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| 23-29.7.17 | **HPC-7:**<br>1. Thawing cells.<br>2. Preparing medium.<br>3. Mixing cytokines.<br>4. Expanding cells.<br><br>**Cloning:**<br>1. Mini prep for second generation tet plasmid |
| 30.7-5.8.17 | **HPC-7:**<br>1. First splitting. |
| 6-12.8.17 | **HPC-7:**<br>1. Splitting.<br><br>**Cloning:**<br>1. Mini prep for PUC 19 and pDisplay-CFP.<br>2. Transformation of tTA –CMV-BgH.<br>3. Mini prep for psb1c3-CMV-GFP-BgH.<br>4. Mini prep to TRE-tight-mCharry.<br>5. Transformation for TRE-mCharry. |
| 13-19.8.17 | **HPC-7:**<br>1. Splitting thawed cell.<br>2. Preparing new medium.<br><br>**HEK-293:**<br>1. Splitting 1:10 and 1:5.<br>2. Seeding for transfection.<br>3. Transfection: CFP, NT, PLA.<br>4. Medium change.<br><br>**Cloning:**<br>1. revPCR for pDisplay –removing the CFP in order to insert PLA+6His tag.<br>2. Purification of PCR products- for pDisplay without CFP.<br>3. Gibson assembly- pDisplat (without CFP ) +PLA- 6 HIStag.<br>4. revPCR for psb1c3 - removing the GFP, and insert the first gBlock (tri- display).<br>5. Mini prep for PUC19, PLA+HIS tag. |

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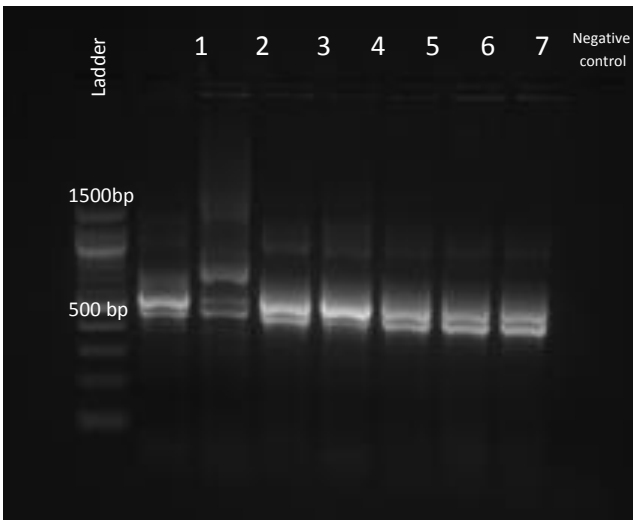
| | 6. ColonyPCR for pDisplay- PLA- his tag.<br>7. gel electroporation for pDisplay- PLA- HIS (~484bp)<br><br>  <br><br><br>**Interlab:** The goal of this experiment was to establish a GFP measurement protocol based on engineering principles using a plate reader.<br>1. Transformation of *Escherichia coli* DH5α with 8 different plasmids (six devices, a positive control and a negative control) were provided in the distribution kit.<br>2. Measurement in OD 600 from the overnight cultures, and analyzing the results. |
| 20-26.8.17 | **HPC-7:**<br>1. Splitting.<br>2. Freezing 6 vials.<br>3. Counting & practicing working with FACS.<br>4. First transfection with CMV m-kate (4 reagents: lipofectamine 2000, LT1, transIT 2020, poly jet).<br>5. FACX test for the transfection.<br>6. Freezing 12 vials.<br><br>**HEK-293:**<br>1. Splitting 1:10 |

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| | **Assay:**<br>1. Thawing the cells.<br>2. Splitting WHEI.<br>3. FACS calibration (before and after centrifuge): HPC-7+WEHI.<br><br>**Cloning:**<br>1. Sequencing for PLA+HIS tag.<br>2. Mini prep for CMV-GFP-BgH.<br>3. Sequencing for CMV-GFP-BgH.<br>4. Mini prep for CMV-GFP-BgH+tTA. |
| 27.8-2.9.17 | **HPC-7:**<br>1. Splitting<br>2. Optimization for the transfection with LIPO and transIT 2020 (we test 2 different DNA -reagent ratios, in 3 different cell concentrations, for each reagent.<br>We also tested 2 different exposure times for each reagent.<br>3. FACS test for the optimization - after 24 hours and 48 hours.<br><br>**HEK-293:**<br>1. Transfection PLA + HIS<br>2. Antibodies - checking HIS tag.<br>3. FACS analysis.<br>4. Freezing (-80).<br>5. Splitting.<br><br>**Assaay:**<br>1. Splitting WHEI.<br><br>**Cloning:**<br>1. ColonyPCR#1 and gel electroporation for psbc13+tTA- didn't succeed, so we repeat the cloning again.<br>2. revPCR for psb1c3 for insert the first gBlock-"tri-display".<br>3. revPCR – insert TRE – MOG1 + MOG3. |

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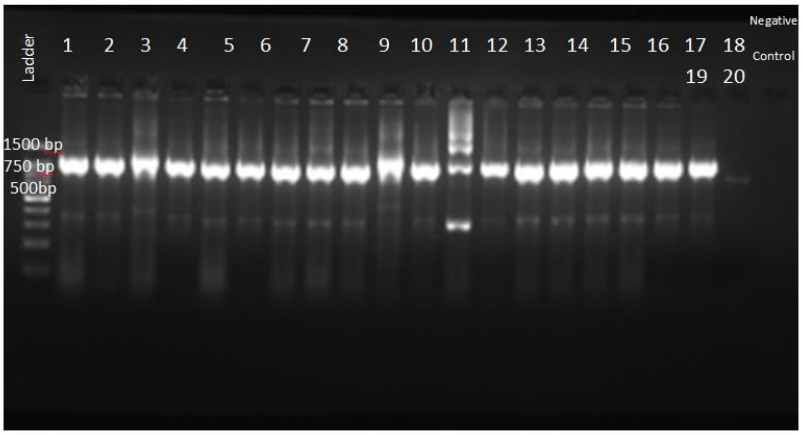
| | 4. Colony PCR#2 for psb1c3+tTA (~750bp)<br>  <br><br>5. Revers-PCR to insert TRE to psb1c3.<br>6. Gibson assembly to insert TRE to psb1c3.<br>7. ColonyPCR for TRE promoter -3 times didn't succeed. ☹ |
| 3-9.9.17 | **<u>HPC-7:</u>**<br>1. Splitting<br><br>**<u>HEK-293:</u>**<br>1. Splitting.<br>2. Transfection: co-transfection of psb1c3+GFP & mkate.<br>3. FACS + antibodies experiment - PLA HIS#2 - checking HIS tag again<br>4. Medium preparation<br>5. Switching medium<br>6. Co-Transfection TTA + TRE from Roni (96 wells).<br>7. Reverse transfection for the "tri-epitopes" (our first gBlock).<br>8. AB experiment+ FACS analysis. |

The following are the results of the experiment:

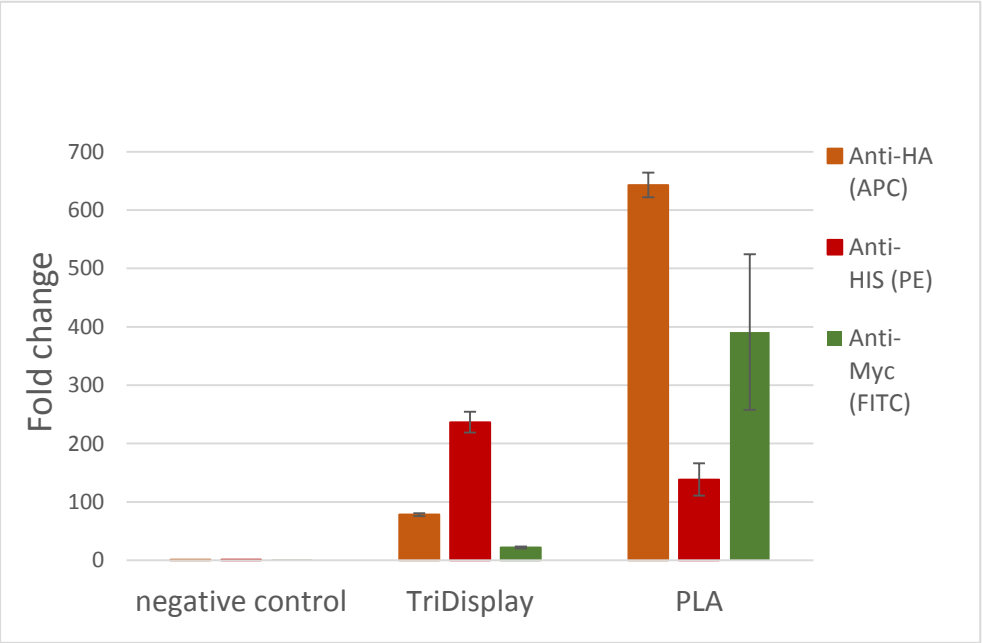



Figure 1: weighted Median of fluorescence intensity - three different antibodies.

From the results shown in Figure 1, we can learn that the TriDisplay works fine, presenting much higher fluorescence than the negative control, while for the HIS fluorescence is even higher compared to PLA.

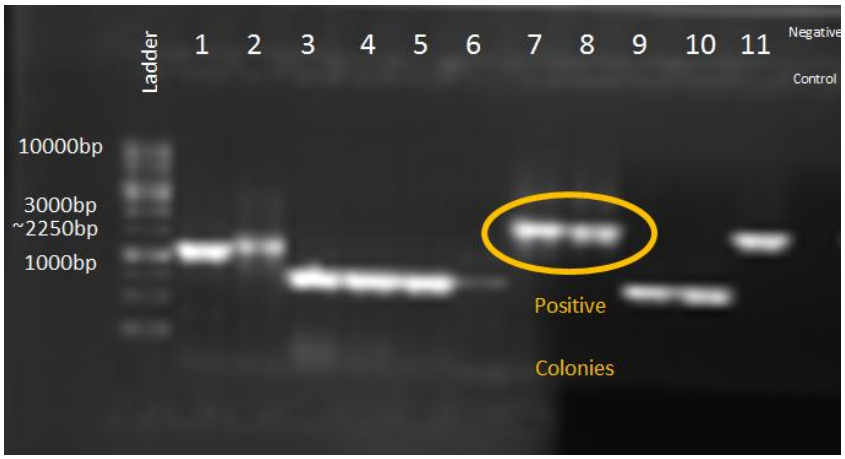
**Assaay:**

1 Defrosting WEHI.
2 Splitting WHEI.

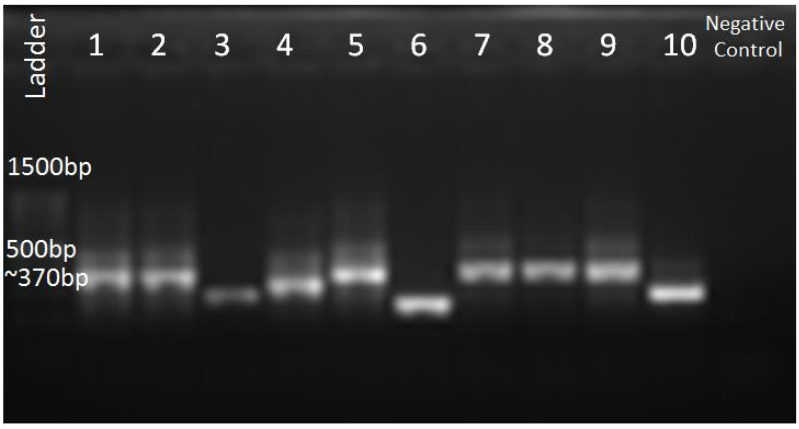
**Cloning:**

1. Gibson for scfv-mono-epitop.
2. revPCR for psb1c3-CMV-GFP-HgH +gel electroporation+ purification of PCR products.
3. PCR for the gBlock (Ef1a+scFv) +gel electroporation+ purification of PCR products.
4. Gibson for gBlock (Ef1a+scFv) into psb1c3.
5. Transformation for Pbs1C3+gBlock (Ef1a+scFv).

6. ColonyPCR for Pbs1C3+gBlock (Ef1a+scFv~2275bp) +gel.




7. Starter for Pbs1C3+gBlock (Ef1a+scFv).
8. Mini prep for Pbs1C3+gBlock (Ef1a+scFv).
9. Sequencing Pbs1C3+gBlock (Ef1a+scFv).
10. Gibson assembly for TRE promoter to psb1C3 (no CMV).
11. ColonyPCR psb1c3+TRE (try number 4, after changing primers)- finely succeed! ☺



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| 10-16.9.17 | **HPC-7:**<br>1. Transfection with pDisplay-PLA.<br>2. Antibodies experiment to the pDisplay-PLA.<br>3. FACS analysis for AB experiment.<br>4. Splitting.<br><br>**HEK-293:**<br>1. Splitting.<br><br>**Assaay:**<br>1. Revers transfection for pbs1C3- first gBlock.<br>2. Splitting WEHI.<br>3. Antibodies experiment.<br>4. FACS analysis.<br>5. Seeding HEK cells for transfection.<br>6. Transfection HEK with the plasmids:<br>a. psb1C3+ Ef1a (mutant promoter) +GFP<br>b. psb1C3+ native Ef1a +GFP<br>c. psb1C3+ scFv.<br><br>**Cloning:**<br>1. revPCR for insert scFv into psb1C3.<br>2. DNA purification<br>3. revPCR psb1C3+Ef1a+GFP<br>4. revPCR pDisplay+ Ef1a<br>5. Gibson for scFv into psb1C3<br>6. ColonyPCR for psb1C3+Ef1a<br>7. ColonyPCR for psb1C3+ scFv<br>8. ColonyPCR for pDisplay+ Ef1a.<br>9. PCR to create overlap regions for native Ef1a.<br>10. Gibson for Ef1a into psb1C3 with GFP no CMV<br>11. ColonyPCR for psb1C3+native Ef1a<br>12. Mini prep for: psb1C3-Ef1a-GFP, pDisplay-Ef1a-CFP,psb1C3-scFV-HGH.<br>13. PCR for scFv.<br>14. PCR for Ef1a.<br>15. Gibson for scFv+psb1C3.<br>16. Gibson for pDisplay+Ef1a.<br>17. Transformation for scFv+psb1C3. |

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| | 18. Transformation for pDisplay+Ef1a.<br>19. Transformation for Ef1a+psc1c3.<br>20. Colony PCR for the scFv+psb1C3.<br>21. Colony PCR for th pDisplay+Ef1a.<br>22. Colony PCR for the Ef1a+psc1c3.<br>23. PCR for native Ef1a. |
| 17-23.9.17 | **HPC-7:**<br>1. First electroporation experiment with NEPA**.**<br>2. Thawing 3 vials.<br>3. Splitting<br><br>**HEK-293:**<br>1. Throw away - cleaning incubator.<br>2. Thawing.<br>3. Splitting<br><br>**Assay:**<br>1. Antibodies experiment for: scFv+psb1C3, pDisplay+Ef1a, Ef1a+psc1c3.<br>2. FACS analysis.<br>3. Splitting WHEI.<br>**Cloning:**<br>1. Sequencing psb1C3-Ef1a and psb1C3-CMV-scFv<br>2. Gibson TRE+(MOG1, MOG2, MOG 3)<br>3. Midi prep for: psb1C3-gfp , tTA, TRE mcherry.<br>4. |
| 24-30.9.17 | **HPC-7:**<br>1. Splitting - 16 plates**.**<br>2. Electroporation –optimization experiment.<br>3. FACS measurement.<br>4. Splitting<br>5. Preparing new medium with IL6.<br><br>**HEK-293:**<br>1. Splitting. |

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| | **Assay:**<br><br>1. Apoptosis test for WEHI cells to distinguish between two populations.<br>2. Seeding HEK-293 for co-culture experiment.<br>3. Seeding of WEHI cells with HEK cells for co-culture (after one day from HEK seeding).<br>4. Apoptosis test time 0 +FACS analysis.<br>5. Apoptosis test time 24+ FACS analysis.<br>6. Apoptosis test of WEHI cells with soluble anti-IgM+ FACS (time 0).<br>7. FACS analysis for apoptosis test of WEHI+anti IgM after 24 hr and 48 hr.<br>8. Splitting WHEI.<br><br>**Cloning:**<br><br>1. PCR for creating scFv with tails into pDisplay.<br>2. Gibson for tri-epitope with: secrecon-T2A, P2A-T2A, Linker-T2A. |
| 1-7.10.17 | **HPC-7:**<br>1. Splitting<br><br>**HEK-293:**<br>1. Reverse transfection TRE+ GFP (which we cloned) +tta.<br>2. Reverse transfection improve "tri- epitope".<br>3. Induction test.<br>4. Switching medium.<br>5. AB experiment + FACS analysis.<br>6. Splitting HEK .<br>7. Throw cells with tet in their genome.<br><br>**Assay:**<br>1. Splitting WHEI.<br><br>**Cloning:**<br>1. Mini prep for mono-epitope –Ef1a-scFv.<br>2. Gibson for native Ef1a-psb1C3.<br>3. Gibson to tri-epitop: T2A-P2A-Ef1a, scFv-fus-Ef1a, scFv-fus-CMV, MOG-T2A-CMV. |

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| | 4. revPCR psb1C3-Ef1a-GFP insert Fab gBlock.<br>5. Midi prep for: EF1a-GFP, EF1a-scFv-fus, CMV-scFv-fus.<br>6. |
| 8-14.10.17 | **HPC-7:**<br>1. Chemical transfection with lipofectamine and transit 2020 + psb1C3 (Ef1a+GFP).<br>2. Electroporation psb1C3 (Ef1a+GFP) linear + cycled<br>3. FACS test to the transfection and Electroporation.<br>4. Splitting.<br><br>**HEK-293:**<br>1. Splitting.<br><br>**Assay:**<br>1. Transfection of HEK cells with scFv fus ratIgG.<br>2. Medium change.<br>3. Antibody experiment with IgM conjugated to fluorophore (FITC).<br>4. FACS analysis<br>5. Splitting WHEI.<br><br>**Cloning:**<br>1. Digestion of CMV-GFP &Ef1a-GFP for linearized plasmid for electroporation HPC-7.<br>2. Gibson Ef1a- for "parts"<br>3. ColonyPCR for MOG-T2A.<br>4. cutting the plasmid psb1C3-Ef1a-GFP with the restriction enzymes: xba1, kas1.<br>5. ColonyPCR for Ef1a- for "parts"<br>6. ColonyPCR- optimal tri-display in psb1C3<br>7. ColonyPCR for tTA-psb1C3<br>8. ColonyPCR – 3 gBlocks (P2A, secrecon, linker).<br>9. revPCR for: psb1C3-TRE-GFP, psb1C3-gBlock-MOG, Gibson TRE+ T2A-P2A. |
| 15-21.10.17 | **HPC-7:**<br>1. Transfection (LIPO) with pDisplay-PLA.<br>2. Antibodies experiment.<br>3. FACS test to pDisplay-PLA.<br>4. Splitting. |

**HEK-293:**

5. Transfection with CMV -P2A (2)
6. Transfection with CMV -P2A (3)
7. Antibodies experiment +FACS analysis.
8. Transfection TRE promoter+ tri-display
9. Revers tansfection - scFv FC fusion
10. Antibodies experiment for tansfection – scFv-FC- fusion.
11. FACS measurement
12. Revers transfection- TRE-tridisplay.
13. Induction test.
14. Antibodies experiment.
15. Splitting.

**Assay:**

16. Revers transfected for HEK+scFv fus ratigG,
17. Seeding of WEHI cells with HEK cells for co-culture experiment+ apoptosis test time 0.
18. Apoptosis test time 24 and 48 hr+ FACS analysis.
19. Splitting WHEI.
20. **Co-culture HEK+ WHEI** + apoptosis test time 0, time 24 hr, and time 48 hr.

The following are the results of the experiment:

**Co-culture results:**

In order to simulate the interactions between the hematopoietic presenting epitopes and immature B cells, we Co-culture our model cells at different ratios- 20:80, 40:60, 60:40, 80:20 (HEK-293:WEHI-231) and checked the vitality of the cells after 0,24,48 hours using Propidium Iodide (PI), a fluorescent intercalating agent that used to stain nucleic acids.

We found that the optimal ratio, the ratio with the lowest percentage of deaths given both types of cells, was 40% HEK-298 (presenting epitopes cells) and 60% WEHI-231 (immature B cells model).

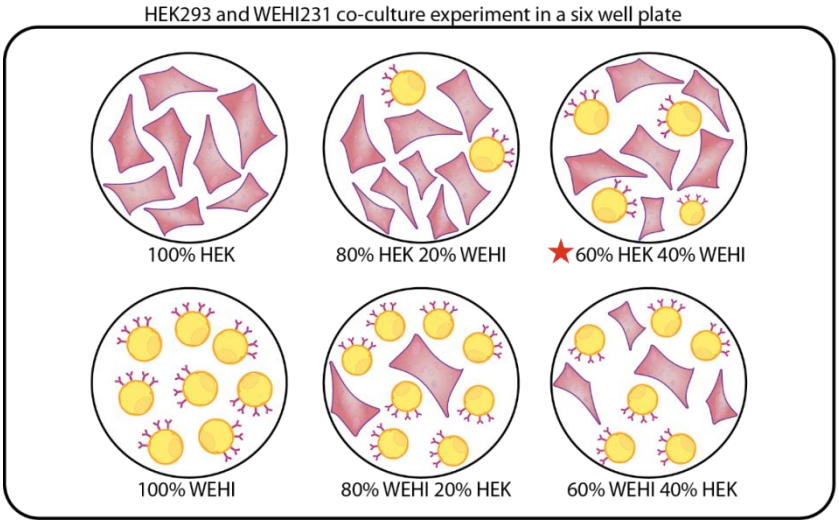


Figure 2- demonstration of the co-culture plate with the different ratios of HEK-293 and WEHI-231