Wrapup 2008-06-17

Priority

General

Plasmids

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

[1] Redo PCR for Ngn1 [Eric picked it up.]

[1] Ngn1 – different digest. Find 2 different enzymes. Hope they work.

[1] Digest pLV-TRE-Ngn1-IRES2-EGFP w/ PacI and ClaI

[1] Run on gel

[1] Sequence pLV-TRE-Ngn1-IRES2-EGFP.

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

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

[3] Midiprep one colony of pLV-TRE-Ngn1-IRES2-EGFP

[1] Set up Ngn1-EYFP-mKate PCR – Need Ngn1

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

[1] Extract PCR products from gel
        -- cut out gel for EYFP (12.6 ng/ul) and mKate (31.4 ng/ul)

[1] PCR together EYFP and mKate

[1] O.D. (72.7 ng/ul)

pLV-TRE-Sox17-Ubc-Bla

[3] Midiprep 2 cultures of pLV-TRE-Sox17-Ubc-Bla (Use 50mL of culture; Spin down and freeze the rest.) – (6) – 792ng/uL; (2-1) – 381ng/uL

[3] Prepare 12 more colonies from Sox17 plates for overnight growth in miniprep.- Pick up tomorrow morning

[3] Miniprep

[3] Restriction Map mini- and midi-preps w/ BamHI and MluI. Evan will come in at 8:30 to put in -20.

[3] Digest ½ ug (or more) of anything above 20ng/ul.

[3] Run gel of digested and undigested and hyperladder and supercoiled ladder.

[Cil] Buy reasonable maxi- and mini-prep kits.- Happy Failure.

              Eppendorf takes longer; QIAgen has more consistent results.

pLV-Ubc-rtTA-2A-Bla

[2] Digest pFUGW with EcoRI and AgeI

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

[2] Band has been found and cut out and is sitting in -20.

[2] Gel Extraction

[2] CIP pFUGW-AgeI-EcoRI

[2] PCR purify

[2] OD

[2] Ligate pFUGW-AgeI-EcoRI-CIP and rtTA-2A-Bla-BspEI-EcoRI

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?)

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