Wrapup 2008-06-20

Priority

General

Plasmids

pLV-TRE-Ngn1-EYFP-2A-mKate-Ubc-Bla

[1] Sequence pLV-TRE-Ngn1-IRES2-EGFP. [Katia took them over – results will arrive by Friday.]

[Cil] Look for another template (another tube of pLV-TRE-Ngn1-IRES2-EGFP).

[1] If another tube of pLV-TRE-Ngn1-IRES2-EGFP is found, do another restriction digest with PacI, ClaI. [Molly will put digest in -20 at 6:20.]

[1] Run the pLV- TRE- Ngn1-IRES2-EGFP digest with PacI and ClaI. (Accurate bands)

[1] Gel extract (Bad OD)

[3] Transform pLV-TRE-Ngn1-IRES2-EGFP; plate.

[3] Midiprep one colony of pLV-TRE-Ngn1-IRES2-EGFP [No colonies, 8 hours was too few.]

[1] Clarify the massive confusion with respect to Patrick's Ngn1 primers.

[1] Use Patrick's primers and template. Run another PCR and use his PCR program. (The band should be around 870bp.)

[1] If the PCR did work, gel extract, measure OD and then SOE. (David and Navin will tonight)

[1] Set up Ngn1-EYFP-mKate PCR

[1] Set up Ngn1-EYFP PCR (in case the Ngn1-EYFP-mKate construct does not work)

[1] Run gel of SOEings

[1] Gradient PCR [Molly will pick up at 9pm]

[1] Run gel on gradient PCR products [come in at 10am tomorrow]

[1] Extract

[1] O.D.

[1] Insert digest with SfiI [double-check that]

[2] PCR purify insert digest.

[2] Measure O.D.

[1] Digest pLV-TRE-Sox17-Ubc-Bla with SfiI [double-check that]

[1] Run vector digest on gel.

[1] Redo digest.

[1] Run on .8%agarose gel at a lower voltage (80V) for a longer time on a longer gel (no middle combs).

[1] Gel extract.

[1] Measure O.D.

[2] CIP vector digest

[2] PCR purify vector digest CIP

[2] Measure O.D.

[2] Ligate

[3] Transform

pLV-TRE-Sox17-Ubc-Bla

[3] Maxiprep colony 2-1. [Negligible OD]

pLV-Ubc-rtTA-2A-Bla

[2] Digest pFUGW with EcoRI and AgeI

[2] Run pFUGW-EcorI-AgeI digest on gel.

[2] Gel Extraction did not work; had to be redone.

Wrapup 2008-06-20 2 of 3

[2] Gel extract try 2; see what the OD is (10.17ng/ul).

[2] CIP pFUGW-AgeI-EcoRI

[2] PCR purify (8.x ng/ul)

[2] OD

[2] Ligate pFUGW-AgeI-EcoRI-CIP and rtTA-2A-Bla-BspEI-EcoRI (take out tomorrow morning). 35ul of Ligation reactions (pFUGW-AgeI-EcoRI-CIP) thrown out by mistake, but still enough left over

[2] Run the pFUGW-AgeI-EcoRI digest again (3rd time) at least 20ug.

[2] CIP vector digest

[2] PCR purify vector digest CIP

[2] Measure O.D. – 90.17ng/ul

[2] Ligate – Cil will take out in the morning.

[3] Transform ligation into ultra-competent cells. – will be taken out tonight

[3] Saturday – Navin will transform today's pLV-Ubc-rtTA-2A-Bla.

[3] Sunday Evan and Lena will pick 40 minipreps (10 from each plate) for growth.

[3] Plate ligation.

[3] Incubate for 12-15 hours

[Cil] Take out tonight.

p148

[3] Transform p148. Grow overnight on plates (Evan). Got ~500 colonies.

[3] Setup for maxiprep – grow overnight in 400ml LB Amp.

[3] Maxiprep

[3] O.D. [~500 ng/uL]

[3] Trust in God that the sequence is correct (Pray?) (Donations?)

p149

[3] Transformed

[3] Pick colony

[3] Grow colony for maxiprep. Take out into +4C Wed. @ 8:30am (Navin)

[3] Maxiprep [1330-1360 ng/ul] (Two O.D. tries)

[3] Trust in God that the sequence is correct (Pray?) (Donations?)

pLV-TRE-PKMz-Ubc-Hyg

[1] Design plasmid

[1] Buy PKMz

pLV-pPKMz-EGFP-Ubc-Bla

[1] Design Plasmid

[1] Buy the promoter (PKMz)

pLV-TRE-Mash1-EYFP-2A-mKate-Ubc-Bla **(in same plasmid as Ngn1?)**

Wrapup 2008-06-20 3 of 3

[1] Design plasmid [do this one first]

pLV-NeuronalPromoter-Neuronal specific CFR

??

Lentivirus

Lenti: pLV-TRE-Ngn1-EYFP-2A-mKate-Ubc-Bla

Experiments

Experiment: Ngn1-EYFP-2A-mKate

- Infect cells
- Add Dox
- Observe differentiation
- Make Yellow/Red artificial brains
- Plan ahead: Camera sensitivity, Dyes, Cil found a dye which could help us see action potentials.
- Buy some neurons, Dyes, potassium and valinomycin, glutamate, GABA, dopamine, ACh, special media
- Start planning microfluidics and also the optical twizzlers
- Bio nanoforce