Wrapup 2008-06-11

Priority

General

Plasmids

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

[1] PCR SOEing: Ngn1-EYFP-2A-mKate – Started PCR amplify for Ngn1, EYFP, mKate

[1] Redo PCR for Ngn1

[1] Make gel

[1] Run gel of PCR products: Ngn1, EYFP, mKate
– Ngn1: didn't do. Need to wait for correct primer
-- EYFP: band around 1kb ??
-- mKate: two bands

[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 (store @-20C, David, 6:30pm)

[2] Parent vector: 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

pLV-Ubc-rtTA-2A-Bla

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

[2] Transformation of ligation. Grow overnight on plates (Andrew). Got ~50 colonies.

[3] Grow 12 minipreps of ligation plate. (Evan to 37C shaker)

p148

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

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

p149

[3] Transformed

[3] Pick colony

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

[3] Maxiprep (check OD)

[3] Restriction mapping (plasmid?)

Lentivirus

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

Experiments

Experiment: Ngn1-EYFP-2A-mKate

Infect cells

Add Dox

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Observe differentiation

Make Yellow/Red artificial brains