Wrapup 2008-07-21

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, DRD5, 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

Primers here

rtTA-2A_rev_LacIKrabOverlap_2008-07-14

Wrapup 2008-07-21 2 of 4

rtTA-2A_fwd_sfiI_Kzk_2008-07-14

LacI/Krab_fwd_rtTA-2A-overlap_2008-07-14

LacI/Krab_rev_stop_SfiI_2008-07-14

S-Ngn1EYFPmKate_midrev_2008-07-11

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 – take down tonight

pLV-TRE-Sox17-Ubc-Bla

[1] Sequencing (use existing primers)

pLV-Ubc-rtTA-2A-Bla

For now, stop working on this.

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 – good OD's 70-100 on average

[3] Design Restriction map

[3] Digested 20 samples with Bsu36I (order more)

[3] Restriction map – run on gel and take picture – five tries looked promising

[3] Re-label tubes and lab notebook files

[1] Sequence the five promising ones

[1] Design / find sequencing primers (ordered)

[1] Order correct primer

pLV-TRE-Ngn1-EYFP-Ubc-Bla

[3] Transform

[3] Grow for miniprep – 24 minipreps

Wrapup 2008-07-21 3 of 4

[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 - FspI

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

[1] Sequence four of the tries that look good – used s-TRE-fwd-2008-06-27 and s-Sox17-rev-2008-06-27

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

[David] order primers by noon

[1] PCR rtTA and LacI/Krab

[1] Ask Patrick for rtTA

[1] PCR SOEing of rtTA with LacI/Krab

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

[David] order primers

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

[David] order primers

pLV-Hef1a/LacO-Mash1-Ubc-Hyg

[David] order primers by noon

pLV-TRE-Lbx1-Ubc-Puro

[David] order primers by noon

pLV-TRE-Lbx1-2A-GLRA1-2A-DRD5-Ubc-Bla

Primers ordered

pLV-TRE-Cav3.1-Ubc-Bla

pLV-TRE-HCN2-Ubc-Bla ? (possibly different selection)

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)

Hardware

Optical tweezers

Missing parts: camera, metal plate, loaded computer (what to load?)

Put together, check about overheating – get Anatolli to help

Get glasses from Craig Arnold

Wrapup 2008-07-21 4 of 4

Surface patterning

E-mail sent to Sigurd Wagner

[Hamza and Evan] – how to get cells to stick to gold?

Experiments

Experiment 1: 293 cell type, pLV-TRE-Ngn1-EYFP-2A-mKate-Ubc-Bla (nonviral)

[MDL] Transfect 293 cells with pLV-TRE-Ngn1-EYFP-2A-mKate-Ubc-Bla and TetON plasmid

[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 (viral)

[MDL] Infect cells 9:30

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