

Wrapup 2008-06-18 1 of 2

Priority

Caroline Sr. needs to remake the gel buffer.

General

Eric is presenting on myelin regeneration tomorrow during wrapin. Navin might also present.

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] Gel extract

[1] If new primers come, pick them up. [David]

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

[3] Midiprep one colony of pLV-TRE-Ngn1-IRES2-EGFP [happening after this meeting – Navin will get it in the morning.]

[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)

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 on undigested while digesting.

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

[3] Maxiprep colony 2-1. [set up after meeting]

[Cil] Buy reasonable maxi- and mini-prep kits.- Happy Semi-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] Gel Extraction did not work; had to be redone. FAILED

[2] Gel extract try 2; see what the OD is. If the OD sucks, start over.

[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]

Wrapup 2008-06-18 2 of 2

[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