Wrapup 2008-08-05

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

Design assays for serotonergic neurons

Buy filters after verifying dye

Buy cell lines (neural stem cell lines)

Exogenous neurotransmitters to test receptors

Design more options for B1, B2

Model

Debugging

Genes that have come in: (blue = have designs for; yellow = need to come up with plasmid designs)

Tlx3(Glutamatergic), Neuregulin1(AChR), Ngn2(Glutamatergic), gephyrin(scaffold for GlyR, GABAR), DRD5, ChAT, Cav3.1, GLRA1, Nurr1

Genes that are coming in:

Helt, Lbx1 (send them an annoying reminder email), Send HCN2 another annoying email,

Plasmids

p148

[3] Maxiprep; OD: ~500 ng/ul

p149

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

pFUGW

[3] Maxiprep; OD: 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] Maxiprep; OD: 680 ng/ul
- [3] Restriction map maxiprep??
- [MKD] Make virus – tomorrow morning
- [MKD] harvest virus – after 40 hours
- [MKD] infect cells

pLV-TRE-Mash1-IRES-mKate

- [D] Design and order primers
- [1] PCR Mash1-IRES and mKate - redo
- [1] Run gel of mKate
- [1] Gel extract
- [1] PCR SOE Mash1-IRES + mKate
- [1] Run gel
- [1] Extract
- [2] Digest vector (pLV-TRE-Mash1-IRES2-EGFP) and insert

Gephyrin

- [3] Midiprep; OD: 12ng/ul

Neuregulin1

- [3] Midiprep; OD: 70ng/ul

Tlx3

- [3] Midiprep; OD: 170ng/ul

Ngn2

- [3] Midiprep; OD: 307ng/ul

PACEMAKER

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

- [David] Design primers not dependent on IRES-Bla plasmid

pLV-Hef1a/LacO-Nurr1-Ubc-Hyg

- [3] Midiprep Nurr1; OD: 98ng/ul
- [2] Restriction map Nurr1 plasmid – run on gel today
- [1] PCR
- [1] Run gel
- [1] Gel extract
- [3] Restriction digest or check to see that this has been done.
- [3] Run on gel – check to see if this has been done.
- Re-make backbone

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

- [D] Design and order
- [1] PCR Mash1 and Nurr1
- [1] Run gel
- [1] Gel extract
- [1] SOE Nurr1 to MashI

[1] Run on gel

[1] Gel extract

[2] Digest pLV-Hef1a/LacO-MashI-Cerulean-Ubc-Hyg (waiting on plasmid) with SfiI and XcmI

[2] Digest Nurr1-2A-MashI with SfiI and XcmI

[2] Ligate

[3] Miniprep; ODs in chart

[3] Restriction Digest

pLV-pLux-ChAT-Ubc-Neo

[3] Midiprep ChAT; OD: 51ng/ul

Put annotated plasmid sequence into Vector NTI

[1] PCR ChAT out of plasmid

[1] Run gel

[1] Gel extract; OD: 36.3ng/ul

[3] If PCR doesn't work - Restriction digest and map – call Open BioSystems tomorrow morning to determine where the gene is in the vector

[David] Design – for pLux, use pMinCMVLux07 (get from Sairam's box – Box 403-position79. Check the other ones in Sairam stock).

[1] PCR ChAT, Ubc, Neo – running now

pLV-Hef1a/LacO-Mash1-Ubc-Hyg

[1] Extract MUH; OD: 47ng/ul

[2] Digest vector and insert with BstEII and SfiI

[2] Digest new vector – serial digest (PCR purify between enzymes)– somebody check vector map and figure out ideal order of enzymes

[2] Run serially digested vector on gel

[2] Gel extract

[2] CIP

[2] PCR purify insert and vector

[2] Ligate table-top (Try 2) - out at 11am

[3] Transform Try 1 Retransform

[3] Grow for miniprep with the few colonies that did show.

[3] Minipreps

[3] Restriction map with Bsu36I – expect bands at 3913 and 8332.

[3] Transform new ligations – coming out at 8pm

Investigate backbone

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

[1] Gel extract MEUH – OD 32 ng/ul

[2] Digest with SfiI

[2] PCR Purify

[2] Digest with BstEII

[2] Ligate

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

[David] Design and order

[1] PCR

[1] Run on gel

[1] Gel extract

[1] PCR SOE Mash1-2A-ChAT

[1] Run on gel

[1] Extract

VECTOR IS pLV-Hef1a/LacO-Mash1-Cerulean-Ubc-Hyg– digest tomorrow with SfiI and BstBI, after miniprep by team 3

[2] Digest Mash1-Cerulean backbone with SfiI and BstBI – bad.

[2] Digest vector again – serial digest (PCR purify between enzymes) – and run on gel and extract

[2] Run digested vector on gel

[2] CIP

[2] PCR purify vector and insert

[D] Design restriction map

pLV-Hef1a/LacO-Ngn2-Ubc-Hyg

[1] PCR Ngn2 – waiting on primers

pLV-Hef1a/LacO-Tlx3-Ubc-Hyg

[1] PCR Tlx3 – waiting on primers

pLV-TRE-HCN2-Ubc-Bla

[efinkels] Contact possible source – waiting for answer

pLV-TRE-Cav3.1-Ubc-Bleo

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

[3] Grow Cav3.1 for midiprep

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

[3] Midiprep; OD: 847 ng/ul

[3] Restriction digest and map – run gel today

[1] PCR

[1] Run on gel

[1] Gel extract; OD: 9.3 ng/ul

[1] Re-PCR

[1] Run gel

[1] Extract

[1] Cut out Cav3.1 with EcoRV (or EcoRI) and NotI

[David] Design and order

pLV-TRE-Cav3.1 [no selection]

[D] Design – order primers

B1 and B2

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

[3] Minipreps

[3] Restriction map – digest with NdeI – not pretty ☹

[3] Grow more colonies and controls

[3] Minipreps

[3] Restriction digest with BsiWI – happening now but waiting for more enzyme

[3] Restriction map – yuck.

[3] Design another restriction map – BsaBI (Bands at 8921 and 2578)

[3] Restriction map parent vector as well (not miniprepped DNA)

[1] PCR SOE rtTA-2A-LacI/Krab with DMSO, in addition to redoing the SOEing again with Betaine as normal – DMSO worked!

[1] Run on gel

[1] Gel extract; OD: 41.6 ng/ul

[2] Digest

[2] Digest more vector if need be

[2] Run on gel-gel bad, start over

Investigating backbone

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

[David] Design primers not dependent on IRES-Bla plasmid

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

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

[1] Run gel

[1] Extract; OD: 12.5ng/ul

[2] Ligate

[3] Transform

[3] Grow for minipreps

[3] Minipreps

[all] Restriction map with Bsu36I – expect bands at 4630 and 8332.

[1] Re-SOE and PCR

[1] Run on gel

[1] Gel extract

[2] Digest vector and insert (because low OD)

[2] Digest more insert if need be

[2] Ligate- waiting on sequencing to determine if necessary

[1] Sequence 2-12 – sent in

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

[David] order primers

pLV-Hef1a/LacO-Ngn1-Ubc-Hyg

[David] Design and order

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] midiprep – 647ng/ul

[3] Restriction digest and map – happening now.

[1] Cut out GLRA1 with EcoRI

[1] Waiting on primers for GLRA1

DRD5 is here!

[3] Transform DRD5 – add 50-100ul solution

[3] Grow DRD5

[3] Midiprep DRD5; OD: ~130

[D] Primers ordered

[1] PCR GLRA1 and DRD5

[Andrew] ask for tracking number for Lbx1

pLV-TRE-Lbx1-Ubc-Bla

[David] order primers by noon

pLV-TRE-GLRA1-Ubc-Neo

[D] Design and order

[1] PCR GLRA1, Ubc, Neo – running now.

pLV-TRE-D5R-Ubc-Bleo

[D] Design and order

[1] PCR DRD5

[1] Run gel

[1] Gel extract

B2

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

Find GABAR gene – Ask Elisabeth Gould from Neuroscience Institute [Andrew] – try to get in touch with a postdoc. Walk over and knock on door.

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

[David] Design and order

pLV-TRE-[GABAR]-Ubc-Neo

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

Think about synthesizing the promoter pPKMz

[1] Design Plasmid

[1] Buy the promoter (PKMz)

pLV-TRE-PKMz-Ubc-Hyg

Buy PKCz

[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, It does overheat, so that's what Craig Arnold and Anatolli are working on right now.

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

Microscope issue

See if it's set up in their lab

Surface patterning

E-mail sent to Sigurd Wagner

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

Wrapup 2008-08-05

Sterilize test patterns

Order dendrimers

First test patterns received – circles and squares

Learned pattern design on L-Edit

Contact Oliver about building masks

Evan made them.

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

[ALC] start another movie – four-day

[ALC] Find molarity of Dox

[ALC] See if movie's still in focus

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.