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Priority

General

Caroline Jr. and Hamza will be presenting on Monday, when Ron and Cil are here.

Plasmids

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

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

[1] Run gel of PCR products

[1] Cut gel in half.

[1] Gradient PCR with betaine for Ngn1 = Did Not Work

[1] Digest pLV-TRE-Ngn1-IRES2-EGFP w/ EcoRI and BamHI

[1] Gel Extract – Done in 10-20min

[1] O.D.

[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

[2] Pick colonies of pLV-TRE-Sox17-Ubc-Bla (tried 3 plates – 2 worked. 10 total, 5 from each of two plates)

[2] Grow 10 minipreps – Prepare growth cultures; 12

[2] Miniprep 10 cultures of pLV-TRE-Sox17-Ubc-Bla (5 surviving, 100 – 300 ng/ul)

[2] Restriction map pLV-TRE-Sox17-Ubc-Bla with PacI, NheI/BsiWI

[2] Run on gel

[2] Gel Picture [Add to lab notebook!] – PacI may have cut correctly, NheI/BsiWI failed.

[2] Redo NheI/BsiWI digest

[2] Image. (Didn't work)

[2] Transform colonies. Plate. Incubate overnight at 37. Transfer to +4 Saturday 10am-2pm [Hamza].

[2] Depending on restriction map, choose one colony to grow at 37 for MaxiPrep. [Caroline Jr. on Sunday]

[3] pLV-TRE-Sox17-Ubc-Bla Colony 6 – set up growth culture at 37 for maxiprep.[Caroline Jr., Sunday, 6-7 PM]

[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] Restriction Map w/ xxx and yyy

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

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

pLV-Ubc-rtTA-2A-Bla

[2] Digest pFUGW with EcoRI and AgeI

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

[2] Gel Extraction

[2] CIP pFUGW-AgeI-EcoRI

[2] PCR purify

[2] OD

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[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