was then incorporated into plasmids to make an E.coli colony library (Cabilly Exhibit No. 11, Bates No. 00941, and Cabilly Exhibit No. 20, Bates No. 00989).

5. I inoculated colonies from the CEA.66-E6 cDNA into microtiter plates containing media (Cabilly Exhibit No. 11, Bates No. 00941). These cultures were stamped onto agar plates and allowed to grow. The colonies were then transferred to duplicate sets of nitrocellulose filters by Michael Rey (a laboratory technician in Dr. Heynecker's lab) transferred to agar plates and allowed to regrow (Cabilly Exhibit No. 15, Bates No. 00502). Mr. Rey also lysed the colonies on each of these filters and

DOCKET ALARM Find authenticated court documents without watermarks at docketalarm.com.

treated them for subsequent probing with radioactive oligonucleotides (Cabilly Exhibit No. 15, Bates No. 00504).

6. I received oligonucleotides from the Organic Chemistry Department at Genentech. I used these oligonucleotides to prepare light chain and heavy chain oligonucleotide probes to hybridize with the filters. After exposure to X-ray film I picked several colonies which hybridized to the light chain or heavy chain oligonucleotide probes. I characterized several of these colonies by PstI restriction endonuclease digestion and fractionation by polyacrylamide electrophoresis (PAGE). Several colonies which hybridized to the heavy chain probe were also digested with both PstI and NcoI and analyzed by PAGE (Cabilly Exhibit No. 11, Bates Nos. 00942, 00946, 00955, 00956).

7. I subcloned these DNA sequences into M13 vectors and Michael Rey sequenced both the light chain and heavy chain DNA inserts. Dr. Herb Heyneker and I analyzed the sequences. The entire coding region of the light chain was found in the cDNA insert of pK17G4. Portions of the nucleotide sequence of the heavy chain were found in two isolated plasmids: pGamma298 and pGamma11 (Cabilly Exhibit No. 11, Bates Nos. 00943, 00947, 00950, 00954, 00957; Cabilly Exhibit 12, Bates No. 00843; Cabilly Exhibit 15, Bates Nos. 00521, 00522, 00525-00528, 00531).

8. The plasmid for direct expression of the anti-CEA light chain gene was made by digesting pHGH207-1* with EcoRI, filling in and digesting with BamHI. Following purification of this fragment, I treated the DNA with bacterial alkaline phosphatase (Cabilly Exhibit No. 12, Bates No. 00830). The large fragment was purified by PAGE (fragment 1).

9. I also digested pK17G4 DNA with PstI, purified the fragment by PAGE, digested with Ava II and isolated the 333 bp PstI-AvaII fragment by PAGE (Cabilly Exhibit No. 12, Bates No. 00825). I used the PstI-AvaII fragment and an oligonucleotide primer in a primer repair reaction to introduce the initiation codon to the light chain gene (Cabilly Exhibit No. 12, Bates No. 00837). The fragment was purified by PAGE, cleaved with Sau3A and the 182 bp fragment isolated by PAGE (fragment 2). The primer repair reaction was confirmed by Michael Rey via sequencing (Cabilly Exhibit No. 15, Bates No. 00530). Dr. Herb Heyneker and I analyzed the sequencing results.

10. Fragments 1 and 2 were ligated together and the ligation reaction transformed into E.coli. The resultant transformants were analyzed by restriction digestion and sequencing to confirm the construction of pKCEAInt1 (Cabilly Exhibit 12, Bates No. 00838).

DOCKET ALARM Find authenticated court documents without watermarks at docketalarm.com.

11. I digested pK17G4 DNA with PstI and purified the fragment by PAGE. This fragment was partially digested with AvaII, filled in and purified by PAGE. This fragment was subsequently digested with HpaII and the 497 bp fragment isolated by PAGE (fragment 3) (Cabilly Exhibit No. 12, Bates No. 00840-00842).

12. I digested the plasmid pKCEAInt1 with AvaI, filled in and digested with XbaI. The large fragment was treated with BAP and isolated by PAGE (fragment 4). The small fragment was digested with HpaII and the 169 bp fragment isolated by PAGE (fragment 5) (Cabilly Exhibit 12, Bates Nos. 00845-00846).

13. Fragment 3, 4 and 5 were ligated and the ligation reaction transformed into E.coli. Resultant transformants were analyzed by restriction digestion to confirm the construction of pKCEAInt2 (Cabilly Exhibit No. 12, Bates No. 00853).

14. I digested pHGH207-1* with AvaI, filled in, digested with BamHI, treated with BAP and purified the large fragment by PAGE (fragment A).

15. I digested pGamma11 with PstI, the fragment was purified by PAGE, digested with AvaII, filled in, and digested with TaqI. The 375 bp fragment was isolated by PAGE (fragment B) (Cabilly Exhibit No. 12, Bates No. 00848-00849).

16. I digested pGamma298 with TaqI, BamHI, and isolated the 496 bp fragment by PAGE (fragment C) (Cabilly Exhibit No. 12, Bates No. 00848).

17. I ligated fragments A, B and C and transformed the ligation reaction into E.coli. The resultant transformants were analyzed by restriction digestion to confirm the construction of pGammaCEAInt (Cabilly Exhibit No. 12, Bates No. 00853).

18. I used a 15 nucleotide DNA primer in a primer repair reaction to introduce the initiation codon into an AluI to RsaI fragment of pGamma298 (fragment D). I digested pGamma298 with PstI, BamHI, HpaII and purified the fragment by PAGE (fragment E). I digested pGammaCEAInt1 with EcoRI, filled in, and digested with BamHI. I then treated this fragment with bacterial alkaline phosphatase and purified the fragment by PAGE (fragment F). I ligated these three fragments (D, E and F) and transformed the ligation reaction into E.coli. The plasmid, pGammaCEAInt2, was confirmed by restriction analysis and sequencing (Cabilly Exhibit No. 12, Bates Nos. 00855, 00852, 00858, 00863; and Cabilly Exhibit No. 15, Bates No. 00534)

19. I prepared the expression plasmid pGammaCEAtrp207-1* as follows: the plasmid pBR322(Xap) was digested with EcoRI, filled in, digested with PstI, and purified by PAGE. I isolated a 1543 bp fragment by treating pGammaCEAInt2 with PstI followed by BamHI and purification by PAGE. I also isolated a 869 bp fragment from pGammaCEAInt2 by digestion with AvaI, filling in, cleavage with BamHI and

DOCKET ALARM

Find authenticated court documents without watermarks at docketalarm.com.

subsequent PAGE purification. I then ligated these fragments, transformed the ligation reaction into E.coli, and analyzed the resultant colonies by restriction analysis to confirm pGammaCEAtrp207-1* (Cabilly Exhibit No. 13, Bates Nos. 00881, 00882 and 00876).

20. Shmuel Cabilly carried out reactions to construct the FAB expression plasmid pGammaCEAFABtrp207-1* (Cabilly Exhibit No. 20, Bates No. 00975-00976, and 00982-00985). Michael Rey, Shmuel Cabilly and I analyzed several tetracycline resistant transformants from the pGammaCEAFABtrp207-1* ligation by restriction cleavage analysis and sequencing (Cabilly Exhibit No. 13, Bates Nos. 00885-00887, and 00889; Cabilly Exhibit No. 15, Bates Nos. 00541, 00543, and 00544, and Cabilly Exhibit No. 20, Bates Nos. 00986-00987).

21. I further declare that all statements made of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

William Holmes, Ph.D.

Dated: 10/28/91

DOCKET ALARM

Find authenticated court documents without watermarks at docketalarm.com.