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Possible Plasmids:

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

A22 Maxiprepped: **sequencing successful!**

pLV-TRE-Ngn1-EYFP-Ubc-Bla

Successfully sequenced and Maxiprepped

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)

Trx3(Glutamatergic), **Neuregulin1**(AChR), **Ngn2**(Glutamatergic), **gephyrin**(scaffold for GlyR, GABAR), **DRD5**, **ChAT**, **Cav3.1**, **GLRA1**, **Nurr1**, **Lbx1**

Genes that are coming in:

Helt, **Send HCN2 another annoying email**

Plasmids

p148

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

[3] Maxiprep OD 1395 ng/ul

p149

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

[3] Maxiprep OD 180 ng/ul

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.

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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

[MKD] Make virus – Friday noonish

[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) with EcoRI and BsrGI – 2nd try still in

[2] Run 2 vectors on gel

[2] Gel extract two tries vector

[2] CIP vector

[2] PCR Purify vector

[2] Digest insert with EcoRI and BsiWI 2nd half of dig still going

[2] PCR purify

[2] Ligate

Gephyrin

[3] Midiprep; OD: 12ng/ul

[3] Restriction map

[1] PCR

Neuregulin1

[3] Midiprep; OD: 70ng/ul

[3] Restriction map

[1] PCR

[1] Run on gel

[1] Extract

Tlx3

[3] Midiprep; OD: 170ng/ul

[3] Restriction map

[1] PCR

[1] Run on gel

[1] Extract

Ngn2

[3] Midiprep; OD: 307ng/ul

[3] Restriction map

[1] PCR

[1] Run on gel

[1] Extract

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GlyT2

[3] Transform

[3] Midiprep

[3] Restriction map

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

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

[D] Design and order

[1] PCR Mash1 and Nurr1

[3] Miniprep Nurr1; ODs in chart

[3] Restriction Digest Nurr1

[1] Run gel on Nurr1

[1] Gel extract

[1] SOE Nurr1 to Mash1

[1] Run on gel

[1] Gel extract

[2] Digest pLV-Hef1a/LacO-Mash1-Cerulean-Ubc-Hyg (waiting on plasmid) with SfiI and XcmI

[2] Digest Nurr1-2A-Mash1 with SfiI and XcmI

[2] Ligate

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

[1] Run on gel

[1] Extract

[1] SOE

[1] Run SOEing on gel

[1] Extract

[2] Digest

pLV-Hef1a/LacO-Mash1-Ubc-Hyg

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- [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 signal to noise 1.5:1
- [3] 18 minipreps
- Investigate backbone
- [3] Maxiprep Ubc-IRES-Bla – retransform – find 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
- [3] Transform and grow [Navin, 1:30am]
- [3] Miniprep
- [3] Restriction Map

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] Digest Insert – 2nd half of digest out at 7pm
- [2] PCR purify vector and insert
- [D] Design restriction map

pLV-Hef1a/LacO-Ngn2-Ubc-Hyg

- [1] PCR Ngn2
- [1] Run on gel
- [1] Extract

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pLV-Hef1a/LacO-Tlx3-Ubc-Hyg

[1] PCR Tlx3

[1] Run on gel

[1] Extract

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

[1] PCR

[1] Run on gel

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

[1] Re-PCR

[1] Run gel

[1] Extract Cav3.1 and Ubc

[1] SOE

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

pLV-TRE-Cav3.1 [no selection]

[1] PCR Cav3.1

[1] Run on gel

[1] Gel extract

[2] Digest backbone (pLV-TRE-IRES2-EGFP) with EcoRI and BsrGI

[2] Gel Extract backbone

[2] CIP

[2] Digest Cav3.1 with EcoRI and BsiWI - 2nd half of digest out at around 7pm

[2] PCR purify CIPed vector and insert

[2] Ligate

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 miniprep 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

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[2] Digest

[2] Digest more vector if need be

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

[3] Restriction digest and run on gel – bad.

Investigating backbone

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

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

[1] PCRs

[1] Run on gel – rtTA, LacI/Krab-IRES, Puro

[1] Extract – rtTA, LacI/Krab-IRES, Puro

[1] SOE

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 and vector (Hef1a with Ngn3)

[2] Gel Extract vector

[2] Cip vector

[2] PCR Purify vector

[2] Digest insert MCUH

[2] PCR Purify MCUH insert

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

[1] Sequence 2-12 – sent in

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

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[3] Midiprep DRD5; OD: ~130

[D] Primers ordered

[1] PCR GLRA1 and DRD5

Lbx1 is here!

[3] Transform Lbx1

[3] Grow Lbx1

[3] Midiprep Lbx1

[1] PCR Lbx1 out of plasmid

pLV-TRE-Lbx1-Ubc-Bla

[David] order primers by noon

pLV-TRE-GLRA1-Ubc-Neo

[D] Design and order

[1] PCR GLRA1, Ubc, Neo

[1] Run GLRA1 on gel

[1] Extract

[1] SOE GLRA1+Ubc+Neo

pLV-TRE-D5R-Ubc-Bleo

[D] Design and order

[1] PCR DRD5

[1] Run gel

[1] Gel extract

[1] SOE D5R+Ubc+Bleo

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 post-doc. 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

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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?](#)

[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](#)

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.

Received genetic dyes and exogenous dyes from Sam Wang's lab – test them.