EXHIBIT H-23

EXPRESS MAIL # 90169539

JJV

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#23

In re Application of:

Fu-Kuen LIN

Serial No: 675,298

Filed: November 30, 1984

For: Production of Erythropoietin

Examiner: A. Tanenholtz

Group Art Unit:

I hereby certify that this paper is being deposited with the United States Postal Service "EXPRESS MAIL" under 37 CFR §1.10 addressed to: Commissioner of Patents & Trademarks, Washington, D.C. 20231, on this date.

October 21, 1987

Pamela A. Simonton
Registration No. 31,060
Attorney for Applicants

TRANSMITTAL

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Enclosed is Fu-Kuen Linn's Declaration Re: Microorganism Deposit for filing in the above referenced case.

Respectfully submitted,

AMGEN INC.

Date 10/21/87

Pamela A. Simonton
Reg. No. 31,060

Amgen Inc.
1900 Oak Terrace Lane
Thousand Oaks, CA 91320

PatFormT

443

451

EXPRESS MAIL # 90169539

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	PRODUCTION OF ERYTHROPOIETIN
FU-KUEN LIN	
Serial No.: 675,298	Group Art Unit
Filed: November 30, 1984	Examiner - A. Tanenholtz

DECLARATION RE: MICROORGANISM DEPOSIT

FU-KUEN LIN, DECLARES AND SAYS:

1. That he is the inventor of the subject matter claimed in the above-identified patent application;

2. That certain cultures have been deposited in the permanent culture collection of the American Type Culture Collection, 12031 Parklawn Drive, Rockville, Maryland, and the culture deposits meet the requirements of the Budapest Treaty for the purposes of patent procedure;

3. That said cultures were deposited, after the filing date of the above-identified patent application, on October 20, 1987 as accession numbers ATCC 40381, for a deposit of a phage clone designated λhE1 at page 42 of the above-identified application, and ATCC 67545, for a deposit of a clone designated pMkE83 in E. coli ("clone 83") as described at page 34 of the above-identified application;

4. That the microorganism in each of said cultures was in his possession prior to the filing of the above-identified application and prior to December 13, 1983, as supported for λhE1 by the identical construction designated "λ21-2a" on attached page 19 of co-worker Sidney Suggs' Notebook No. 439, dated October 28, 1983 and page 30 dated November 7, 1983 and identified as Exhibit 11, and for pMkE83 by the identical construction designated "p83" on attached page 37 of co-worker Sidney Suggs' Notebook No.439, dated November 11,

452

449

[illegible]3 and identified as Exhibit 12;

5. That he declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief and 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, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

KIRIN-AMGEN, INC.

BY: Fu-Kuen Lin
Fu-Kuen Lin
Inventor

Oct. 21, 1987.

445

453

EXPRESS MAIL # 90169539

Project No. 4

TITLE Southern blots of E-D ... Book No II 19

From Page No. — F.K. Lin has isolated 4 clones that "light up" with oligo probes designed from
10/27 2 different regions of EPO. Charon 4A phage have been grown up and banded in CsCl. These DNA's were dialyzed (in Eppendorf tubes) vs 50 mM $T_{25}E_{10}$ for ~ 1 hr. I will prepare DNA from these.

			20% SDS	5 M NaCl	proteinase K (15 mg/ml)
Measure volumes:	16-3a'	0.52 ml	5 µl	175 µl	10 µl
	21-2a	0.52	5 µl	175 µl	10 µl
	27-6a	0.51	5 µl	170 µl	10 µl
	44-1a	0.34	3.5 µl	115 µl	7 µl

Add the amounts of the salts indicated to bring SDS to 0.2%, 1/30 volume 5M NaCl, proteinase K to 100 µg/ml
Incubate 50°/30'
ϕ-OH/$CHCl_3$ extract (0.7 ml) 2X → $CH:Cl_3$ extract (0.7 ml) → divide each sample into 2 tubes → other extract (~1 ml)
Put at 4°/ON

10/28 I vortexed the samples yesterday during the phenol extraction. This should probably not have been done. Should gently extract.
Put in spun vac for 5'.
Add ~1 ml EtOH to each tube. Most tubes had viscous globs of DNA.
- Put on dry ice/5'
Spin 5' in Eppendorf. Large salt pellets for 7 of the 8 tubes.
Resuspend the 7 pellets @ in 300 µl H_2O & Add 700 µl EtOH. -20°/1.5 → dry/K
Spin. Wash Dry
Resuspend in 75 µl $T_{10}E_{0.1}$
Measure OD profiles: Will just 27-6a look like clean DNA profiles. 27-6a appears to contain protein (contamination at OD_{270}).
The concentrations are: 16-3a' 1.13 mg/ml
21-2a 700 µg/ml
27-6a 460 µg/ml
44-1a 333 µg/ml

Dilute DNA's:	Stock DNA solution	H_2O
16-3a'	2.5 µl	34 µl
21-2a	4 µl	32
27-6a	6.2 µl	30
44-1a	9.3 µl	31
From Dave Fremont-Smith → 16-3a	18 µl	18

Put in frig/ON

To Page No. 20

Witnessed & Understood by me,	Date	Invented by	Date
M Alton	11/10/83	Recorded by Ed Sharp	28 Oct 83

EXHIBIT 11 - LAB No. 439 1/46

454

Project No. 1

30 Book No. IV TITLE M13 sequencing of putative EPO clones

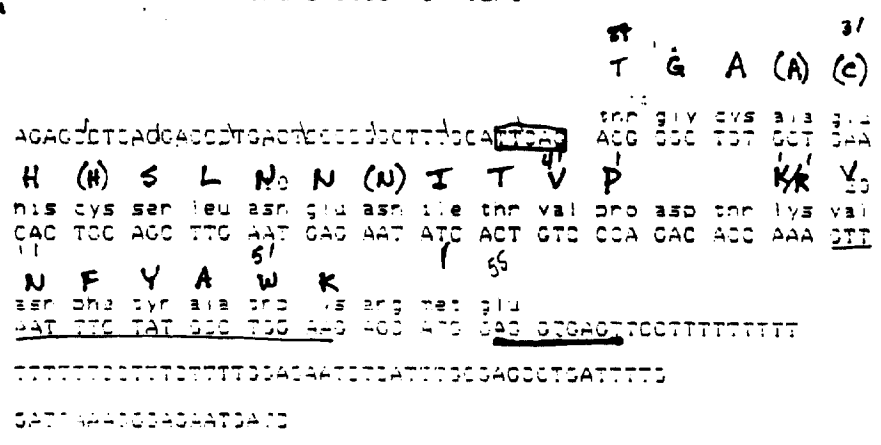
From Page No. 29

quence that is a perfect match for one of the sequences in the EpV probes. The sequence is one of the 16 sequences in EpV 6. This means that the clone was detected with a 1 bp mismatch.

20 m

Trans ation of .Dra

clone 9,11,12



iii) Above is shown the reverse complement of the sequence. The probe hybridization site is underlined in red. The letters written in blue above the nucleotide sequence are the amino acid sequences of EPO frag obtained by Por Lin. The first stretch of amino acid sequence is starting with residue 27 from the N-terminus. The second stretch of sequence corresponds to the tryptic peptide from which the 20-mer (EpV) probes were designed. The nucleotide sequence enclosed in the box matches well with a consensus splice acceptor site. The underlined sequence matches extremely well with a consensus splice donor site.

iv) Clones 1,3,4 are the same as clone 17 that Mike Fox sequenced. My sequence did not work as well as his, mainly because I have some "n-1" mers in the sequence. This is probably caused by s/o contamination.

v) Mike sequenced clone 18 & 19 as well. These clones had different sequences from each and different sequences from 17 as well. He did a C-test and determined that 18 is the opposite orientation from 17.

vi) The sequence of all the frag to date are on the next page. We have been unable to find the 17-mer hybridization site in the sequence of clones 17 & 18.

vii) From the sequence of clones 9,11,12, there is no doubt that clones 21-2a (and 17-6a as well) is a clone for EPO.

To Page No. 31

Witnessed & Understood by me,	Date	Invented by	Date
M K Alton	11/10/83	Recorded by Sid Suggs	7 Nov 83

441

Project No. 7

TITLE Sequencing of EPO cDNA clone #83 Book No. IV 37

From Page No. —

11/10 I want to try sequencing directly out the plasmid using the 20-mers (EpV) as primers. This is the procedure we used at City of Hope. F.K.L. believes that the EpV_2 mixture contains the sequence found in the monkey cDNA clone.

Yesterday, I received ~1 μg of rapid plasmid DNA (alkaline method) that was prepared by F-K.L. He added an equal volume of 4 M NH_4OAc and then 0.6 volumes iso-propanol (room temp), and then gave the DNA to me. I let it sit at room temp for ~1 hr.

Spin. Wash. Dry.

Resuspend in 1.75 μl H_2O
0.25 μl 10× ISRB
0.2 μl 1 M tris, pH 7.4
0.2 μl Eco RI (12 u/μl)
37°/1 hrs → −20°/ON

Today, set up std sequencing rxns

t/p: 2.5 μl DNA soln (B3 cut with Eco RI)
4 μl H_2O
0.75 μl 10× H buffer
0.5 μl EpV_2 (1 pmol/μl) — of each sequence
100°/3′ → plunge into ice water bath and use immediately.

A/dN: see p. 33

Rxns were run at room temp.

Run on a 10% PA-8 M urea gel.
Expose 2 hrs/−80°/2 screens

CONCLUSIONS:

i) Clone B3 definitely is an EPO clone. There is considerable sequence homology with the human genomic clone sequence.

ii) I will be able to read more sequence tomorrow when I have an exposure without screens.

11/11 iii) On the computer, I have translated all 3 frames of the reverse complement of the sequence. I can find 3 stretches of sequence that match well with the human genomic sequence and/or the human amino acid sequence 43 88

To Page No. —

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by Sid Suggs	11 Nov 83

456