| 1  | Specimen   | Status     | Location  | T C    | I D        | Τ   |         |        | PM/     | A Batche | S          |            |  |  |
|--|--|------------|-----------|--------|------------|---|---------|--------|---------|----------|------------|------------|--|--|
| well   |  |            | Location  | Conc   | Date       | Tasks   | Volume  | Ly     | Ly+Mon  | Total    | 3E+6       | .3E+6      |  |  |
| A  | NDOOG  | Adult      |           | ?      | 01/27/2    | je de la companya de | 2       | 5.29   | 7.68    | 20.68    |            |            |  |  |
| 401/   | ND006  | Adult      | 20.51     | 7      | 01/27/21   |   | 2       | 500    | 7.02    | -        | 5          |            |  |  |
| · Car p  |  |            | 20.511    |        | 7000       |   | liano . | 3, 03  | 1.02    | 4.136    | 721/1      |            |  |  |
|  |  |            |           |        |            |   |         |        |         |          |            | =          |  |  |
|  | 7  |            |           |        |            |   |         |        |         |          |            |            |  |  |
|  |  |            |           |        |            |   |         |        |         |          | 4          |            |  |  |
|  |  | at.        |           |        |            |   |         |        |         |          |            |            |  |  |
|  | 23 AM Tha  |            |           | 10     |            |   | ~       |        |         |          |            | 6          |  |  |
|  | SIAM Co.   |            |           | 72     | <u> </u>   | Unstained   | caps    | 7      | Way     | Jap.     | LATEN CEPE | No stopped |  |  |
| (0   | :20 km I   | incubation | an start  |        | 51         | Unshou sm   | outs    |        |         | as uder  | ens (      | No. 19 6A. |  |  |
| L  | :27 BM   | Call 5     | 1 c01074  | )      |            |   |         |        |         | 1        |            | Mr. 5      |  |  |
| 4  | :22 pm   | Cars,      | PRC       | Han .  | ince ba    | for .   |         |        |         |          |            | V. Klay    |  |  |
|  |  |            | (103 Spin | 1 (inc | luding     | Sc's)   |         |        |         |          |            | SION OF    |  |  |
|  | Hot se's @ source = 1  |            |           |        |            |   |         |        |         |          |            |            |  |  |
|  | Cold Scis @ 506pm - 336pm Rbc yse @ 5:39 pm  |            |           |        |            |   |         |        |         |          |            |            |  |  |
|  | Cold Sols @ 514 pm > 5:46 pm Abor ] - Into Some Spm  Hot 372 @ @5:24                   |            |           |        |            |   |         |        |         |          |            |            |  |  |
|  | Hof 3  | 72 e       | @5.2H     | -, 65  | 7 5:3      | 54pm.   | 1-1/4   | Saur   | ic spin |          |            |            |  |  |
|  | Het sets chilling Ridge e 5:57pm. Table out CD161-Tet sets and place by samples nextle |            |           |        |            |   |         |        |         |          |            |            |  |  |
| Gillon = 1   |  |            |           |        |            |   |         |        |         |          |            |            |  |  |
| min Telamore had to                                |  |            |           |        |            |   |         |        |         |          |            |            |  |  |
| 439°C @ 6:24pm > 6:54pm [RBCYSe!]                  |  |            |           |        |            |   |         |        |         |          |            |            |  |  |
| Scistated a Gizspan -> 38 pm -> 48 pm Unstand cold |  |            |           |        |            |   |         |        |         |          |            |            |  |  |
|  | (  | Sc. 5      |           | 0.280  | m _        | 738pm   | -> 48/  | om     |         | W 312    | maca o     | 20101      |  |  |
|  |  |            |           | 1 1    | Trong MART | V to the star I   |         |        |         |          | → Gener    |            |  |  |
|  | Samp   | les @      | 7:14p.    | 7-7    | 242        | 2741  |         |        |         | Uhsh     | aned h     | 16t-7      |  |  |
|  |  | So         | 7:14p.    | ae e   | 100        |   |         | ·<br>· |         |          | Menoyl     | · ·        |  |  |
|  | - 01=  | 7.540      | 24        | 1      | 17.5       | > 7/20.   | 15/-    | Done?  | 8: ८१   |          | crst.      | ennecl     |  |  |
|  | Samples<br>Fatrace   | 2          | , -7      | 6.34   | pm/        |   | ·       |        |         |          |            |            |  |  |
|  |  |            | _         |        | -          | ~   |         |        |         |          |            |            |  |  |
|  |  |            | De        | no by  | 4580       |   |         |        |         |          |            |            |  |  |

| 25th, 2023 |   | uary  |
|------------|---|-------|
| h, 2023    |   | 25t   |
| 023        |   | h, 2( |
|            | 0 | 223   |
|            |   |       |

PMA WORK GODAMN IT!

|  | 56 |           | 60.7  | 0         | 66                     | lume /sample | Pippette draw volume /sample            |      | 19.5 |              | /sample  |              |                       |              |             |           |          |
|--|----|-----------|-------|-----------|------------------------|--------------|---|------|------|--------------|----------|--------------|-----------------------|--------------|-------------|-----------|----------|
| Defect   D   |    | ŏ         |       |           | 1006341                | Stain        | Brilliant                               | 43.5 | 14.5 | draw volume  | Pippette |              |                       |              |             |           |          |
|  |    | .1        | l iss |           |                        | A local      | 211111111111111111111111111111111111111 | 1 6  | 4 6  | y lotal      | R10 Mer  |              |                       |              | ITROLS IIII | AINED CON | And UNST |
|  |    |           | S CE  |           |                        | Table        | Antibodi                                | 18   | 60   |              |          |              |                       |              |             |           |          |
| # File: Sneglector(u) fed cit. Unnine of Manor-Norme Manor M |    |           |       | 6         |                        |              |   |      |      |              |          |              | 1                     | 1186         |             | K &       | 14       |
| Recompleted   September   Se   |    |           |       |           |                        |              | <1:2500>                                |      |      | 32           |          |              |                       | 1.61         | 5           | R7        | 26       |
| # Filer Single-coor(u) Red cht Umarine # 16000 divisoria (12.0) (2.000 divisor |    |           |       |           |                        |              |   |      | 6.0  | 3)           |          |              |                       | 100          | Term        | R6        | 25       |
| # Filet Steple color (u) fed chr Umanne Hall Marker Cone (1916) 5.00 (27) (27) (27) (27) (27) (27) (27) (27)   |    |           | _     | <b>^:</b> |                        |              |   |      |      | 91           |          |              |                       | -            | 9           | R4        | 24       |
| ## Filer Sugle color (u) Red crit Unniving ctrit Anne Nation (Color)    1  |    | .1        |       |           |                        |              |   |      |      |              | MR1      |              | A                     |              |             | R2        | 23       |
| # Filter Single-color (d) Red card Unmining and Annee Walleys Caree (1974)  1 U/2    1 U/2   1/2 |    | .5        |       |           |                        |              |   |      |      |              | GD16     |              |                       |              |             | R1        | 29       |
| # Filet Single.coor (a) Ref cit Unnaine (Marker Clone Cone (Marker Clo |    |           |       |           |                        |              |   |      |      |              |          |              |                       |              | 2.7         | B13       | 22       |
| # Filter Single-color(u) Red cital Unaniang cital Page 2002.   Dollica Signature of Clone   Dollica Sig |    |           |       | 3.6       |                        |              |   |      |      | 311)         |          |              |                       | THE STREET   | E           | B10       |          |
| # Filter Single-color(u) Red ctrl Name   Place Single-color(u) Red ctrl Name   Riversity   Care    |    |           |       | 3.6       |                        |              |   |      |      | 2511         |          |              |                       | 1183         | * 60        | 88        | 20       |
| # Filer Single-color (u) Ref ctrl Unmining ctrl I Annue Fluorochome Marker Colone (Distance Service Se |    |           |       | 1.5       |                        |              |   |      |      | 261)         |          |              |                       | F            |             | 86        | 3   5    |
| # Filter Single color (u) Rel ctrl Unmixing att Iname (and partie) Ruorochrome (burse) Clone (burse) (BALTS) ( |    | 6         |       |           | 1 1000                 |              |   |      |      |              |          |              |                       |              |             | 5 4       | 1 6      |
| # Filter Single color (u) Ref ctrl Unmixing ctrl I pane to the Single color (u) Ref ctrl I pane to pan |    |           |       | ^         |                        |              |   |      |      | 7)           |          |              |                       | ACT IN       |             | RA S      | 1 2      |
| # Filter Single color (u) Referrit Unmixing continume Marker Colone (A)  |    |           |       |           |                        |              |   |      |      |              |          |              |                       |              |             | B. F      | 17       |
| ## Filter Single-color(u) Ref ctrl Inname  |    |           |       |           |                        |              |   |      |      | A9)          |          |              |                       | 1111         |             | R3        | 16       |
| Filter Single color (u)   Ref ctrl   Unmixing ctrl   Pluorchome   Marker   Clone   Color   Stimili   Sti   |    |           |       | u         |                        |              |   |      |      | 27]          |          | BV750        |                       |              |             | V15       | 15       |
| Filter Single color (u)   Ref ctrd   Dumixing ctrd   Dumixin   |    |           |       | u         |                        |              |   |      |      |              | CD7      | BV711        |                       |              |             | VIV.      | 10       |
| Filter   Single color (u)   Ref ctrl   Unmixing ctrl   Ruovachome   Marker   Clone   Vial Lot #   Stimilli   3   U.D.15min   Tetramer 40   MolStain   3      |    |           |       | u         |                        |              |   |      |      |              | CCR7     | BV650        |                       |              |             | V12       | 28       |
| Filter   Single color (u)   Ref ctrl   Unmixing ctrl   Fluorochrome   Marker   Clone   Vial lot #   Stimit!   3     (RT)   min @RT   3   3   30 mm @LC   3   3   3   30 mm @LC   3   3   3   3   3   3   3   3   3   |    |           |       | ,         |                        |              |   |      |      |              | CD56     | BV605        |                       |              |             | VII       | #   H    |
| Filler   Single color (ul)   Ref ctrl   Dunnixing ctrl   Pluorochrome   Marker   Clone   Vial lot #   Stimili   3   Vial Stim   |    |           |       |           |                        |              |   |      |      |              |          | 87570        |                       |              |             | V10       | 17       |
| Filter   Single color (ul)   Ref ctrl   Dunnixing ctrl   Pluorochrome   Marker   Clone   Vial lot #   Stimili   3   Vial Stim   |    |           |       |           |                        |              |   |      |      | 100)         |          |              |                       | 1            | E           | 4         | :        |
| Filter   Single color (ul)   Ref ctrl   Dunnixing ctrl   Pluorochrome   Marker   Clone   Vial lot #   Stimili   3   Vial Stim   |    | 3.0       |       |           |                        |              |   |      |      | <u>1631)</u> |          |              |                       | 100          |             | 5 8       | = :      |
| Filter   Single color (ul)   Ref ctrl   Dunniking ctrl   Pluorochrome   Marker   Clone   Vial lot #   Stim!!!   Stim!!   Stim!!!   Stim!!   Stim!!!   Stim!!!   Stim!!!   Stim!!   Stim!!!   Stim!!!   Stim!!!   Stim!   |    | 3.0       |       |           |                        |              |   |      |      | B19)         |          | Pacific Blue |                       |              |             | <b>Y</b>  | 10       |
| Filter   Single color (ul)   Ref ctrl   Dunniking ctrl   Pluorochrome   Marker   Clone   Vial lot #   Stim!!!   Stim!!   Stim!!!   Stim!!   Stim!!!   Stim!!!   Stim!!!   Stim!!   Stim!!!   Stim!!   Stim!!   Stim!!    |    |           |       | 1.0       |                        |              |   |      |      | 5E2)         |          | Pacific Blue |                       |              | DA.         | \S        |          |
| Filter   Single color (ul)   Ref ctrl   Dunnixing ctrl   Pluorochrome   Marker   Clone   Vial lot #   Stimili   3   U/0.15 min   Tetramer 40   Hotstain   3   Goldstain   3   2   2   2   2   2   2   2   2   2  |    | 1         |       | 1 :0      |                        |              |   |      |      | 1905)        |          |              |                       |              |             | ک         | 9        |
| Filter   Single color (ul)   Ref ctrl   Dunnixing ctrl   Pluorochrome   Marker   Clone   Vial lot #   Stimili   3   Vial 5 min   Tetramer 40   Hotstain   3   3   30nmin @4C   3   3   3   3   3   3   3   3   3   |    | 7         | 1     | л         |                        |              |   |      |      | K3)          |          |              |                       |              |             | <u></u>   | œ        |
| Filter   Single color (ul)   Ref ctrl   Dunnixing ctrl   Pluorochrome   Marker   Clone   Vial lot #   Stimili   3   UD 35 min   Tetramer 4D   Hotistain   3   Goldstain   3   20min@4C   3.6   |    | 5         |       |           |                        |              |   |      |      | CR3)         |          | ICIANG       |                       |              |             | UV16      | 7        |
| Filter   Single color (ul)   Ref ctrl   Dunnixing ctrl   Fluorochrome   Marker   Clone   Vial lot #   Stimili   3   UD 15 min   Tetramer 4D   Hotistain   3   Somin @4C   3.6  |    | 2.1       |       |           |                        |              |   |      | 1    | C6/          |          | BINTOT       |                       |              |             | U14       | 6        |
| Filter   Single color (u)   Ref ctrl   Dunnixing ctrl   Fluorochrome   Marker   Clone   Vial Lot #   During   Stim!!   3   U/D 15 min   Tetramer 40   HotStain   3   30min @4c   3   3   3   30min @4c   3   3   3   30min @4c   3   3   3   3   3   3   3   3   3   |    |           |       | 6         | 2.0                    |              |   |      | 1    | B6)          |          | BUV661       |                       | 8            |             | UV11      | U        |
| Filter   Single color (u)   Ref ctrl   Unmixing ctrl   Fluorochrome   Marker   Clone   Vial Lot #   During   Simil!   3   L/D 15 min   Tetramer 40   HotStain   3   300min @40   3   3   3   300min @40   3   3   3   3   3   3   3   3   3  |    | 1.5       |       |           |                        |              |   |      | ŀ    | 161)         |          | BUV615       |                       | -            |             | UV10      | 4 .      |
| Filter   Single color (u)   Ref ctrl   Unmixing ctrl   Fluorochrome   Marker   Clone   Vial Lot #   During   Simility   3   L/D 15 min   Tetramer 40   HotStain   3   30min @4c   3   30min    |    | 2.1       |       |           | LOTE CONTRACT          |              |   | 1    | 1    | N50)         |          | BUV563       |                       |              |             | 640       | .        |
| Filter   Single color (u)   Ref ctrl   Durnixing ctrl   Fluorochrome   Marker   Clone   Vial Lot #   Stim!!   3  |    |           |       |           |                        |              |   | 1    |      | A-T8)        |          | BUV496       |                       |              | 1           | 100       | .        |
| Filter Single color (u) Ref ctrl Unmixing ctrl Fluorochronne Marker Clone Vial Lot # Stim!!! 3 L/D 15 min Tetramer 40 HotStain 30min @37C 3 30min @4C 3  |    | 3.6       |       |           |                        |              |   |      | +    |              |          | AF           |                       |              |             | IN7       | 2        |
| Filter Single color (ul) Ref ctrl Unmixing ctrl name Fluorochrome Marker Clone Vial Lot # Stim!!! 3 L/D 15 min Tetramer 40 HotStain ColdStain 3 30min @4C 3  |    |           |       |           |                        |              | 210000000000000000000000000000000000000 |      |      | (EG-56)      |          | BUV395       |                       | The state of |             | 240       | .        |
|  |    | RBC Lyse, |       |           | HotStain<br>30min @37C |              | L/D 15 min<br>(RT)                      |      |      |              |          | Fluorochrome | Unmixing ctrl<br>name | ) Ref ctrl   |             | Filter    | .   #    |

## Simplified Protocol

Thaw cells, DNAse, count.

Collect, count, aliquot cells 3.0E+6 Cells R1(
Bring volume upto 1 ml R10, add 2 ul PMA/C
Cap and incubate at 37°C for 6 hours

Wash with 2 ml PBS, spin down 1300 rpm 8n 800 ul of LiveDead mix (1:2500) @RT for 15n Wash 2 ml 5% PBS-FBS, spin 1300 rpm, 8ml

Add HotStain mix, incubate @37C for 30 mir Wash 2 ml 5% PBS-FBS 1300 rpm, 8 min

Add ColdStain mix, incubate @ 4C for 30mir Add 300-500 ul 1x RBC Lysis for 3 minutes Wash 2 ml 5% PBS-FBS 1300 rpm, 8min Add Tetramers, incubate @RT for 10 min

300 ul BD FixPerm, incubate @ 4C for 20mi (vortex every

First PermWash: Second Perm Wash:

1 ml PermW.
1 ml PermW.

Add Intracellular Stain, incubate @ RT for 40 First PermWash: 2 ml PermW.

Resuspend in 100 ul 0.4% PFA-PBS
Cap tubes, wrap rack in foil, store at 4\*C

Notes:

| New         | All e03<br>Udz | THE 15 | 3.5<br>23.1 |
|-------------|----------------|--------|-------------|
| old         | co3<br>vdz     | 15.3   | 7.3         |
| Current     | 28°            | 14.4   | 7.8         |
| Perm<br>Nev |                | 23.4   | 24.8        |
|             |                | 22.6   | 20.7        |

ILT & NK SFC Panel

|                |              |            |               |          |                                       |            |       |   | 10.00         |          |               |       | _   |                | -    |         |                                       |        | _        | -      |      | _        |
|----------------|--------------|------------|---------------|----------|---------------------------------------|------------|-------|---|---------------|----------|---------------|-------|-----|----------------|------|---------|---------------------------------------|--------|----------|--------|------|----------|
|                |              |            | 750<br>760    |          |                                       |            |       |   |               |          |               | 542   | 525 | 514            | 508  | 473     | 458                                   | 443    | 428      | 388    | 373  | opecuum  |
|                | UV16         | <u> </u>   | UV14          |          | UV13                                  | UV12       |       | UV11                                    | UV10          |          | 6AN           | 8VU   |     | UV7            |      | DV6     | UV5                                   | UV4    | UV3      | UV2    | LVU  |          |
|                | BUV805       |            | <b>BUV737</b> |          |                                       |            |       | BUV661                                  | <b>BUV615</b> |          | BUV563        |       |     | BUV496         |      | AF-UV6  |                                       |        |          | BUV395 |      | V        |
|                | Ξ            |            | <u> </u>      |          |                                       |            | 3     | ≅ 3                                     | <u></u>       | 3        | <u></u>       |       | :   | [2]            | - 10 |         |                                       |        | 1        | [2]    | +    |          |
|                | CD4          |            | CXCR3         |          |                                       |            | 4     | 2 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 | CCRA          | 000      | CDee          |       | Ü   | CDs            |      | ΔF      |                                       |        |          | CD62I  |      |          |
| - 6            | V15          |            | V14           | 5        | \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ | 3          |       | V10                                     | <u> </u>      | <u> </u> | <u> </u>      | í ó   | ś   | <b>S</b>       | 4 7  | £ 6     | \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ | S =    | <u> </u> |        | +    |          |
|                | BV786        |            | BV750         | 04711    |                                       |            | 0.690 | BV605                                   |               | BV5/0    | OLCAG         |       |     | BV480          |      | racbine | Daselina                              | 0447   | 0        |        | 1000 | Violet   |
|                | 3            |            | [2.5]         | 4        |                                       |            | [3.5] | 3                                       |               |          | [1.5]         |       |     | <u> </u>       |      | 3       | 3                                     | 4      | :        |        | T    | 1        |
|                | CCR6         |            | IFNg          | CD7      |                                       |            | CCR7  | CD56                                    |               |          | CD45RA        |       |     | CD161          |      | CD14/19 |                                       | 721.00 |          |        |      |          |
| B14            | B13          | B12        | B11           | B10      | B9                                    | B8         | B7    | B6                                      | B5            | B4       | B3            | B2    |     | B <sub>1</sub> |      |         |                                       |        |          |        | H    | 1        |
|                | Pe-Vio770    |            |               |          | PerCP-Cy5.5                           | PE-Cy5     |       | PE-CF594                                |               | PE       | SparkBlue 550 | AF488 |     |                |      |         |                                       |        |          |        | Blue | ?!       |
|                | <u> </u>     |            |               |          | [2]                                   | [4.5]      |       | 4                                       |               | [4]      | Ξ             | [1.5] |     |                |      |         |                                       |        |          |        |      |          |
|                | PD1          |            |               |          | TNFa                                  | CD25       |       | CD26                                    |               | NKG2D    | CD3           | hCD1d |     |                |      |         |                                       |        |          |        |      |          |
| R <sub>8</sub> | R7           | D<br>D     | R5            | R4       | R3                                    | R2         | 刄     |   |               |          |               |       |     |                |      |         |                                       |        |          |        |      | ١        |
| APC-Fire 810   | APC-Fire 750 | Zombie NIR |               | APC-R700 |                                       | AF647      | APC   |   |               |          |               |       |     |                |      |         |                                       |        |          |        | Red  | 22021111 |
| <u>a</u>       | [2]          | 3          |               | <u></u>  |                                       | [3.5]      | [3.5] |   |               |          |               |       | _   |                |      |         |                                       |        |          |        |      | ٥        |
| CD38           | CD27         | -<br>5     |               | CD107a   |                                       | hMR1/Va7.2 | CD16  |   |               |          |               |       |     |                |      |         |                                       |        |          |        |      |          |
|                |              |            |               |          |                                       | -          |       |   |               | -        |               | -     | -   |                | -    |         | _                                     |        |          |        |      | l        |

1/11/2023