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 MICHAEL MUMFORD

1. I, Michael Mumford, declare and state that I am a citizen of the United States residing in Bellevue, Washington. My curriculum vitae is attached as Cabilly Exhibit No. 16.

2. From June, 1981 until November, 1987, I was an employee of Genentech, Inc. (Genentech) located at 460 Point San Bruno Blvd., South San Francisco, California 94080. During the time spanning June, 1981 to April, 1985 I was responsible for microbial fermentation optimization which was performed to confirm, as well as maximize expression of gene products. I am presently employed by Immunex as Vice President of Pilot Plant Operations, in Seattle, Washington.

3. Genentech Notebook No. 1246 (Cabilly Exhibit No. 17) contains handwritten records or samples received by myself of others under my supervision in the Fermentation Research and Process Development Department. These samples were used to make DMSO stocks of microbial cultures for frozen storage to be utilized later in fermentation runs. It was my normal practice to begin fermentation research and optimization within a few weeks after I received a new recombinant organism that a researcher wanted to be grown.

4. The procedure I used to make DMSO stocks was to purify the strains that were received. I achieved purification by streaking a small aliquot of the liquid cultures or a colony from a plate, onto an agar plate containing selective media. After incubation, I would pick a single colony to inoculate into selective liquid media. This would be grown to an optical density of approximately 1.0 at a wavelength of 500nm. The culture would then be diluted to a final concentration of 10% DMSO.

Following addition of DMSO, the cultures would be aliquoted in 1.0 ml quantities into pre-sterilized cryovials and frozen at approximately -60°C or below.

5. On December 13, 1982, Neal Clayton recorded receipt of W3110/pGGR1+L from Dr. Herb Heynaker's laboratory (Cabilly Exhibit No. 17, Bates No. 00615). This organism is an E.coli strain (W3110), contains a plasmid for the expression of the light chain of an antibody directed against human carcinoembryonic antigen (CEA) antibody.

6. On December 20, 1982, I recorded the receipt of W3110/pGGheavy from William Holmes (Cabilly Exhibit No. 17, Bates No. 00617). This organism is an E.coli strain containing a plasmid for the expression of the heavy chain of an anti-CEA antibody. The liquid culture received was used to prepare DMSO stocks, although due to the expedited nature of this project, the liquid culture was used to initiate 10L research fermentations prior to the completion of the DMSO stocks.

7. On February 2, 1983, I recorded the receipt of W3110/p10$_2$ and W3110/p6$_2$ from Dr. Herb Heynaker's laboratory (Cabilly Exhibit No. 17, Bates Nos. 00626-00627). These two organisms are E.coli strains, which had been co-transformed with two plasmids for the co-expression of heavy and light chain of an anti-CEA antibody. These samples were used to prepare the DMSO stocks 1246-31 and 1246-32, respectively.

8. On March 31, 1983, I recorded the receipt of W3110/pXAPtetGammaFABII from William Holmes (Cabilly Exhibit No. 17, Bates No. 00645). This E.coli strain contained a plasmid for the expression of the FAB portion of the heavy chain of an anti-CEA antibody. I renamed this sample pCEA3. This sample was used to prepare the DMSO stock 1246-50.

9. Notebook No. 1247 (Cabilly Exhibit No. 18) contains handwritten records and brief summaries of fermentations. The fermentations were conducted by myself or someone under my supervision. The 10L research and development fermentations followed a general format. The DMSO stock of interest would be thawed and 0.1 ml would be used to inoculate approximately 500 ml of LB liquid broth containing appropriate antibiotics. This culture would be incubated for 8 hours at 37°C with an agitation rate of approximately 250 rpm. Inorganic salts would be brought to approximately 8L with water in a fermentation vessel. After sterilization, several additions would be made. These would include antibiotics, vitamins and a carbon source. The inoculum would be aseptically transferred to the bioreactor by gravity feed from the inoculum flask. During the fermentation, the temperature would be kept constant at 37°C, the agitation at 1000 rpm and aeration at 10 liters per minute.

As the fermentation progressed, samples would be taken hourly after inoculation. Product expression would be induced by the addition of IAA (50 ug/ml). The fermentation would continue for four to six hours after induction. The culture would be harvested by centrifugation, the wet biomass scrapped from the centrifuge container into a plastic bag and frozen at approximately -60°C or below for at least 24 hours.

10. On January 12, 1983, I recorded the fermentation of W3110/pGGheavy from DMSO stock No. 1246-22 (Cabilly Exhibit No. 18, Bates Nos. 00697-00698). This fermentation conducted December 21, 1982, was assigned identification number GG-1I. The product that was expected from the fermentation run was the heavy chain of the anti-CEA antibody. This was the first immunoglobulin gene we worked with in the Fermentation Research and Process Development Department. I recorded in my notebook that refractile bodies were seen. Refractile bodies are indicative of recombinant protein over expression. Analysis by SDS-polyacrylamide gel electrophoresis (PAGE) shows a band in the 55kD region which is the expected molecular weight of the heavy chain product.

11. On January 12, 1983, I recorded fermentations of W3110/pGGheavy from DMSO stock No. 1246-22 (Cabilly Exhibit No. 18, Bates Nos. 00699-00701). These fermentations were assigned identification numbers GG-2I and GG-3J and were conducted January 3, 1983. The product that was expected from the fermentation run was the heavy chain of an anti-CEA antibody, as in fermentation run GG-1I. However, in these fermentation runs there were variations of the fermentation format such that some fermentation nutrients were removed and product induction occurred at a later time point in the process. Analysis by SDS-PAGE shows a band in the 55kD region which is the expected molecular weight of the heavy chain product. I recorded that the fermentation run GG-3J was harvested and given to Dr. Ron Wetzel for purification.

12. On January 20, 1983, I recorded in my notebook multiple fermentations of W3110/pGGR1+L from DMSO stock No. 1246-20 (Cabilly Exhibit No. 18, Bates Nos. 00726-00729). These fermentations were assigned identification numbers GG-2H, GG-3I and GG-4J and was conducted January 6, 1983. The product that was expected from these fermentation runs was the light chain of an anti-CEA antibody. The fermentation designated GG-3I, producing the light chain anti-CEA antibody, was examined microscopically at the completion of the fermentation run in which refractile bodies were seen, signifying product expression. SDS-PAGE analysis (Cabilly Exhibit No. 18, Bates Nos. 00728-00729) indicated product expression at the

23.5 kD molecular weight range which was expected for light chain protein. This fermentation was harvested (486 grams) and given to Dr. Ron Wetzel for purification (Cabilly Exhibit No. 18, Bates Nos. 00727).

13. On February 14, 1983, I recorded the fermentations of the co-transformed strains, W3110/p10$_2$ (GGLH-1) and W3110/p6$_2$ (GGLH-2) from the DMSO stocks No. 1246-31 and 1246-32, respectively (Cabilly Exhibit No. 18, Bates Nos. 00730, 00733 and 00734). These fermentations were assigned identification numbers GGLH-1 and GGLH-2, and were grown in bioreactors No. 2 and 3 on February 8, 1983. The products that were expected from these fermentation runs were both the light and heavy chain of an anti-CEA antibody. SDS-PAGE shows protein expression from the time course samples. I recorded that the fermentation run No. GGLH-1 was harvested and that the 80 grams of wet biomass paste was given to Dr. Ron Wetzel. Fermentation GGLH-1 demonstrated refractile bodies upon microscopic analysis.

14. 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.

Michael Mumford

Dated: 10-28-91