Wrapup 2008-07-17

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

Order: Nurr1, D5R, VIAAT, Lbx1, HCN2, GABAR

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)

Buy filters after verifying dye

Buy cell lines (neural stem cell lines)

Chemical that allows us to see action potentials

Exogenous neurotransmitters to test receptors

Design more options for B1, B2

Start working on surface patterning

Model

Debugging

Plasmids

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

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[1] if sequences look good (or we can't tell), use the rest of the sequencing primers.

[1] EYFP mid reverse sequence A22

pLV-TRE-Sox17-Ubc-Bla

[1] Sequencing (use existing primers)

pLV-Ubc-rtTA-2A-Bla

Check status [Navin]

pFUGW

[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

This Mash1 has a stop codon!

[3] Grow for minipreps this evening, coming out at 11pm-Evan – 25 from table top, ask how many from overnight

[3] Miniprep

[3] Design Restriction map

[3] Digest with xxx and yyy

[3] Restriction map

[1] Order correct primer

pLV-TRE-Ngn1-EYFP-Ubc-Bla

[3] Transform

[3] Grow for miniprep – 24 minipreps

[2] Design restriction map-PstI

[3] Miniprep- awesome- avg 200 OD

[3] Restriction map- Cut with PstI- gel is running

[David and/or Caroline] Design new restriction map

[3] Digest and restriction map the ones with the lower double band

pLV-Ubc-rtTA-2A-LacI/Krab-IRES-Puro

[Cil or Patrick] check construction

[David] order primers by noon

pLV-Hef1a/LacO-Mash1-GFP-Ubc-Hyg

[Cil or Patrick] check construction

[David] order primers by noon

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pLV-Hef1a/LacO-Mash1-Ubc-Hyg

- [Cil or Patrick] check construction
- [David] order primers by noon

pLV-TRE-Lbx1-Ubc-Puro

- [Cil or Patrick] check construction
- [David] order primers by noon

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

- [MDL] Plasmid transfection (read about it!)
- [MDL] Virus production
- [MDL] Virus harvesting

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

Experiments

Experiment 1: AINV cell type (contains rtTA-Puro), pLV-TRE-Ngn1-EYFP-2A-mKate-Ubc-Bla

- [MDL] Transfect AINV cells with pLV-TRE-Ngn1-EYFP-2A-mKate-Ubc-Bla
- [MDL] Induce with Dox
- [ACK] Observe for fluorescence using microscope
- [ACK] Take images; show during wrap-in

Experiment 2: AINV cell type (contains rtTA-Puro), pLV-TRE-Ngn1-EYFP-2A-mKate-Ubc-Bla

- [MDL] Infect cells
- Grow
- Add Dox
- Make movie
- 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