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 SHMUEL CABILLY

1. I, Shmuel Cabilly, declare and state that I am a citizen of the state of Israel, residing in Haifa, Israel. My Curriculum Vitae is attached as Cabilly Exhibit No. 19. I am a co-inventor of United States Patent 4,816,561 entitled "Recombinant Immunoglobulin Preparations".

2. From December, 1980 until October, 1984, I was a Post Doctoral Fellow in Dr. Art Rigg's laboratory at the City of Hope, Duarte, California. I am presently employed by the Weizmann Institute of Science as a Scientist, in Rehovot, Israel.

3. I began my involvement in the project to express antibodies in bacteria on or about December, 1980. In response to an invitation from Dr. Art Riggs to join his laboratory, I came to the City of Hope.

4. In September, 1981, I received CEA.66-E3 cells from Dr. John Shively at the City of Hope (Cabilly Exhibit No. 20, Bates No. 00970). I used the CEA.66-E3 cells from Dr. Shively's lab to extract total RNA. PolyA mRNA was purified from the total RNA by using an oligo-dT cellulose column (Cabilly Exhibit No. 20, Bates No. 00970-00974). Following the isolation of the mRNA, I gave a sample of it to William Holmes at Genentech for the preparation of an E.coli colony cDNA library (Cabilly Exhibit No. 11, Bates No. 00941 and Cabilly Exhibit No. 20, Bates No. 00989).

5. I modified pKCEAtrp207-1* by cleaving out the PstI-PvuI fragment from the ampicillin resistance gene, filling it in and religating the blunt ends (Cabilly Exhibit No. 20, Bates Nos. 00995-00997) to yield the plasmid pKCEAtrp207-1*delta. The plasmid is resistant to tetracycline but sensitive to ampicillin.

6. I transformed competent E.coli cells with pKCEAtrp207-1*delta. I then isolated successful transformants, treated them with calcium chloride and retransformed these competent cells with pGammaCEAInt2 which confers resistance

to ampicillin but not to tetracycline. These cotransformed cells can be grown and identified by their resistance to both ampicillin and tetracycline (Cabilly Exhibit No. 20, Bates No. 00997-00998).

7. I grew the co-transformed cells in minimal media containing ampicillin and tetracycline. These cultures were induced with indoleacrylic acid (IAA) and samples were taken and allowed to grow over night. The cells from these cultures were collected by centrifugation and refractile body preps made. One of these samples was given to Jeanne Perry for analysis by SDS- polyacrylamide gel electrophoresis (PAGE) (Cabilly Exhibit No. 8, Bates Nos. 00149-00151). I analyzed several samples by SDS-PAGE which were subsequently silver stained or subjected to Western blot using anti-mouse IgG for the identification of light and heavy chain protein (Cabilly Exhibit No. 20, Bates Nos. 00994, 00998-01001).

8. I conducted a reconstitution experiment with material from the co-transformed heavy and light chain E.coli cells. I grew the co-transformed cells, lysed them by sonication and solubilized the pellet with guanidine hydrochloride (Cabilly Exhibit No. 20, Bates Nos. 00991-00992). This material was incubated overnight at room temperature. I, then, dialyzed the reaction mixture against urea buffer at room temperature followed by dialysis into phosphate buffered saline (PBS). I performed an assay to detect active anti-CEA antibody. I found that the heavy chain and light chain protein had recombined to yield antigen binding activity significantly higher than background (Cabilly Exhibit No. 20, Bates Nos. 00992-00993).

9. I was also involved in the construction of pGammaCEAFABtrp207-1*, a plasmid vector for the direct expression of the FAB fragment of the heavy chain gene (Cabilly Exhibit No. 20, Bates No. 00975). I digested pBR322 with HindIII, filled in, digested with PstI and treated with bacterial alkaline phosphatase (Cabilly Exhibit No. 20, Bates Nos. 00982-00983). I isolated the vector fragment by PAGE (fragment I). I received a sample of pGammaCEAtrp207-1* from William Holmes (Cabilly Exhibit No. 13). I digested this plasmid with BamHI and PstI and isolated the fragment by PAGE (fragment II). Another sample of this plasmid was digested with NcoI and NdeI. I isolated the 260 bp DNA fragment by PAGE. I used a 13 bp oligonucleotide primer in a primer repair reaction in order to introduce a termination codon (Cabilly Exhibit No. 20, Bates No. 00976). The fragment was then digested with BamHI, the 179 bp fragment isolated by PAGE, and filled in (fragment III) (Cabilly Exhibit No. 20, Bates No. 00976). Approximately 100 ng of fragment I, II, and III were ligated and transformed into E.coli (Cabilly Exhibit No. 20, Bates Nos. 00983-00985). These

transformants were analyzed by Michael Rey, William Holmes and myself (Cabilly Exhibit No. 13, Bates Nos. 00885-00887 and 00889; Cabilly Exhibit No. 15, Bates Nos. 00541, 00543 and 00544; Cabilly Exhibit No. 20, Bates Nos. 00986-00987) which verified the construction of pGammaCEAFABtrp207-1* by sequencing and restriction cleavage analysis. These transformants were later co-transformed with the light chain plasmid pkCEAtrp207-1* (Cabilly Exhibit No. 20, Bates Nos. 01013-01014).

10. 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.

Cabilly Shmuel

Shmuel Cabilly, Ph.D.

Dated: Oct 28 1991