Wrapup 2008-07-16

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

End of week – clean out boxes, come up with a system to keep track of where everything is.

Complete Genes.xls – antibodies, ordering, sources

Dyes for action potentials; dyes vs. marker spectra (check for toxicity)

Design plasmids that do not have fluorescent reporters

Design assays for serotonergic neurons

Request PKMz

Design learning (Thursday)

Debugging

Plasmids

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

- [1] if sequences look good (or we can't tell), use the rest of the sequencing primers.
- [1] EYFP mid reverse sequence A22
- [MDL] Plasmid transfection (read about it!)
- [MDL] Ask Cil about transfecting cells with rtTA and this plasmid to see if we see colors
- [MDL] Virus production
- [MDL] Virus harvesting
- [MDL] Infection

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

Wrapup 2008-07-16

[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] PCR SOEing to make the plasmid – we did MC, CU, UH, MCU, CUH, MCUH

[1] Run gel on PCR SOEing products

[1] Gel extract

[1] O.D.

[1] Additional PCR SOEing if necessary: MC+UH, MCU+H, M+CUH, MCU+CUH

[1] Run gel on MCUH SOEing products

[1] Gel extract MCUH

[1] O.D. – 14 and 16ng/ul

[2] Digest parent vector pLV-Hef1a/LacO-IRES2-DsRed2 with SfiI and BstEII

[2] Digest MCUH with SfiI and BstEII – digests coming out at 6:15

[2] PCR purify digested insert and parent vector

[2] Ligate MCUH into Hef1a/LacO backbone – to be set up tonight – two-hour table-top ligation and overnight ligation

[3] Transform

pLV-TRE-Ngn1-EYFP-Ubc-Bla

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

[1] Run gel

[1] Extract – 41ng/ul

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

[2] Digest Ngn1-EYFP with SfiI

Wrapup 2008-07-16 3 of 3

[2] PCR purify

[2] Ligate Ngn1-EYFP into pLV-TRE-Sox17-Ubc-Bla-SfiI

[3] Transform – done by 11

[3] Grow for miniprep – ask Ron and Cil how many minipreps to do

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

Lenti: pLV-TRE-Nkx2.2-IRES2-EGFP (already made)

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

pLV-TRE-Nkx2.2-IRES2-EGFP – thaw infected cells, find out if they become serotonergic

Grow, induce, and infect TRE-Mash1-IRES2-EGFP cells with pLV-TRE-Nkx2.2-IRES2-EGFP.

Change EGFP to mKate