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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

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

Tlx3, Neuregulin1, Ngn2, gephyrin, 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] 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

[3] Transform

[3] Growing – take out at midnight

[3] Maxiprep – 680 ng/ul

[3] Restriction map maxiprep??

[MKD] Make virus – ask Cil when

[MKD] harvest virus – after 40 hours

[MKD] infect cells

pLV-TRE-Mash1-IRES-mKate – Ask Why (David knows and needs to share)

[D] Design and order primers

[1] PCR Mash1-IRES and mKate – setting up now.

[1] PCR SOE Mash1-IRES + mKate

PACEMAKER

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

[D] Design and order

Waiting on pLV-Ubc-rtTA-2A-LacI/Krab-Ubc-Bla

pLV-Hef1a/LacO-Nurr1-Ubc-Hyg

[3] Midiprep Nurr1 – OD = 98ng/ul

[2] Restriction map Nurr1 plasmid

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[1] PCR – set up now.

[3] Restriction digest or check to see that this has been done.

[3] run on gel – check to see if this has been done.

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

[D] Design and order

[1] PCR Mash1 and Nurr1 – being set up now

[1] SOE Nurr1 to MashI

[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

[3] Restriction Digest

pLV-pLux-ChAT-Ubc-Neo

[3] midiprep ChAT – 51ng/ul

Put annotated plasmid sequence into Vector NTI

[1] PCR ChAT out of plasmid – being set up now.

[1] Cut out ChAT with EcoRI – happening now just in case

[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).

[Cil] Buy Neo, potentially with other useful things on the plasmid

pLV-Hef1a/LacO-Mash1-Ubc-Hyg

[David] order primers by noon

[1] PCR SOE Mash1-Ubc-Hyg

[1] Run gel

[1] Extract – 47ng/ul

[2] Digest vector and insert with BstEII and SfiI – vector came out bad

[2] Run digested vector on gel

[2] Digest new vector – serial digest (PCR purify between enzymes) – second half of digest comes out at 6:30 – 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)

[3] Transform Try 1 Retransform

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

[3] Minipreps

[3] Restriction map – design!

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

[1] PCR Mash1, EGFP

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

[1] Run gel

[1] Gel extract – got MEU and EUH but not MEUH

[1] PCR SOE to get MEUH

[1] Run gel

[1] Gel extract – OD 24

[1] Redo best PCR SOEing of MEUH [higher volume - double]

[1] Run gel

[1] Gel extract

[2] Digest with SfiI

[2] PCR Purify

[2] Digest with BstEII

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

[David] Design and order

[1] PCR – being set up now.

[1] Run on gel

[1] Gel extract

[1] PCR SOE Mash1-2A-ChAT

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-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 – 847 ng/ul

[3] Restriction digest and map

[1] PCR – being set up now

[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

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

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[1] PCR SOE rtTA with LacI/Krab again

[1] Run gel

[1] Gel extract

[2] Digest insert – coming out at 5:45

[2] PCR purify insert [Hamza]

[2] Ligate [Hamza]

[3] Transform

[3] Grow for minipreps

[1] PCR SOE rtTA-2A-LacI/Krab

[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

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

[David] order primers – waiting for Bla

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

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

[1] Run gel

[1] Extract - 12.5ng/ul

[2] Ligate

[3] Transform

[3] Grow for minipreps

[3] Minipreps

[1] Re-SOE and PCR

[1] Run on gel

[1] Gel extract

[2] Digest vector and insert (because low OD) – halfway done [Hamza]

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

DRD5 is here!

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

[3] Grow DRD5

[3] Midiprep DRD5 – OD ~130

Primers ordered

Andrew ask for tracking number for Lbx1

pLV-TRE-Lbx1-Ubc-Bla

[David] order primers by noon

pLV-TRE-GLRA1-Ubc-Neo

[D] Design

pLV-TRE-D5R-Ubc-Bleo

[D] Design

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

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

Surface patterning

E-mail sent to Sigurd Wagner

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

Sterilize test patterns

Order dendrimers

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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

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.