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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

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

pLV-TRE-Mash1-IRES-mKate – Ask Why

[D] Design and order primers

[1] PCR Mash1-IRES and mKate – waiting on mKate reverse primer

[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

[D] Design

Nurr1 here!

[3] Transform Nurr1

[3] Grow – done at 6pm

[3] Midiprep – OD = 98ng/ul

[1] PCR – waiting on primers

[3] Restriction digest

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

[D] Design and order

pLV-pLux-ChAT-Ubc-Neo

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

[3] Grow ChAT for midiprep

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

[3] midiprep – 51ng/ul

Put annotated plasmid sequence into Vector NTI

[1] PCR ChAT out of plasmid – waiting on primers

[1] If PCR doesn't work - Cut out ChAT with EcoRI

[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

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

[2] Run digested vector on gel

[2] Gel extract

[2] CIP

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

[2] PCR purify insert and vector

[2] Ligate table-top (Try 2)

[3] Transform Try 1

[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

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

[David] Design and order

[1] PCR – waiting on primers

[1] Run on gel

[1] Gel extract

[1] PCR SOE Mash1-2A-ChAT

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

[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

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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] 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
- [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] Run on gel
- [1] Gel extract

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

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[1] Gel extract

[2] Digest vector and insert

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

[1] Cut out GLRA1 with EcoRI

DRD5 is here!

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

[3] Grow DRD5

[3] Midiprep DRD5

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

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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

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.