Wrapup 2008-08-08

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)

- Tlx3(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
- [2] Run 2 vectors on gel
- [2] Gel extract two tries vector
- [2] CIP vector
- [2] PCR Purify vector
- [2] Run on gel
- [2] Digest insert with EcoRI and BsiWI 2nd half of dig still going
- [2] PCR purify
- [2] Ligate table-top

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

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-MashI-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 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

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

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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 signal to noise 1.5:1
- [3] 18 minipreps
- Investigate backbone
- [3] Maxiprep Ubc-IRES-Bla – retransform

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

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 instert

[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 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

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- [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
- [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 Pufiy 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

[3] Midiprep DRD5; OD: ~130

[D] Primers ordered

[1] PCR GLRA1 and DRD5

Lbz1 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.