Wrapup 2008-07-25

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

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

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

LacI/Krab_rev_stop_SfiI_2008-07-14

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S-Ngn1EYFPmKate_midrev_2008-07-11

Plasmids

p148

[3] O.D. [~500 ng/uL]

p149

[3] Maxiprep [1330-1360 ng/ul] (Two O.D. tries)

pFUGW

[3] Maxiprep – O.D. = 250ng/ul

pLV-TRE-Sox17-Ubc-Bla

[1] Sequencing (use existing primers)

pLV-Ubc-rtTA-2A-Bla

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

BAD. (the insert appears to be missing altogether)

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

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

The A22 maxiprep sequence is a perfect match!

pLV-TRE-Ngn1-EYFP-Ubc-Bla

[3] Miniprep- awesome- avg 200 OD

[1] Sequence four of the tries that look good

[1] Analyze sequencing data

PACEMAKER

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

pLV-Hef1a/LacO-Nurr1-Ubc-Hyg

[D] Design

Nurr1 here!

[3] Transform Nurr1

[3] Grow

[3] Midiprep

pLV-Hef1a/LacO-Nurr1-2A-Mash1-Ubc-Hyg

[D] Design

pLV-pLux-ChAT-Ubc-Zeo

ChAT is here! Figure out how it was inserted into the backbone. [David and Junior]

[3] incubating, pick up at 8:30am [Lena]

[3] Set up for midiprep growth – Navin taking out at 11pm

[3] Midiprep – bad, redo.

[3] Grow ChAT for midiprep

[3] Spin, freeze in -20 tomorrow [Navin]

[3] midiprep

[David] Design

pLV-Hef1a/LacO-Mash1-Ubc-Hyg

[David] order primers by noon

[1] PCR SOE Mash1-Ubc-Hyg

[1] Run gel

[1] Extract

[2] Digest vector and insert

pLV-Hef1a/LacO-Mash1-2A-ChAT-Ubc-Hyg

[David] Design

pLV-TRE-HCN2-Ubc-Bla

[efinkels] Contacted possible source

pLV-TRE-Cav3.1-Ubc-Bleo

Cav3.1 is here! Figure out how it was inserted into the backbone. [David and Junior]

[3] incubating, pick up at 8:30am [Lena]

[3] Set up for midiprep growth Navin taking out at 11pm

[3] Midiprep – bad, redo

[3] Grow ChAT for midiprep

[3] Spin, freeze in -20 tomorrow [Navin]

[3] midiprep

pLV-TRE-Cav3.1 [no selection]

[D] Design

B1 and B2

pLV-Ubc-rtTA-2A-LacI/Krab-IRES2-Bla

[1] PCR SOEing of rtTA with LacI/Krab

[1] Run gel

[1] Gel extract – O.D. = 16ng/ul

[1] Retrieve Ubc-IRES2-Bla in Sairam's box – box 401 # 58

[2] Digest insert with SfiI

[2] Digest vector with SfiI

[2] Run gel for vector

[2] Gel extract vector – OD = 20ng/ul

[2] CIP

[2] PCR purify vector and insert [Hamza] – O.D. 12 and 3 ng/ul

[2] Ligate – only enough DNA for overnight ligation

[3] Transform

[3] Grow for mini- or midi-preps (find out!)

[1] PCR SOE rtTA with LacI/Krab again

[1] Run gel

[1] Gel extract

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

[David] order primers

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

[1] PCR SOE Mash1-Cer-Ubc-Hyg

[1] Run gel

[1] Extract

[2] Digest vector and insert

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

[David] order primers

pLV-Hef1a/LacO-Mash1-Ubc-Hyg

[1] Design

[1] PCR Mash1

[1] Run on gel

[1] Gel extract – OD = 41

[1] PCR SOE Mash1-Ubc-Hyg

[1] Run on gel

[1] Extract

pLV-Hef1a/LacO-Ngn1-Ubc-Hyg

[David] Design

B1

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] pick up at 8:30am [Lena]

[3] Set up for midiprep growth Navin taking out at 11pm: pellet, label, store in -20C

[3] Midiprep

[3] Grow ChAT for midiprep

[3] Spin, freeze in -20 tomorrow [Navin]

[3] midiprep

Primers ordered

DRD5 on its way…

pLV-TRE-Lbx1-Ubc-Bla

[David] order primers by noon

pLV-TRE-GLRA1-Ubc-Zeo

[D] Design

pLV-TRE-D5R-Ubc-Bleo

[D] Design

B2

pLV-TRE-GLYT2-2A-VIAAT-2A-D5R-IRES-[GABAR]-Ubc-Bla

pLV-TRE-GLYT2-2A-VIAAT-Ubc-Bla

pLV-TRE-[GABAR]-Ubc-Zeo

pLV-TRE-D5R-Ubc-Bleo [see B1]

Learning

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-TRE-PKMz-Ubc-Hyg

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Buy PKMz

[1] Design plasmid

[1] Order primers

[1] Buy PKMz

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: load computer with proper software

Put together, check about overheating – Euan will be back sometime Monday

List of missing parts: e-mailed to Euan

Get glasses – we'll know after meeting with Steve Elwood Monday morning

E-mail Craig Arnold to set up

Surface patterning

E-mail sent to Sigurd Wagner

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

Sterilize test patterns

Order dendrimers

First test patterns received – circles and squares

Learn pattern design on AutoCAD or L-Edit [Evan]

Experiments

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

[MDK] Infect cells 10pm 7-21

Grow

Replace media

Add Dox

Image

[ALC] Make movie with +Dox cells if undifferentiated

Observe differentiation

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

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

Order BsiWI