Wrapup 2008-06-27

Priority

General

Plasmids

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

- [2] Measure O.D. 48.94ng/ul
- [2] Ligate – coming out at 6pm and 9am
- [3] Transform – signal to noise 1:1 (tabletop) and 1:2 (16 hour)
- [3] Miniprep
- [2] Design restriction digest (pick AscI and BsmBI) – expect bands at 8315 and 3553.
- [3] Restriction digest with xxx and yyy
- [1] PCR Ngn1-EYFP-2A-mKate (did not trust)
- [1] PCR SOEings of Ngn1 with EYFP-2A-mKate, Ngn1-EYFP with EYFP-2A-mKate, Ngn1-EYFP with mKate
- [1] Run gel - good bands!
- [1] Gel extract
- [1] O.D. - 117.2470ng/ul; 260/280 2.4508
- [1] Sequence new Ngn1 – Designed and ordered.

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.
- [3] Digest with BamHI and MluI
- [3] Run on gel – make sure you have the right ladder and supercoiled ladder and undigested.
- [1] Give some to Cil; sequence some.
- [1] Design sequencing primers.
- [1] Order primers.
- [1] Sequencing

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 now.
- [3] Set up for midiprep tonight 11pm [Navin] – comes out at noon
- [3] Midiprep – O.D.'s 266 and 415ng/ul
- [2] Design sequencing primers
- [1] Sequence
- [3] Restriction map with MluI – expect bands at 6386 and 3933.

pFUGW

- [3] Make more pFUGW – transformed

[3] Maxiprep

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

[1] Buy PKMz

pLV-pPKMz-EGFP-Ubc-Bla

[1] Design Plasmid

[1] Buy the promoter (PKMz)

pLV-Hef1a-LacO-Mash1-Cerulean-Ubc-Hyg

[1] PCR SOEing to make the plasmid

[1] determine what the backbone's going to be – Hef1a/LacO or Ubc-Hyg

pLV-TRE-Ngn1-EYFP-Ubc-Bla

[1] PCR Ngn1

[1] Run gel

[1] Extract

pLV-NeuronalPromoter-Neuronal specific CFR

??

Vector NTI Stuff

Learn how to design sequencing primers for TRE-Sox17-Ubc-Bla and pLV-TRE-Ngn1-EYFP-2A-mKate-Ubc-Bla

What we have in the database / load database

Look over primer design for Ngn1-EYFP

Put primer design manual in public folder

Design PCR SOEing primers for pLV-Hef1a-LacO-Mash1-Cerulean-Ubc-Hyg

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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