

February 13th, 2023

HEU: D17 γδ CBMC panel #1 - 2023

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly(150)	Ly+Mon	Total	.66E+6	.5E+6
62001	Inf 182	IL-2	16μl			6	6.4 3.2		18μl	206μl	156μl
71041	Inf 182	Zot	16.7μl			6.5	6.2 3.1				315μl
82041	Inf 182	BCG	16.2μl		no leftovers	6.5	6.3 3.15			213μl	161μl
94041	Inf 135	IL-2	40μl		more cells than T cells? no leftovers	6	3.2 1.6		9.6	412μl	312μl
44041	Inf 135	Zot	32.8μl			6.5	3.4 1.7			388μl	294μl
35041	Inf 135	BCG	20μl		no leftovers	7	5.1 2.55			259μl	196μl
34041	Info39	IL-2	24.8μl			6	4.3 2.15		13.5	307μl	232μl
36041	Info39	Zot	32μl			6.5	3.6 1.8			366μl	277μl
37041	Info39	BCG	57.0μl		no leftovers	6	2.2 1.1			600μl	454μl
39501	NYU58	IL-2	23μl			2.5	4.6 2.3			287μl	217μl
2000	NYU58	Zot									437μl

Antibody prep @ 10:27 am

Growth on @ 11:07 am

Cell collection @ 11:39 am

12:05 pm collected

13:13 cells spinning down 1ml RIC (200 rpm/10min)

Plate spin @ 13:58 pm < Cells baell into the incubator >

Cells out again @ 3:11 pm

Cells aliquoted, extras spinning @ 3:54 pm

Cells spin tubes @ 4:09 pm

DM50-FBS prepped @ 4:14 pm

SC's spin @ 4:30 pm

4:42 pm (cells frozen ~~50~~ ^{ugh} 20%)

4:48pm SC's vortexed & melted.

V/D @ 4:58 pm

Samples Surface @ 5:49 pm → 6:09 pm

Spin @ 6:14 pm

P1 / P4b stacked @ 6:36 pm

clotin
tray (still across
screen) 3 2.1
1.35 no leftovers ?

9μl in
9μl

LD Horseradish

27μl
x .8
21.6 mls → 45.2μl up

→ step @ 6:40 pm → 6:55 pm

Hot Wash @ 7:06 pm

SC's @ 7:17 pm → 7:37 pm

8:35 pm Perc spindown

4D @ 8:45 pm → 9:00

40μl per tube Inf 182 Col Inf 182 B.C.E splashed w/ 201

4D Spin @ 9:04 pm

P_2/P_{4b} intrac @ 9:12 pm \rightarrow 10:52 pm

stacked cu @ 9:15 pm

< spinning cu abs @ 9:20 pm >

CH surface @ 9:40 pm \rightarrow 10:00 pm

10:15 pm P_2/P_{4b} done (50%)

10:16 pm

Intrac

stacked cu

stacked cu

2.0

stacked cu

0

avg 11:18 o higher than 11:00

February 13th, 2023

D17 Well Census

Antibody prep @ 9:00 AM

$$\begin{array}{r} 18 \\ \times .800 \\ \hline 15200 \text{ mls } 4/0 \times 3 = 341 \text{ ml } 15\text{ml} \\ \hline \end{array}$$

182 B66 ↑ to CD56
Inf 35 62L → BUS10

AB steep @ 5:41 pm

Note 5:55 pm → 6:25 pm

Hot scs @ 60 rpm \rightarrow 37 μm
Cold scs @ ...

Cold SCS @ 2:28 pm

$H_{\text{eff}}^{\text{sc}}$ spin $\in 6446 \text{ pm}$

AB's stashed @ 6:48pm

Combination

Hot scs stashed @ 6:55pm → 7:08pm

Tetrameric complex

AB cold \odot 7:00pm \Rightarrow 7:40 pm
7:10pm \Rightarrow 7:40 pm

strep SC CD16(AB) 2:23 → 7:38 PM

Fix Perm @ 7:38 pm → 48 pm → 58 pm → Frost Perm Wash @ 8:10 pm

All the FBS washes @ 7:53 pm

Everythus stashed! @ 8:11 pm

Pm stayed @ 8:22 pm

2nd PermWash @ 8:47 pm (25 min in cold)

ILT rec @ 8:51 pm
→ 9:21 pm

Abs Street @ 9:07 pm → 9:22 pm

Final FBS Wash (rec'd 9:25 pm)

Majority sc's resuspended in 30/1

sc's Intra @ 9:36 pm → 10:36 pm

Final spin @ 10:36 pm

10:16 pm

10:20 pm FullPerm → 10:30 → 10:40

Second PermWash 11:04 pm

Intra @ 11:24 pm → 12:04 pm

~~3.2~~ 3.4
~~1/1~~ 1/1

~~3.6~~
~~1/1~~

Final spin @ 12:08 pm

Done @ 00:29 am

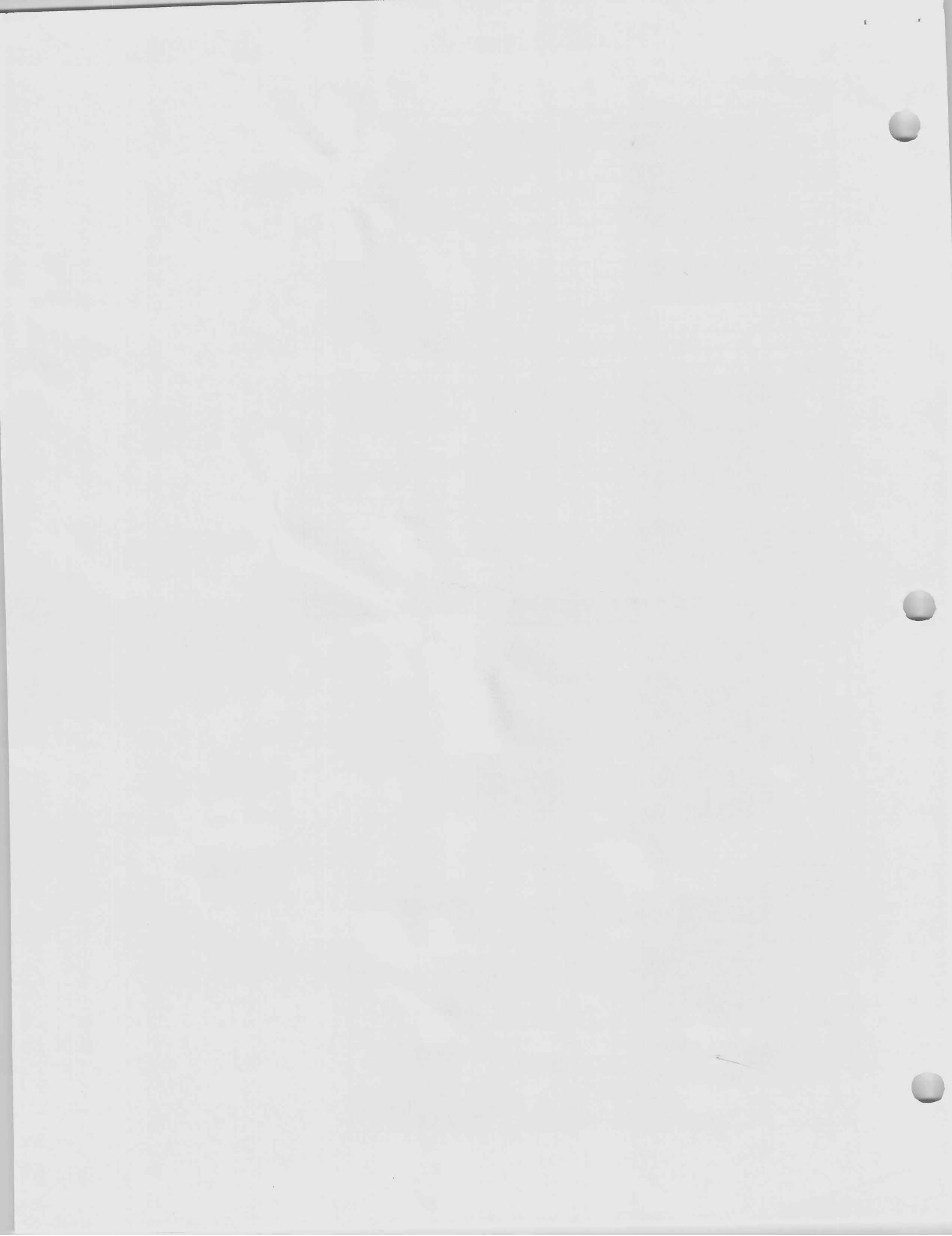
February 13th, 2023

D17 Gd-P4b									
#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	9.5	Intracellular 40min @RT	9.5
1	V450	BV421	PD1*			2	19		
2	V525	BV510	L/D Aqua		<1:500>				
3	V670	BV650	CD56*			1.8	17.1		
4	B530	Alexa 488	Perforin					3	28.5
5	B710	PerCPeF710	CD3			1.5	14.25		
6									
7	Y780	PE-Vio770	NKG2A			0.6	5.7		
8	R670	APC	Vδ2			1	9.5		
9	R780	APC-Fire750	CD16			1	9.5		
Antibody Total					7.9	75.05	3	28.5	
PBS					12.6	119.7	17.5	166.25	
Pipette draw volume / sample					19.5		19.5		

D17 Gd-P2										
#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	9.5	Streptavidin	Intracellular 40min @RT	9.5
1	V450	BV421	Vδ2			1	9.5			
2	V525	BV510	CD3			1.5	14.25			
3	V670	BV650	NKG2D			2	19	1.5		
4	B530	Alexa 488	Perforin						3	28.5
5	B710	PerCPVio700	CD56			1	9.5			
6	Y590	PE	PD1			1.5	14.25			
7	Y780	PE-Vio770	NKG2A			0.6	5.7			
8	R670	APC	Vδ1			1	9.5			
9	R780	APC-Fire750	L/D Horizon		<1:1000>					
Antibody Total					8.6	81.7	14.25	3	28.5	
PBS					11.9	113.05	180.5	17.5	166.25	
Pipette draw volume / sample					19.5		19.5	19.5		

D17 Gd-P1							
#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	9.5
1	V450	BV421	PD1			2	19
2	V525	BV510	L/D Aqua		<1:500>		
3	V670	BV650	CD16			1.5	14.25
4	B530	FITC	Vδ2			1.2	11.4
5	B710	PerCPeF710	CD25			2	19
6	Y590	PE	CD28			2	19
7	Y615	PE-Dazzle	CD27			1.5	14.25
8	Y780	PE-Vio770	CD3			0.5	4.75
9	R670	APC	Vδ1			1	9.5
10	R780	APC-Fire750	CD45RA			1.8	17.1
Antibody Total					13.5	128.25	
PBS					7	66.5	
Pipette draw volume / sample					19.5		

D17 gd-CK: α-γδTCR									
#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	9.5	Intracellular 40min @RT	9.5
1	V450	BV421	PD1			2	19		
2	V525	BV510	L/D Aqua		<1:500>				
3	V670	BV650	CD56			1.5	14.25		
4	B530	Alexa 488	CD107a					<3 uL>	
5	B710	PerCPeF710	TNFα				0	2	19
6	Y590	PE	Vδ2			1	9.5		
7	Y780	PE-Vio770	IFNγ					0.6	5.7
8	R670	Alexa 647	CD27			1.5	14.5		
9	R780	APC-Fire750	CD45RO			1.8	17.1		
Antibody Total					7.8	74.1	2.6	24.7	
PBS					12.7	120.65	17.9	170.05	
Pipette draw volume / sample					19.5		19.5		



#	Filter	SingleColor (all) Ref ctrl type	Unmixing ctrl name	Fluorochrome	Marker	Clone	Vial lot#	During stim!!!	9	U/D	RT	RT for 10 min	HotStain 30min @37C	9	ColdStain 30min @4C	9	4°C for 15 min	RBC Lyse, then FixPerm	Intracellular Stain 40 min @RT	9
1	UV2			BUV395	CD62L	(DRG-G-56)											1.2	10.8		
2	UV7			AF	AF-UV6															
3	UV9			BUV496	CD8	(RPA-TB)														
4	UV10			BUV563	CD69	(FR150)														
5	UV11			BUV615	CCR4	(1G1)														
6	UV14			BUV661	V02	(E6)														
7	UV16			BUV737	CXCR3	(1G6/CXCR3)														
8	V1			BUV805	CD4	(5H3)														
9	V3			BUV821	CD127	(A01905)														
10	V5			Pacific Blue	CD14	(M5E2)														
11	V7			Pacific Blue	CD19	(HIE19)														
12	V10			BUV480	CD161-Biotin	(REA631)														
13	V11			BUV510	CD45RA	(H1200)														
14	V13			BUV605	CKR05	(125D4)														
15	V14			BUV650	CCR7	(G043H7)														
16	V15			BUV736	IFNG	(3C10)														
17	B3			FITC	For R3/Val															
18	B4			Spark blue 550	CD3	(SK7)														
19	B5			PE	PD-1	(EH12.2H7)														
20	B6			PE-CF594	CD26	(M-A251)														
21	B8			PE-CY5	CD25	(M-A251)														
22	B10			PerCP-Q5.5	TNF-a	(MAB11)														
23	B13			PE-vio770	HLA-DR	(I243)														
24	R1			APC	CD39	(A1)														
25	R2			Alexa Fluor 647	IL2	(MQ1-)														
26	R4			APC-R700	CD107a	(H4A3)	<6>	#####	<1:2500>											
27	R6			Zombie NIR	L/D															
28	R7			APC/Fire 750	CD27	(O125)														
29	R8			APC/Fire 810	CD38	(H112)														
And UNSTAINED CONTROLS !!								Antibody Total	0.0											
								R10 Media	20.5											
								PIPette draw volume / sample	19.5											
								PIPette draw volume / sample	65.7											

Simplified Protocol

<Resuspend at 100ul of R10, rest cells for <12hrs in a 6-well plate, >

Collect, count, aliquot cells 2.0E+6 Cells 5ml polypropylene tube

Add antibodies, stir antibodies, and Duriglism antibodies in 500 ul R10

Cap and incubate at 37C for 2 hrs hours

Add goji block, and continue to incubate for <6> hours

Wash with 2 ml PBS, spin down 1300 rpm 8min

Wash 2 ml 5% PBG-FBS, spin 1300 rpm, 8min

Add 300-500 ul 1x RBC Lysis for 3 minutes

Wash 2 ml 5% PBG-FBS 1400 rpm, 6min

Add Streptavidin Mix, incubate @ 4C for 15min

Wash 2 ml 5% PBG-FBS 1400 rpm, 6min

1 ml Nuclear FixPerm, incubate @ 4C for 30min

(vortex every 10 minutes)

2 ml NuclearWash 1500 rpm 6 min

2 ml NuclearWash 1500 rpm 6 min

Add intracellular Stain, incubate @ RT for 40min

First PermWash:

2 ml NuclearWash 1500 rpm 6 min

Second Perm Wash:

2 ml NuclearWash 1500 rpm 6 min

First and Second Perm Wash:

2 ml NuclearWash 1500 rpm 6 min

Add intracellular Stain, incubate @ RT for 40min

First PermWash:

2 ml NuclearWash 1500 rpm 6 min

Second Perm Wash:

2 ml NuclearWash 1500 rpm 6 min

Add intracellular Stain, incubate @ RT for 40min

First PermWash:

2 ml NuclearWash 1500 rpm 6 min

Second Perm Wash:

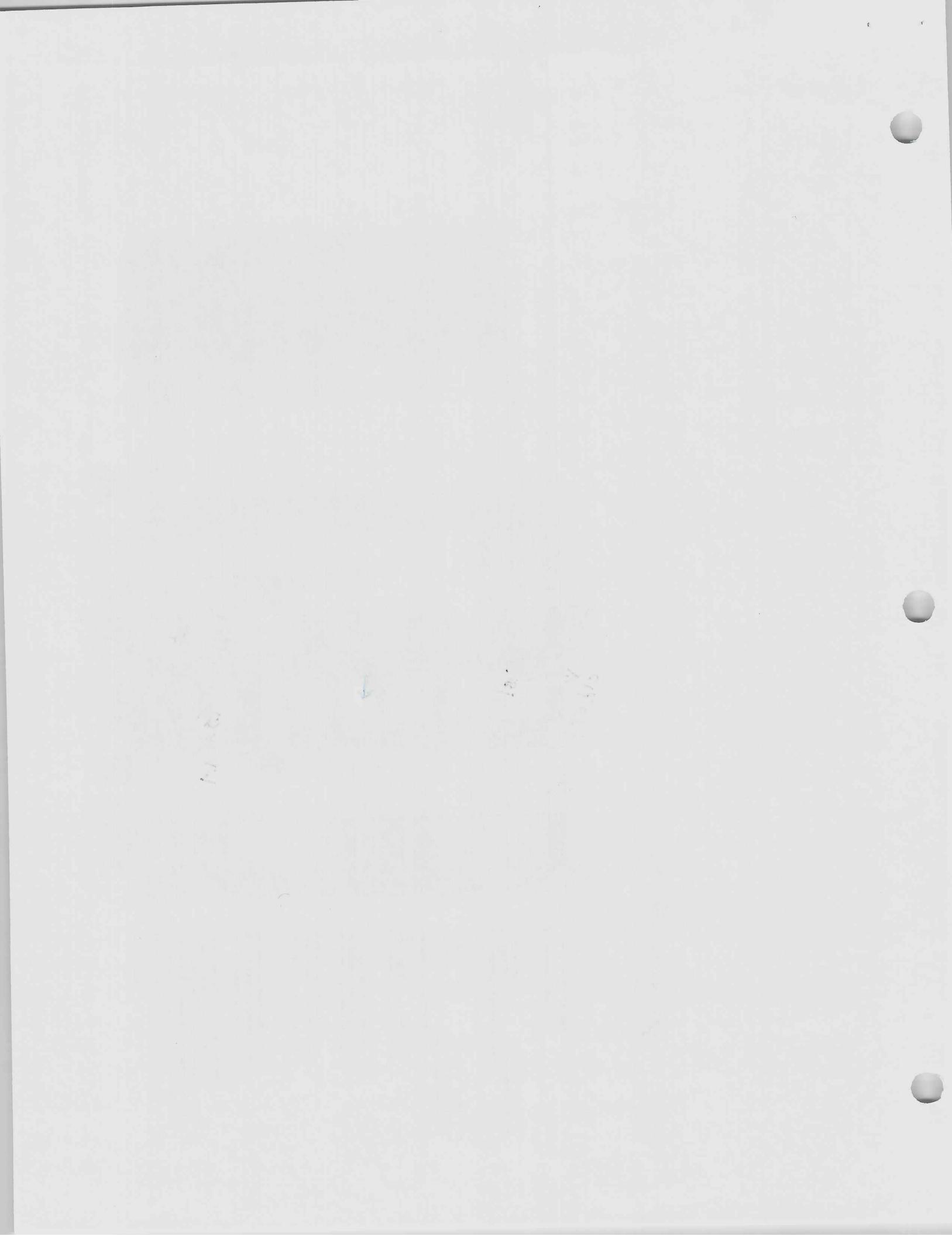
2 ml NuclearWash 1500 rpm 6 min

Notes:

Cap tubes, wrap rack in foil, store at 4C

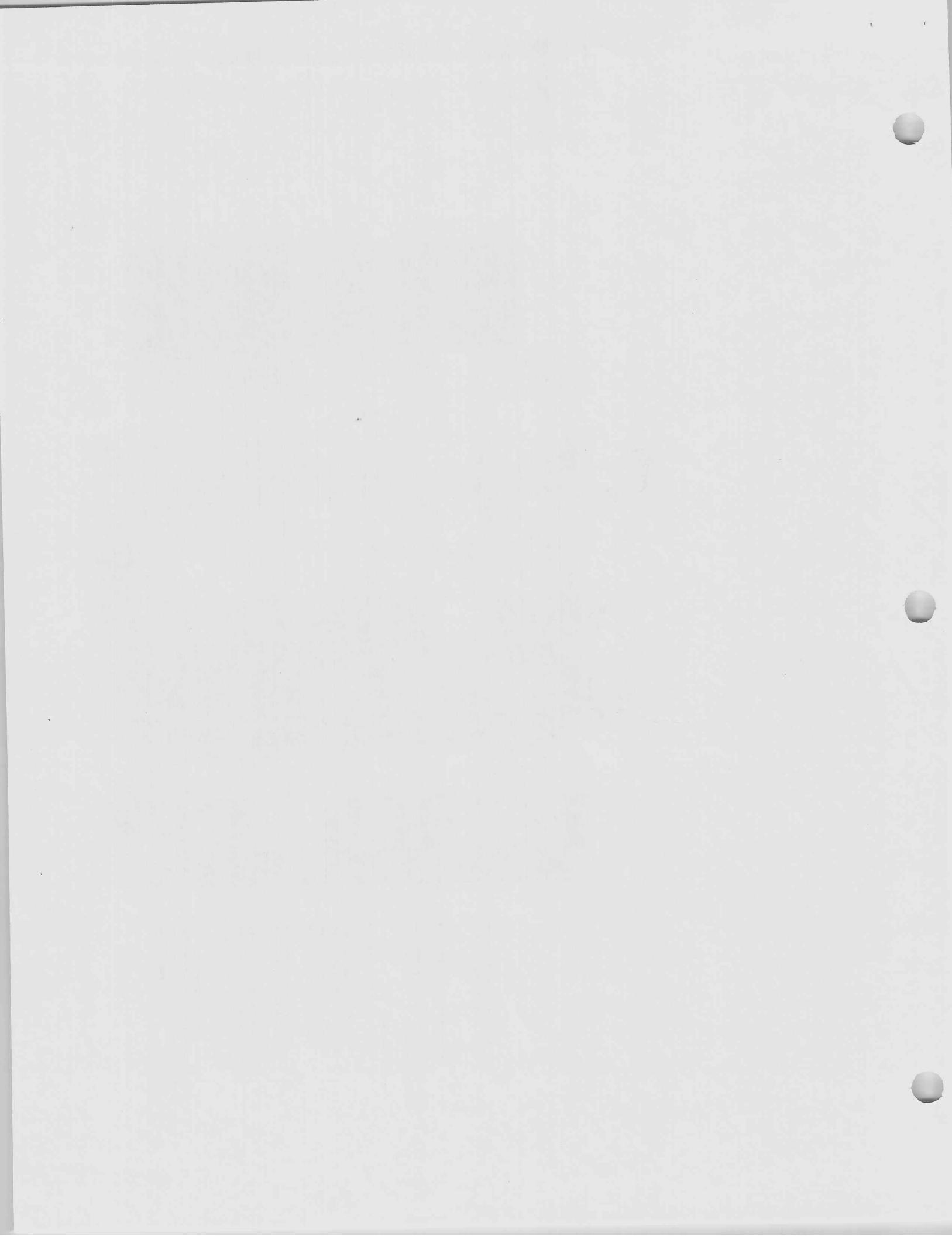
No intra-cell

No intra-cell



Notes:

New C No intra CERT



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~1:36pm LSR-II^o

FSC 610 → 620

SSC 280

R670 510

R780 490 → 520

B530 495 ← 480

B710 420 500

V450 380 410

V525 550

V670 480

Y590 630 ← 595

Y615 640 ← 630

Y780 490 ← 480

SC's done, CR acquisition @ 1:56pm

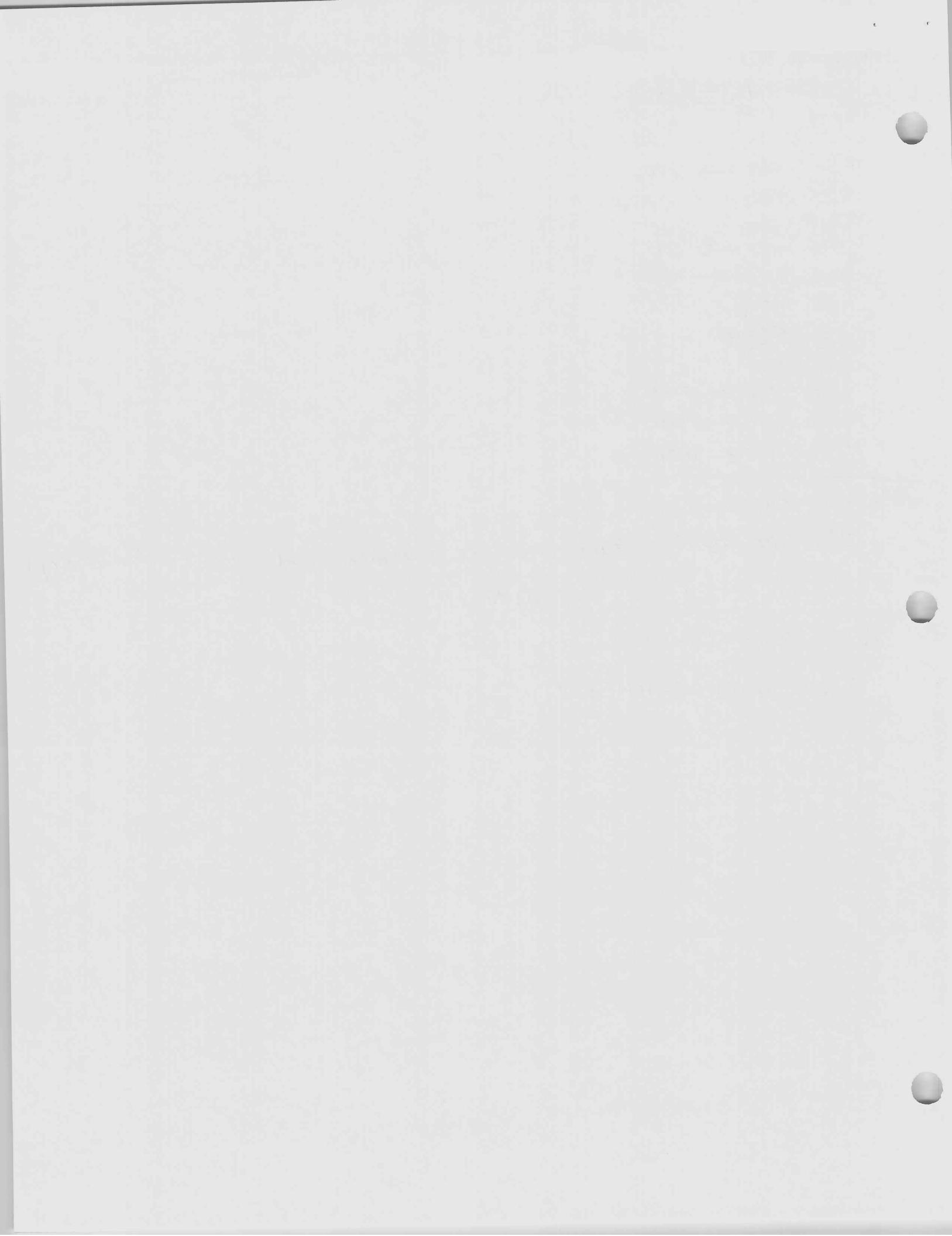
CR @ 700 FSC ~ 2400 events/sec @ medium speed

2½ minutes @ 50fL

* * 182 BCG (accidentally
201 spilled) → CR

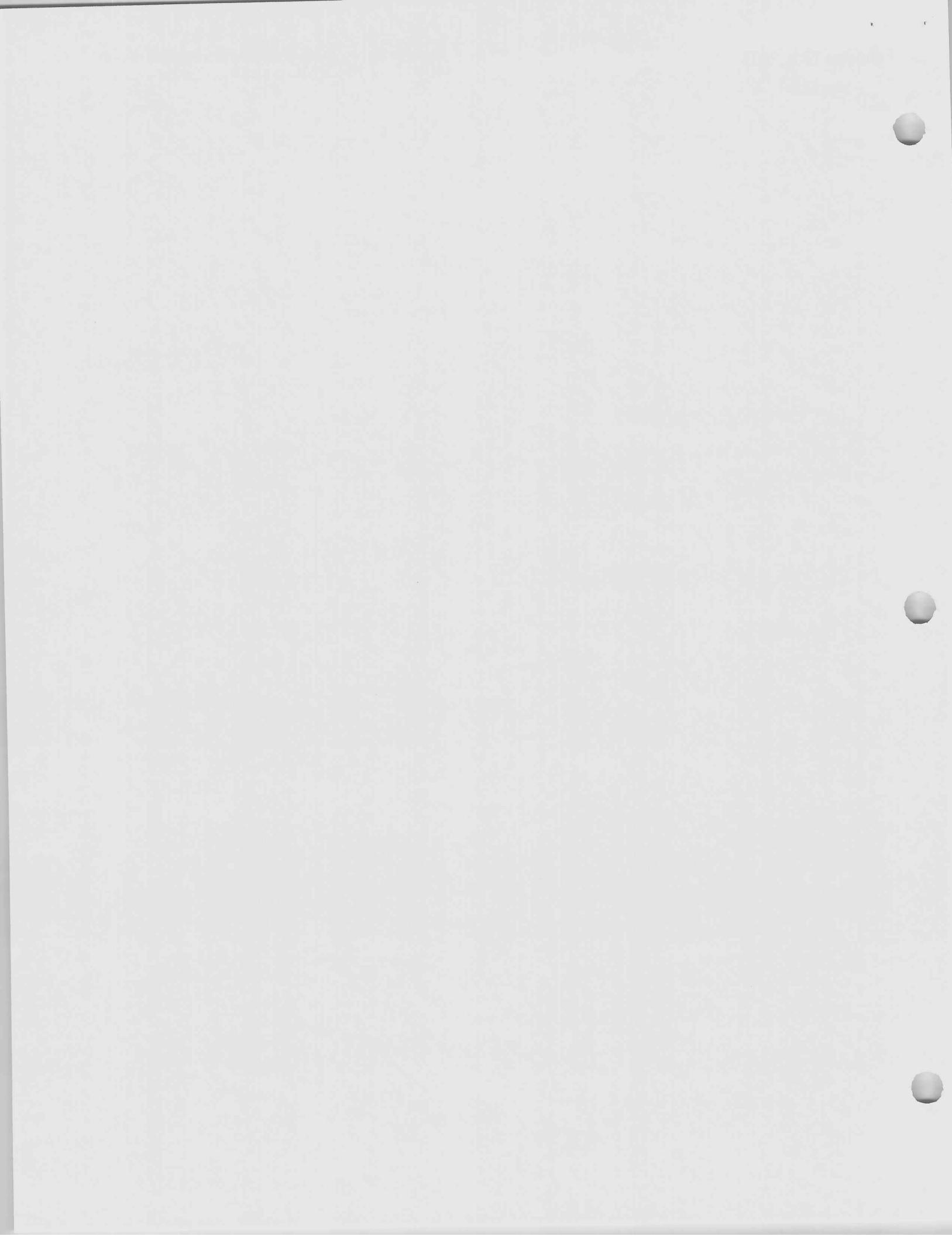
Preb @ 3:10pm (P1 done)

Final sample @ 4:45pm



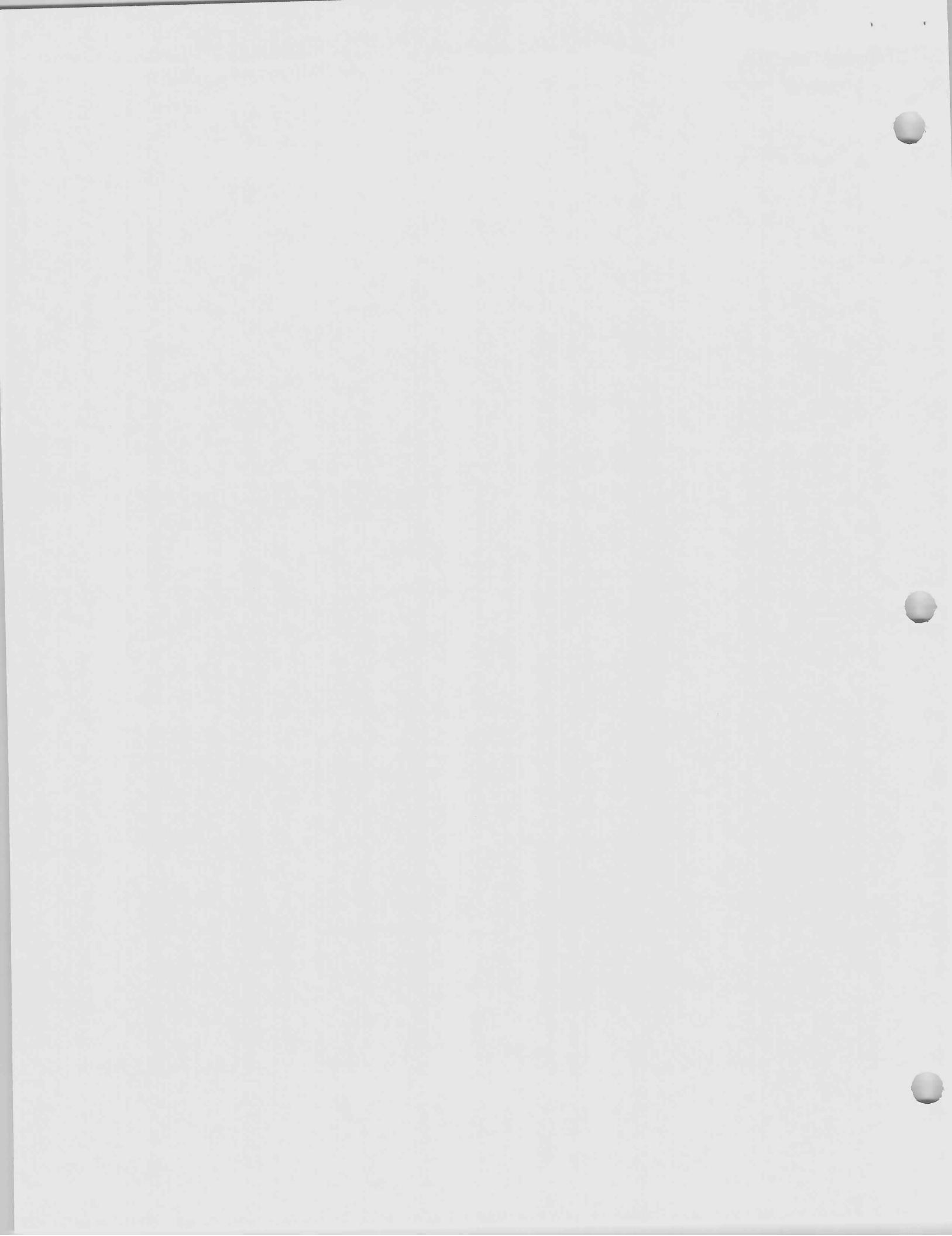
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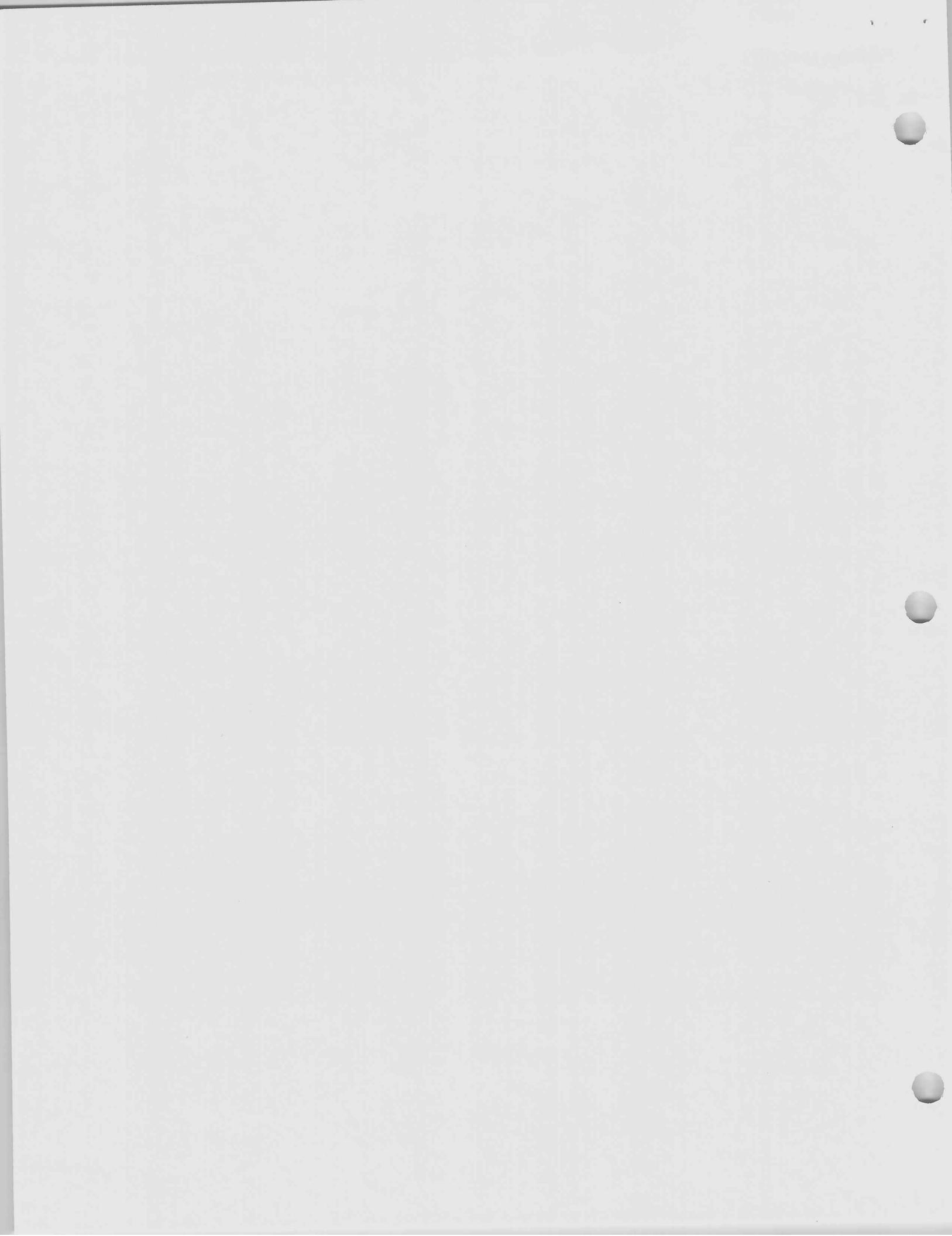
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HEU: D17 $\gamma\delta$ CBMC panel #1 - 2023



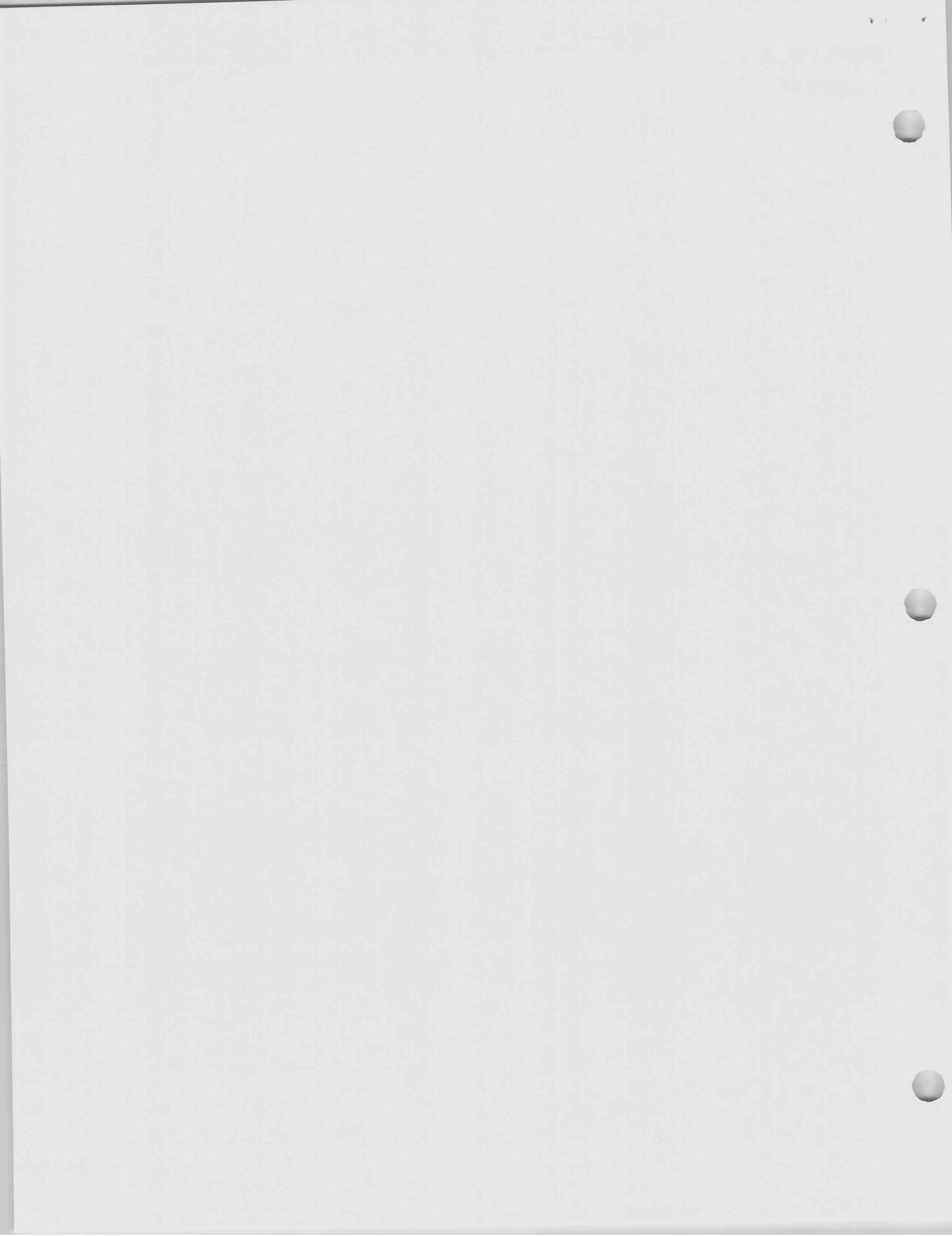
February 13th, 2023

HEU: D17 $\gamma\delta$ CBMC panel #1 - 2023



February 13th, 2023

D17 Well Census



February 13th, 2023

D17 Well Census

No CD14/CD19

- Artificial dead monocyte signal
dead monocytes vs small es

¶ CXCR5

6:44pm 5-laser Aurora Acquisition (post Cris):
running faster? (\downarrow 55PL volume instead of 60PL)

Inf135 201 ILTs \sim 13,000 events @ medium speed.
Threshold 300,000
FSC 35 ssc 120 ssc-B 115

Saves automatically?

Sc's \rightarrow 40PL; 350,000 cells for CD62L

* A lot of CD85. (80%)

62L, CD69, CC24, CXCR5,

* Good CXCR3 expression.

Some CD4, Vd2, CD161, Va7.25

Weak CD127.

60% CD4RRR -

γ 3 S6 + most CC27 -

decent CC27

25% CD3 neg

Strong CD26 expression

Some CD25

Some HLA-DR

A LOT of CD31

Final Sample sc @ 9:00pm //

Φ35 \rightarrow unstained ctrl
182 \rightarrow act card unst

" Fluores something turned out "

318