Wrapup 2008-07-08

Priority

General

Debugging

Plasmids

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

- [3] Minipreps – 25 from each plate (50 total)
- [2] O.D. – 4 – 108ng/ul; mostly 20-60ng/ul
- [2] Finish Restriction digests with AscI and BsmBI
- [2] Run restriction digest on gel for last 18. Restricted with Bsu36I; expect bands at ~3000 and ~9000bp.
- [1] Sequence new Ngn1 – Designed and ordered
- [1] Sequence insert – A22, A25, [David is taking it down tonight]
- [1] Sequence B14 – print papers; Andrew will take them over to LTL tonight.
- [3] Midiprep (with smaller elution volume) A6, B4, A22, A23 (Transform, Grow, Midiprep) – Navin will grow tonight
- [3] Midiprep B14, B18 - transform
- [2] Restriction digest with Bsu36I (the ones that looked promising but hadn't been digested with Bsu36I yet) (waiting for midipreps)
- [2] Design another restriction map

pLV-TRE-Sox17-Ubc-Bla

- [1] Sequencing (use existing primers)

pLV-Ubc-rtTA-2A-Bla

- [1] Sequence (wait for primers)
- [3] Restriction map with MluI – expect bands at 6386 and 3933 – gel is running.
- [3] Restriction digest and map again with remaining minipreps
- [1] Find a single-cut enzyme - PacI
- [1] Digest
- [1] restriction map
- Bad sequencing results.
- [2] PCR purify 20ug
- [1] Design new sequencing primers
- [1] Sequence the PCR purified DNA

pFUGW

- [3] Make more pFUGW – transformed
- [3] Maxiprep – O.D. = 250ng/ul

p148

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

p149

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

pLV-TRE-PKMz-Ubc-Hyg

Wrapup 2008-07-08 2 of 3

[Cil] Request gene from lab X

[1] Design plasmid

[1] Buy PKMz

pLV-pPKMz-EGFP-Ubc-Bla

[Cil and Team #1] PCR PKMz gene and promoter out of chromosome

[1] Design Plasmid

[1] Buy the promoter (PKMz)

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

[1] PCR SOEing to make the plasmid – add Ubc-Hyg to PCR SOEing

Design SOEing primers for Mash1-Cerulean-Ubc-Hyg

[1] The backbone's going to be Hef1a/LacO

pLV-TRE-Ngn1-EYFP-Ubc-Bla

[1] Gradient PCR EYFP by itself from the original plasmid [Eric and Caroline Jr.]

[1] Run gel

[3] Transform and midiprep pEYFP – set up for midiprep tonight

[1] Design digest (50-250 bp on either side of EYFP) and digest pEYFP with (5') HindIII or BsaXI and (3') SpeI or BsmBI

[1] PCR EYFP

[1] PCR Ngn1

[1] SOE Ngn1-EYFP.

[1] Run gel

[1] Extract

pLV-NeuronalPromoter-Neuronal specific CFR

??

Vector NTI Stuff

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

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