EXHIBIT 4

LABORATORY NOTEBOOK

№ 633

AMGEN, INC.

NOTEBOOK NO. 633

ISSUED TO dEjmé

ON 7/10 19 84

DEPARTMENT

RETURNED 19

SCIENTIFIC NOTEBOOK CO.
5007 WEST DONNA DRIVE
STEVENSVILLE, MICHIGAN 49127

≡ 9/19-9/21/84 Western Analysis

Project No.
Book No.

65

Page No.

Large Western analysis = 2 gels to look at the following:

(1) Comparison of Bill Hu EPO B Lot 82 to illustrate similarity in size
ENDO F treatment of 2 as well

(2) Goldwasser EPO ± ENDO F

(3) α Therapeutics EPO ± ENDO F

} ∴ Comparison of site of glycosylated + deglycosyl EPOs from 3 diff. human urinary preps.

(4) Comparison of ab-reactive EPO in cycles 1 → 9 RB collections + their ENDO F digestion prods.

(5) Bill pool for 1st scale human EPO purification ± ENDO F

(6) ~~Sean~~ Cycle (2) Bill RB Sample 1st either treated or not treated ± ETOH pptn before ~~either~~ running on gel

(7) Use α C terminal peptide in Western ± ± ENDO F treated EPOs

(8) GEL, TRANSFER + AB PROTOCOL

Run 12.5% SDS PAGE - during the day.
Transfer to 0.2 μm Nitrocellulose at 30 v/ON = 16 hours
Block ± either 10% horse or goat sera in PBS

Abs - (1) for part of 1 gel use 208 prep #9 1.05 mg/ml ab at conc of 12 μg/ml - ON
then RT in 5% horse sera
Develop ± avidin/biotin reagents - 1.5 hr α mouse Abs, 1.5 hr ABC

(2) α NH2 peptide ab 1:300 dilution of SC196 #4 4/25/84 test bled in 5% goat sera
other conditions as in #1

(3) α C terminal peptide ab - 1:200 dilution of SC 278 #4625 in 5% goat sera
other conditions as in #1

ETOH Precipitation Protocol

From Jeff's Coomassie-stained gel it looked like there was only 1 ENDO F digestion product + not the usual doublet that we've seen. Difference betw his protocol + ours is that he used ETOH pptn to concentrate sample after ENDO F ± before gel loading. The undigested sample was treated the same way.

Shot ± sample = 15 λ cycle (2) + 5 λ ^{125}I EPO (230K cpm) Add 250 λ -80° ETOH & 100%
Let sit at -80° for 20 min.
Centrifuge 2' in Eppendorf. Remove sup. See no pellet, but have faith.
Count pellet + sup (in new tube)

To Page No. 66

Read & Understood by me,	Date	Invented by	Date	AM 17 009853 CONFIDENTIAL SUBJECT TO PROTECTIVE ORDER
		Recorded by		

FRITSCH v. LIN
INTER NOS 102,096
102,097, 102,334
LIN DOC NO L01070

Project No.
66 Book No. TITLE

From Page No. 65

Sup: 1383 cpm = 6.8% recovery of counts
pellet: 19061 cpm = 93.2% " " " in pellet.
20444 total cpm

Let pellet tube stand overnight in hood & fluorescent light on + blowers blowing.
In AM all residual EtOH gone
Dissolve in 3x Laemmli Sample buffer (LSB) + boil 2' to get into solution
20 µl

ENDO-F treated sample - cycle ② EtOH ppt'n = SAMPLE # 11
15 λ cycle ② + 5 λ ^{125}I-EPO (23,520 cpm) + 20 λ ENDO F buffer + 0.5 µl ENDO F
Incubate 5 hrs, 37°
Cool on ice
Add 500 µl 100% EtOH final [EtOH] = 92.53%
Keep at -80° for 20'. Centrifuge + count as above
Sup - 1419 - 6.1% cpm recovered
Pellet - 22004 93.9% cpm recovered.
23,423

ENDO F. from NEN - Lot #

FRISCH v. LIN
INTER NOS 102,096
102,097, 102,334
LIN DOC NO L01071

AM 17 009854
CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER

To Page No. 67

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by	

LE

Project No.

Book No.

67

m Page No. 66

EL # 1

for Western	#	SAMPLE	Sample Preparation Info.
	1	Hi MW BRL prestained Mrk. = 11 µl	
	2	LOT 82 1:20 stock in 0.025% BSA - 6λ - 52.5ng	
	3	LOT 82 " " - 3λ 26.3ng	
CA- ProtA	4	CHO Cycle ② - 2λ = 88.3ng	
#1	5	CHO Cycle ② - 1λ - 44.2 ng	
µg/ml	6	LOT 82 +ENDO F - 6λ LOT 82 1:20 52.5ng	see ①
	7	LOT 82 + ENDO F - 3λ LOT 82 1:20 26.3ng	see ①
	8	CHO cycle ② - 2λ +ENDO F 88.3 ng	see ②
	9	CHO Cycle ② - 1λ +ENDO F 44.2ng	see ②
	10	Goldwasser 1λ 1:10 for blots = 30ng	
	11	Goldwasser 1λ 1:10 for blots + ENDO F = 30ng	see ③
	12	α therapeutic "OLD" - 23µl = 2.3 U = 33ng	
	13	α therapeutic "old" + ENDO F - 23µl = 33ng	see ④
	14	Lo MW BRL Prestained Mrk = 11 µl	
	15	Goldwasser - 1λ 1:10 for blots - 30ng	
oo dil'n.	16	Goldwasser - 1λ 1:10 for blots + ENDO F - 30ng	See sample ③ above in box
mmal ab	17	CHO - Cycle ② - 2µl = 88.3ng	
it 8C 278	18	CHO - cycle ② - 2µl + ENDO F 88.3ng	See sample ② above in box
1265	19	CHO cycle ② - 2µl + ENDO F - 88.3 ng	
	20	Lo MW BRL Prestained Mrk - 11 µl	

① LOT 82 - 10λ of 1:20 in 0.025% BSA + 40λ ENDO F BUFFER + 0.5λ ENDO F = 50.5λ - incubate 5 hrs, 37°
Add 6 µl LSB - 10x - boil = 57 µl approx. total vol.
Use 38λ for well 6 + 19λ for well 7

② CHO cycle ② - 12λ + 48λ ENDO F buffer + 1λ ENDO F = 61λ TOTAL. 5hr 37°. Cool.
+ 9λ 10x LSB = 70λ TOTAL Boil
for 1λ CHO sample, use 5.5λ mix.
for 2λ CHO sample, use 11λ mix

③ 33λ G.C. 1:10 + 66λ ENDO F buffer - 5hr/37°C 0.5µl ENDO F = 100λ
Add 15λ 10x LSB = 115λ - Use 38λ for wells 11 + 16.

④ 23 µl α therapeut. + 23 µl ENDO F buffer + 0.25λ ENDO F - 5hr 37°
= 46 µl total vol. + 5λ 10x LSB - boil. load total sample

FRITSCH v. LIN
INTER NOS 102,096
102,097, 102,334
LIN DOC NO L01072

AM 17 009855
CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER

To Page No. 68

ssed & Understood by me,	Date	Invented by	Date
		Recorded by	

Project No._______

[redacted]8 Book No._______ TITLE __________

n Page No. 61

Used	#	SAMPLE	SAMPLE PREPARATION
↑	1	BRL LoMW prestained markers 11 μl	
	2	Cycle ① - B11 - 1.5 μl = 60 ng	
	3	Cycle ③ - B11 - 1.5 μl = 71 ng	
	4	Cycle ⑥ B11 3 μl = 73 ng	
	5	Cycle ⑨ B11 - 4.5 μl = 66 ng	
[redacted] 300 dilu. 4b51B	6	B11 pool for lg scale purif. - 2 μl = 53 ng	
[redacted] 196#,	7	" " " " - 4 μl = 106 ng	
[redacted] nit d amino	8	E coli EPO fr. K.Z. E - 2 μl amt EPO unknown	Make a 1:10 diln. fr. Kris' 20 μl stock + use 2λ. 2λ 1:1 → good Coomassie band. Orig. is from EK bin. Orig. is in LSB + dye.
[redacted] inal abs	9	Cycle ① - 1.5 μl = 60 ng + ENDO F ← ⑤	15λ cycle ① + 10λ ENDO F buffer + 0.25λ ENDO F, 5 hrs 37°, + 2λ 10x LSB
↓	10	Cycle ③ - 1.5 μl = 71 ng + ENDO F ← ⑥	1.5λ cycle ③ + 10λ " + 0.25λ " " + 2λ 10x LSB
	11	Cycle ⑥ - 3 μl = 73 ng + ENDO F ← ⑦	3λ cycle ⑥ + 10λ " + 0.25λ " " + 2λ 10x LSB
	12	Cycle ⑨ - 4.5 μl = 66 ng + ENDO F ← ⑧	4.5λ cycle ⑨ + 10λ " + 0.25λ " " + 2λ 10x LSB (Load whole sample)
	13	B11 pool - lg scale purif. 2λ = 53 ng + ENDO F ⑨	B11 pool 12 μl + 48λ ENDO F buffer + 0.5λ ENDO F - 5 hrs, 37° ~~+ 7λ 10x LSB → 68λ~~
	14	B11 pool. lg scale purif. 4λ = 106 ng + ENDO F	Use 11.3λ for the 2λ sample + 22.6λ for the 4λ sample
	15	BRL LoMW prestained markers - 11 μl	
	16	Cycle ② + EtOH - 2 μl equiv = 88 ng	⑫ See description on p. 65-66. Use 2λ for the 1λ sample. Use 4λ " " 2λ sample
	17	Cycle ② + EtOH - 1 μl equiv = 44 ng	
	18	Cycle ② + ENDO F + EtOH - 2 μl equiv = 88 ng	⑪ See description on p. 65-66. Use 2λ for the 1λ sample. ~~Use 4λ for the 2λ sample~~
	19	Cycle ② + ENDO F + EtOH 1 μl equiv = 44 ng	
	20	BRL LoMW prestained markers - 11 μl	

FRITSCH v. LIN
INTER NOS 102,096
102,097, 102,334
LIN DOC NO L01073

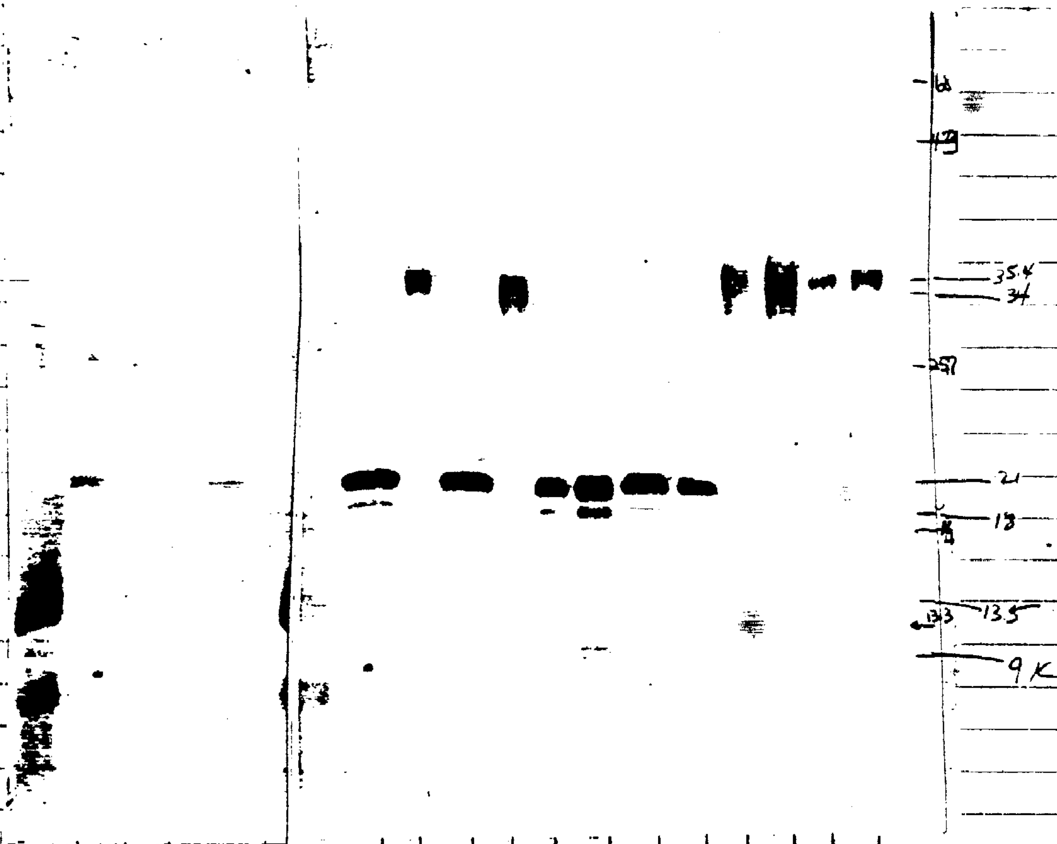
AM 17 009856
CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER

To Page No.____

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by	

Project No.________
TITLE ______________________ Book No.________ 69

From Page No. 68 Gel #1



CHO ② +ENDO F 86ug 44ug | CHO Gene ② 86ug | GC +ENDO F 30ug | GC 30ug +ENDO F | α therapeutic | GC +ENDO F 30ug | GC 30ug | CHO B11 Cycle ② +ENDO F 44ug 86ug | LOT 82 +ENDO F 26ug 52ug | CHO B11 Cycle ② 44ug 86ug | LOT 82 26ug 52ug

Develop ē α C term. rabbit sera 1:200

Develop ē MCA - 12 μg/ml

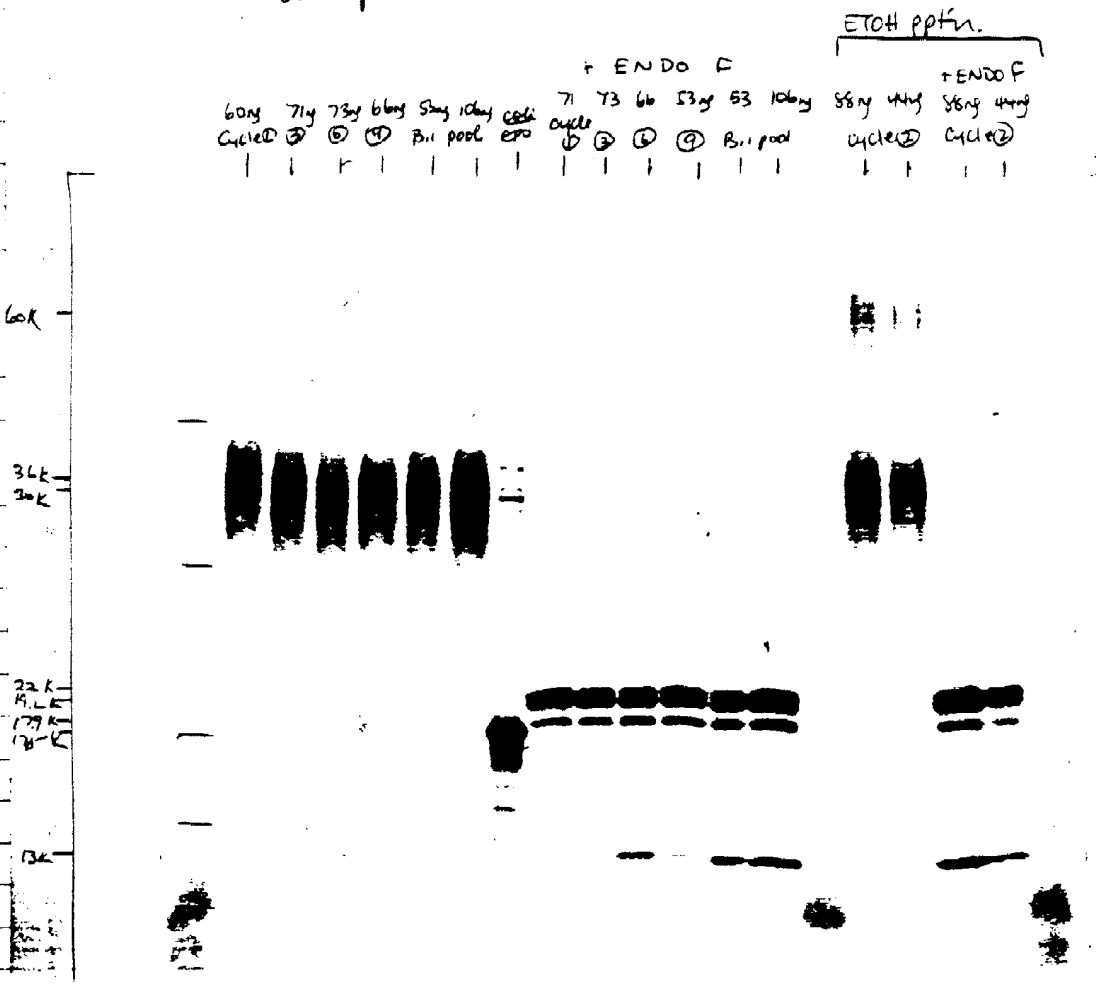
Conclusions - CHO ② + lot 82 same size, although CHO is very heterogeneous. On +ENDO F, lot 82 gives 1 band + only 1 faint lower MW band, whereas CHO ⟹ 3 bands. Intensity lower one is less than higher MW band, but darker than correspond. band in lot 82
- Gene's EPO - behaving as usual - glycoform = ↓ MW fr. Lot 82, although ENDO F digestion prod - MW. So faint lower band + 1 degrad. prod.
- α therapeutic - ~ same size as CHO + lot 82. Digestion prod = lot 82 + lower band is lighter than in CHO, but darker than lot 82
- α C terminal sera - light bands - need to repeat - See only top band of digestion prod. but must repeat ē ~10x sample load.

To Page No. 70

Project No._____
Book No._____ TITLE _____

Page No. 69

Develop ē 1:300 Rabbit α NH2 Terminus Sera



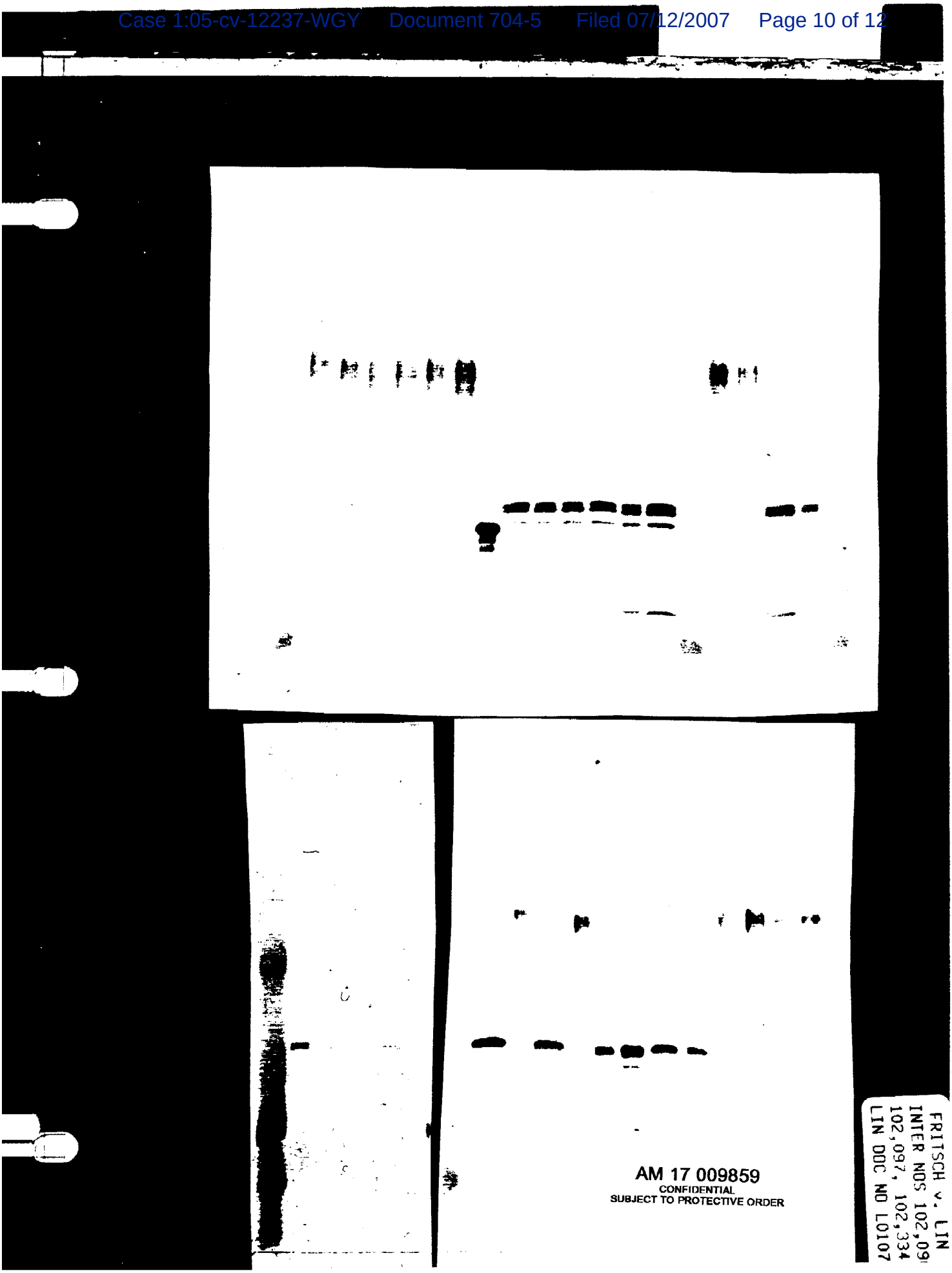
Conclusions ① Think there is a tendency towards ↓ MW in ↑ cycle # of CHO samples
② See 3 ENDO F major bands - 2=weighted doublet
(why seeing more bands now? Previously we needed double ab procedure for ENDO F of recombinant material) ⇒ 1 lower MW band ⇒ 1 degradation prod
③ EtOH pptn. → aggregation + no ↓ in # ENDO F (Ethanol) bands
④ E. coli EPO has MW (on migration) even lower than the smallest of the ENDO F fragments of CHO EPO. Why such a discrepancy?

AM 17 009858
CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER

To Page No.____

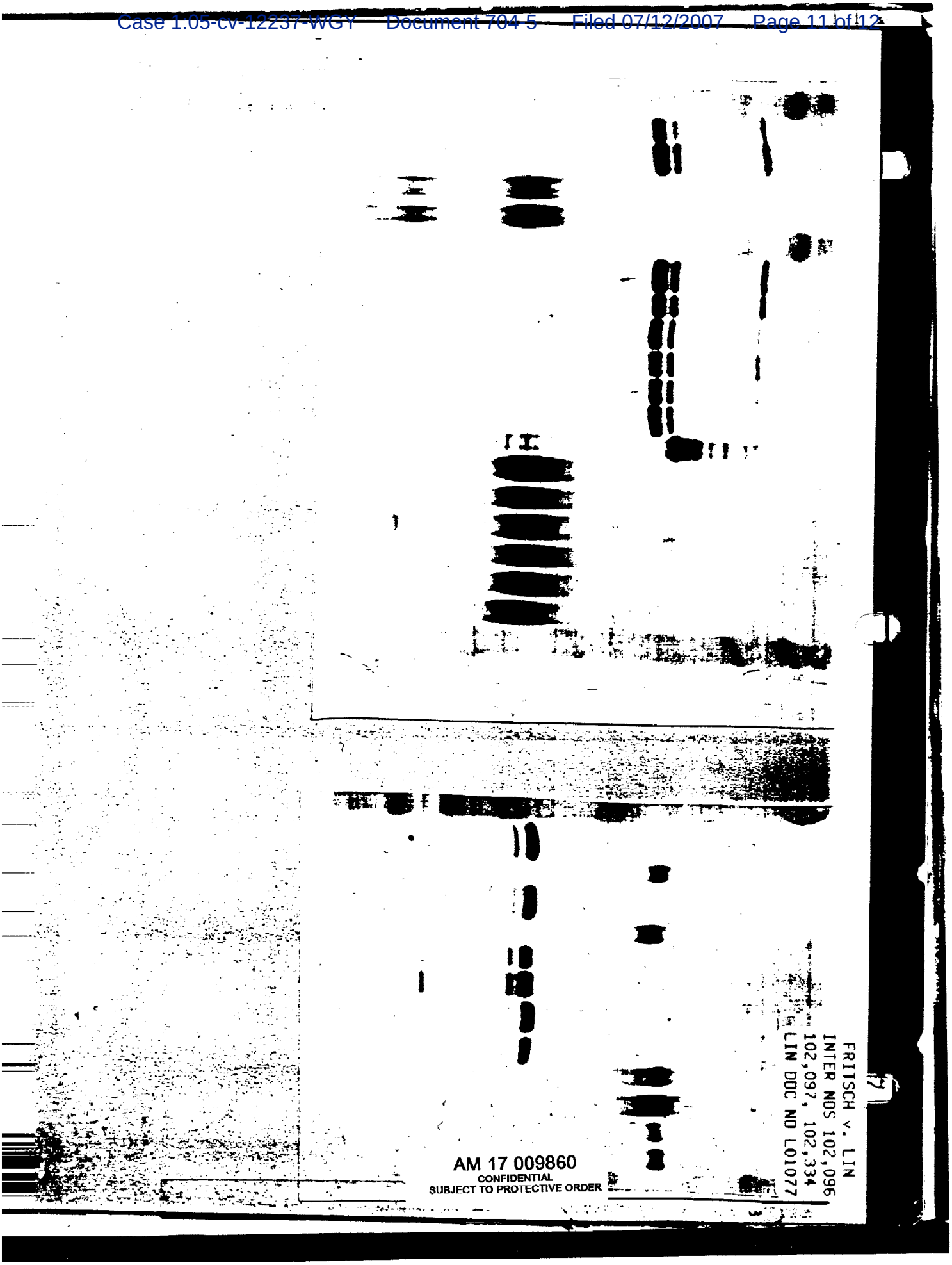
FRITSCH v. LIN
INTER NOS 102,096 LIN DOC NO L01075 102,097, 102,334

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by	



AM 17 009859
CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER

FRITSCH v. LIN
INTER NOS 102,09
102,097, 102,334
LIN DOC NO L0107


FRITSCH v. LIN
INTER NOS 102,096
102,097, 102,334
LIN DOC NO L01077
AM 17 009860
CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER

Project No.

TITLE Book No. 71

From Page No.

FRITSCH v. LIN
INTER NOS 102,096
102,097, 102,334
LIN DOC NO L01078

AM 17 009861
CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER

To Page No.

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by	