OriginALS notebook:

Week 13 – 6/5/18 – 12/5/18

6/5/18:

Who's at the lab:
MorP, Liat

Today's goals:

1. mini-prep for 3 different plasmids, all from DH5a (pGL3-F4/80, pX601, pX458)
2. PCR, amplify F4/80p from pGL3-F4/80
3. Restriction of F4/80p (PCR product) and pX601
4. Gel extraction
5. Ligation of pX601 with F4/80p
6. Transformation of ligation product to DH5a competent cells
7. Primers arrived (#34, #38, #49, #48) prepare stock and working solutions.

Description:

1. Mini prep for 3 starters (DH5a pX458, DH5a pX601, DH5a pGL3-F4/80) was done according to Mini prep(x2 each). I used 4ml for each reaction.
   results:

plasmid	conc. [ng/µl]	260/230	280/230
pGL3-F4/80 #1	87.5	1.9	2.1
pGL3-F4/80 #2	37.5	1.9	1.7
pX458 #1	303	1.84	2.23
pX458 #2	367	1.8	2.2

pX601 #1	128	1.8	1.4
pX601 #2	91.5	1.8	2.2

pGL3-F4/80 plasmid conc. are very low!!

2. PCR reaction was done to amplify F4/80p from pGL3-F4/80 plasmid according to PCR hotstart ready mix.
   I ran 25µl and 50µl reaction, to see if we can save material:

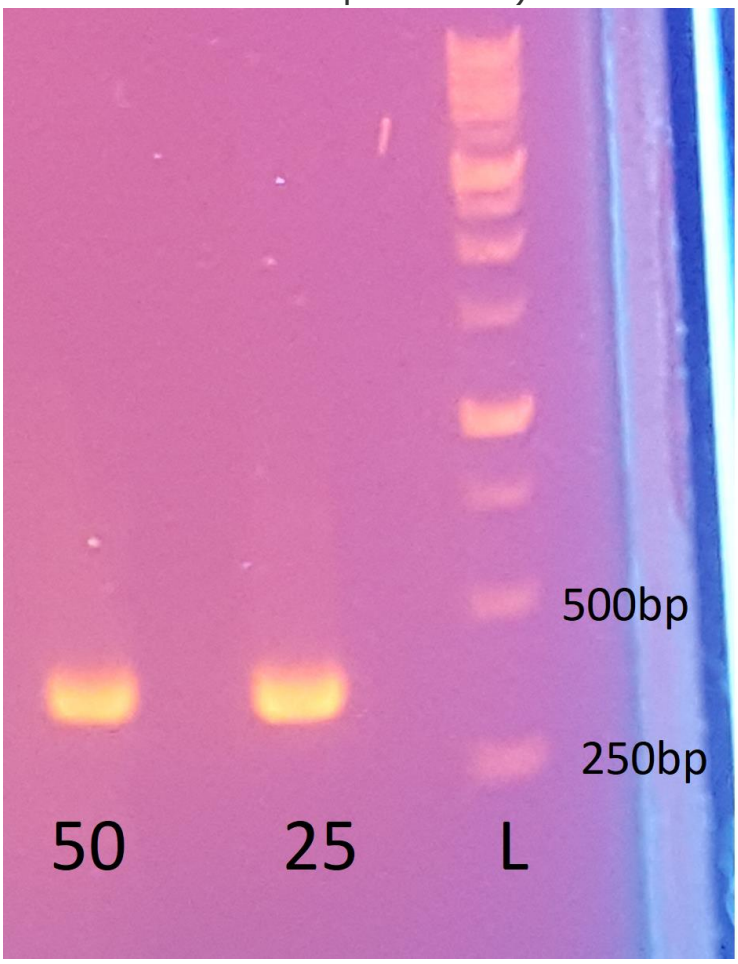
	50µl	25µl
DDW	21.5µl	10.5µl
KAPa HIFI HorStart ReadyMix	25µl	12.5µl
Primer F #20	1.5µl	0.75µl
Primer R #19	1.5µl	0.75µl
pGL3-F4/80	0.5µl	0.5µl

The plasmid I used was pGL3-F4/80 in conc. of 163ng/µl (260/230=1.8, 280/230=2.1)

Reaction set up:

Step	**Temperature**	**Duration**	**Cycles**
Initial denaturation	95 ºC	3 min	1
Denaturation	98 ºC	20 sec	30
Annealing	60 ºC	15 sec	
Extension	72 ºC	15 sec	
Final extension	72 ºC	30 sec	1
	4 ºC	∞	1

The samples were loaded to 2% agaros gel (0.7gr agarose +50ml TAE + 2 drops of EtBr). results:



since the samples look good in the gel, I used PCR and DNA Fragment Extraction
in order to purify the samples:

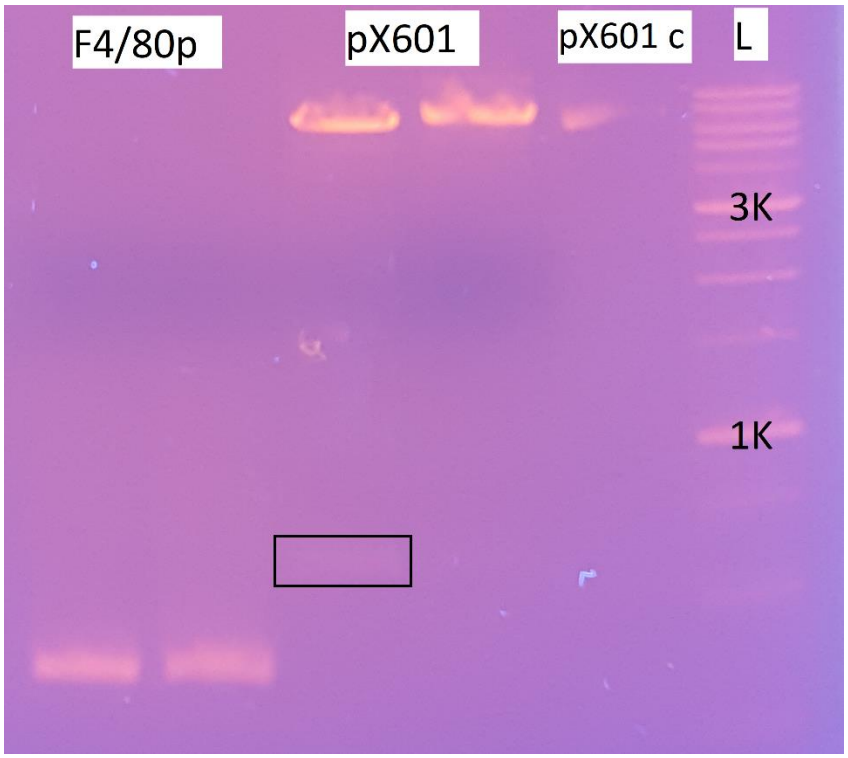
sample	F4/80 50µl	F4/80 25µl
conc. ng/µl	56.5	49.5
260/230	1.823	1.74
280/230	1.948	2.02

3. Restriction of F4/80p (PCR product) and pX601:

reaction name:		F4/80p 50ul	F4/80p 25ul	pX601 x2
DNA type		PCR product	PCR product	plasmid DNA
FD Buffer		3	3	2
DNA		6.5	7	8
FD Enzymes	XbaI	1	1	1
	AgeI (BshTI)	1	1	1
DDW		18.5	18	8
total volume		30	30	20
	DNA Conc.	56.5	49.5	128
	Actual ng	367.25	346.5	1024
	product size	349	349	7449

Reaction - 37°C for 1hr
Followed by deactivation 5min 80°C
Samples were loaded to 2% agarose gel (0.7gr agarose +50ml TAE + 2 drops of EtBr).

Results:



The two wells of F4/80 looks good and the left well of pX601 - these

two samples (red circle) were taken to the next step (F4/80p sample are combined to one sample).

4. Gel extraction was done to two samples from previous step according to Gel extraction HyLab
   I melted the gel in 50°C for 15min (every 2min - vortex)

	F4/80p	pX601
eppendorf weight	0.3096gr	0.1644gr
conc.	12.5 ng/µl	11 ng/µl
260/230	1.92	1.89
280/230	0.086	0.06

5. Ligation of pX601 with F4/80p according to the next protocol:

Invitrogen Buffer	4µl
Vector	7.55µl
insert	1.36µl
T4 ligase (Invitrogen)	1µl
DDW	6.09µl

   Total reaction is 20µl
   Insert to vector ratio is 1:4 (both from the previous step)
   Total DNA in reaction is 100ng
   Reaction-> 20min in 37°C

6. Transformation of ligation product to DH5a competent cells according to New DH5a competent cell transformation protocol.

7. Primers arrived (#34, #38, #49, #48), I prepared stock and working solutions according to IDt instructions.

Tasks for next time:

1. Freeze 3 starters of DH5a pAC-GFP in -80 glycerol stock
2. Prepare starters from transformation plate
3. Gibson

7/5/18:

Who's at the lab:
Nitzan, Liat

Today's goals:

1. Transfection of GFP- **Liat**
2. Splitting BV2 cell line- **Liat**
3. Continuing the promoter assay- To do transfection of the cells in the 2 24 wells plates.

Description:

1. Transfection of the PUC-GFP and px458-GFP plasmids to the BV2 cell line:
    a. Dilute 0.5 µg of DNA into 10 µl of 150 mM NaCl. Vortex gently and spin down briefly.
    b. Dilute 1.6 µl of jetPEI®-Macrophage into 10 µl of 150 mM NaCl. Vortex gently and spin down briefly.
    c. Add the 10 µl jetPEI®-Macrophage solution to the 10 µl DNA solution at once (Avoid the reverse
    d. order).
    e. Vortex-mix the solution immediately and spin down briefly.
    f. Incubate for 15 to 30 minutes at room temperature.
    g. Add the 20 µl jetPEI®-Macrophage/DNA complexes to each well and homogenize by gently
    h. Swirling the plate.
    i. Transfection experiments are usually analyzed after 24 hours and reporter gene activity is measured.
    j. 2 wells were transfected with PUC-GFP
    k. 2 wells were transfected with px458-GFP
    l. control- 1 well with 10 µl jetPEI®-Macrophage solution, 1 well only with cells

m. 4 hours after the transfection the medium was changed

2. Splitting BV2 cell line:

a. warm Microglia medium
b. write the date and cell line name and add 8.5ml/8ml of new medium
c. take out the medium from the old flask
d. add 4ml of new medium and scrape gently with sterile scraper
e. pipet well
f. transfer 0.5ml/1ml of medium with cells from the old flask to the new flask

3. Transfection for promoter assay:

a. Dilute 0.5 µg of DNA into 10 µl of 150 mM NaCl. Vortex gently and spin down briefly.
b. Dilute 1.6 µl of jetPEI®-Macrophage into 10 µl of 150 mM NaCl. Vortex gently and spin down briefly.
c. Add the 10 µl jetPEI®-Macrophage solution to the 10 µl DNA solution at once (Avoid the reverse
d. order).
e. Vortex-mix the solution immediately and spin down briefly.
f. Incubate for 15 to 30 minutes at room temperature.
g. Add the 20 µl jetPEI®-Macrophage/DNA complexes to each well and homogenize by gently
h. Swirling the plate.
i. Transfection experiments are usually analyzed after 24 hours and reporter gene activity is measured.

We performed the transfection to the cells according to the protocol of transfection in the folder in the cell culture room.
The transfection was according to the table:

pGL3 + F4/80 & Renilla	pGL3 + F4/80 & Renilla	pGL3 + F4/80 & Renilla	pGL3 + F4/80 & Renilla	pGL3 + F4/80 & Renilla	pGL3 + F4/80 & Renilla
pGL3 no promoter & Renilla	pGL3 no promoter & Renilla	pGL3 no promoter & Renilla	pGL3 no promoter & Renilla	pGL3 no promoter & Renilla	pGL3 no promoter & Renilla
No transfection/ just Renilla	No transfection/ just Renilla	No transfection/ just Renilla	No transfection/ just Renilla	No transfection/ just Renilla	No transfection/ just Renilla

4 hours after the transfection the medium was changed- just for plate number 1. the medium of plate number 2 will be change tomorrow- (because I made it an hour later and 4 hours should be between the transfection and the changing of the medium)

Tasks for next time:

1. GFP- **Liat**
2. Splitting BV2 cell line- **Liat**
3. Freezing BV2 Cells- **Liat**
4. Promoter assay- To see what is the condition of the cells.
   If tomorrow the condition is bad, perform the illuminometer assay. If the condition is good, you can wait for Wednesday with the illuminometer assay.