Wrapup 2008-07-22

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; antibodies, chemicals, cells – Sent e-mails for DRD5, Nurr1, Helt

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

rtTA-2A_fwd_sfiI_Kzk_2008-07-14

Wrapup 2008-07-22 2 of 4

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

pLV-TRE-Sox17-Ubc-Bla

[1] Sequencing (use existing primers)

pLV-Ubc-rtTA-2A-Bla

[3] Restriction map again – MluI and NdeI (separately)

BAD.

Stop trying. Just do rtTA-LacI/Krab-Bla.

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

Buy PKMz

[1] Design plasmid

[1] Order primers

[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

Wrapup 2008-07-22

- [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 - 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] Run gel
- [1] Gel extract – O.D.'s 20ng/ul, 30ng/ul
- [1] PCR SOEing of rtTA with LacI/Krab
- [1] Run gel
- [1] Gel extract
- [1] Look for Ubc-IRES-Bla in Sairam's box – box 401 # 58
- [2] Digest insert with SfiI
- [2] Digest vector with SfiI
- [2] CIP

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

- GLRA1 is here! Figure out how it was inserted into the backbone. [David and Junior]
- [3] Midiprep GLRA1- incubating, pick up at 8:30am [Lena]
- Primers ordered

pLV-Hef1a/LacO-Cav3.1-Ubc-Hyg

- Cav3.1 is here! Figure out how it was inserted into the backbone. [David and Junior]
- [3] Midiprep Cav3.1 – incubating, pick up at 8:30am [Lena]

pLV-TRE-HCN2-Ubc-Bla

pLV-Hef1a/LacO-ChAT-Ubc-Puro

- ChAT is here! Figure out how it was inserted into the backbone. [David and Junior]
- [3] Midiprep ChAT – incubating, pick up at 8:30am [Lena]

pLV-NeuronalPromoter-Neuronal specific CFR

Wrapup 2008-07-22 4 of 4

??

Vector NTI Stuff

What we have in the database / load database

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: metal plate, load computer with proper software

Put together, check about overheating – get Anatolli to help

Get glasses – find out whether Steve has ordered them.

Tomorrow – look for metal plate, if not then order one.

Let Anatolli know when we have everything; set up.

Surface patterning

E-mail sent to Sigurd Wagner

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

Talk to Cil about ordering dendrimers

Sterilize test patterns

Experiments

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

[MDL] Infect cells 10pm 7-21

Grow

Replace media

Add Dox

Image tomorrow

Make movie if we see differentiation

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