Wrapup 2008-07-14

Possible Plasmids:

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

A6 Midiprepped, PCR purified, waiting for forward and middle sequence; 68 ng/ul Bad restriction map; give up!

B4 Midiprepped, PCR purified, waiting for forward and middle sequence; 45 ng/ul

A22 Maxiprepped: Ngn1 sequencing successful! ; waiting for sequence

A23 Midiprepped, PCR purify today; 62 ng/ul, taken for sequencing.

A25 (sequenced) Excess DNA????

B14 midiprepped 53ng/ul, Eric took down for sequencing.

B18 midiprepped 97ng/ul, Eric took down for sequencing.

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]
- [3] Midiprep (with smaller elution volume) A6 (54ng/ul), B4 (17ng/ul)
- [2] PCR purify maximum volume of A6 (68 ng/ul), B4 (45 ng/ul) that fits into a single tube [100 or 120ul?], elute with 40ul
- Molly and Eric will find a good concentrator to use and will be very kind to the head of said lab so that we can use it the rest of the summer.
- [1] Sequence A6, B4, rtTA [Molly]
- [1] A22 sequence of Ngn1 successful by BLAST; ALIGNX verification in Vector NTI
- [3] Maxiprep A22 – O.D. 120ng/ul
- [1] Sequence A22 Maxiprep properly
- [1] Design/order correct sequencing primer (instead of UBC forward, order a reverse primer)
- [1] Order another reverse primer for EYFP
- [3] Grow and midiprep A23 (152ng/ul), B4 (54ng/ul)
- [1] if sequences look good (or we can't tell), use the rest of the sequencing primers.
- [3] Midiprep B14 (53ng/ul), B18 (97ng/ul)
- [2] PCR purify B14 (978ng/ul), B18 (635ng/ul), A23 (195.5ng/ul)
- [1] Sequence B14, B18 – taken down.
- [2] Restriction digest A6, B4 with Bsu36I – B4 looked like it might be good; NOT A6.

pLV-TRE-Sox17-Ubc-Bla

- [1] Sequencing (use existing primers)

pLV-Ubc-rtTA-2A-Bla

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[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 or 120ul, whichever is larger

[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

[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] Order primers

[1] PCR Mash1, Cerulean, Ubc, and Hyg (get Cerulean from Patrick, rest from Cil)

[1] Extract Mash1, Hyg, Ubc

[1] PCR Mash1, Hygro

[1] Run Mash1 and Hygro on gel

[1] Extract Mash1 and Hygro [Eric]

[1] Gradient PCR for Cerulean, Ubc

[1] Run Cerulean on gel

[1] Gel extract Cerulean [Eric]

[1] Run Ubc on gel

[1] Gel extract

[1] PCR SOEing to make the plasmid [Molly and Eric start tonight]

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 (360ng/ul)

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[1] Design digest (50-250 bp on either side of EYFP) and digest pEYFP with (5') HindIII [37C] and (3') BsmBI [55C]

[1] PCR EYFP

[1] PCR Ngn1

[1] Extract Ngn1

[1] Extract EYFP

[1] SOE Ngn1-EYFP [Molly and Eric start tonight]

[1] Run gel

[1] Extract

[2] Check that we still have digested parent vector (pLV-TRE-Sox17-Ubc-Bla w/ SfiI)

[2] Digest Ngn1-EYFP with SfiI

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