Wrapup 2008-06-24

Priority

General

Plasmids

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

[1] O.D. 192 ng/ul for Ngn1-EYFP-2A-mKate; ~300ng/ul for Ngn1-EYFP

[2] Insert digest with SfiI

[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 – 10ug (or 20ug if we have it). [David will re-digest tonight].

[1] Run on 1%agarose gel at a higher voltage (110V) for 2 hours on a longer gel (no middle combs). [try to get rid of u-shaped bands – ask VectorNTI]

[1] Gel extract. (if not good separation, take the bottom part of the band.)

[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] Grow two colonies overnight [Evan and Navin]

[3] Midiprep. – O.D. 250 and 270ng/ul

[3] Restriction map with BamHI and MluI; expect bands at 7498 and 3461.

pLV-Ubc-rtTA-2A-Bla

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

[3] Extract DNA

[3] O.D. 30-40ng/ul

[2] Design restriction map.

[2 and 3] Restriction map – cut with MluI. Do as many as you can as soon as you can.

[3] If you find a good one – transform today to maxiprep tomorrow

[3] On the three decent-looking samples [7-2, 7-8, 7-6], transform, midiprep, sequence, restriction map again.

p148

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

p149

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

pLV-TRE-PKMz-Ubc-Hyg

[1] Design plasmid

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[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?)**

[1] Design plasmid [do this one first]

pLV-TRE-Mash1-Cerulean

pLV-TRE-Ngn1-EYFP

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