Wrapup 2008-08-13

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

T-shirt design “coming along nicely”, according to Evan.

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.

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

[3] Miniprep – good OD's!

[3] Restriction map – digest with Cla1 and BbvCI

Gephyrin

[3] Midiprep; OD: 12ng/ul

[3] Restriction map

[1] PCR

[1] Run gel

[1] Extract

Neuregulin1

[3] Midiprep; OD: 70ng/ul

[3] Restriction map

[1] PCR

[1] Run on gel

[1] Extract [OD: 35 ng/ul]

Tlx3

[3] Midiprep; OD: 170ng/ul

[3] Restriction map

[1] PCR

[1] Re-PCR

[1] Run on gel

[1] Extract [OD: 59 ng/ul]

Ngn2

[3] Midiprep; OD: 307ng/ul

[3] Restriction map

[1] PCR

[1] Run on gel

[1] Extract OD: 28.75

GlyT2

[3] Transform

[3] Midiprep growth culture

[3] Grow cells for 3x minipreps

[3] 3xMiniprep

[3] Restriction map

PACEMAKER

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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
- [3] Restriction digest
- [1] PCR
- [1] Run gel
- [1] Gel extract
- [2] Digest Nurr1 and backbone with SfiI and XcmI
- [2] Run backbone on gel
- [2] Gel extract
- [2] PCR Purify Nurr1
- [2] Ligate

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-EGFP-Ubc-Hyg with SfiI and XcmI
- [2] Digest Mash1-2A-Nurr1 with SfiI and XcmI
- [2] Run backbone on gel
- [2] Gel extract
- [2] PCR Purify Mash1-2A-Nurr1
- [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 ChAT-Ubc+Neo and Ubc+Neo
- [1] Run SOEings 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

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
- [3] Miniprep
- [3] Restriction Map – Looks good.
- [3] Transform promising sample.
- [3] Midiprep 4 samples
- [3] 3x miniprep
- [1] Sequence the 4 samples

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

[2] Digest Ngn2 and backbone with SfiI and XcmI

pLV-Hef1a/LacO-Tlx3-Ubc-Hyg

[1] PCR Tlx3

[1] Run on gel

[1] Extract

[2] Digest Tlx3 and backbone with SfiI and XcmI

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

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

[3] Transform

[3] Miniprep – good ODs!

[3] Miniprep 12 more

[3] Restriction Digest with xxx and yyy

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

[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] Gradient PCR Puro and LacI/Krab-IRES

[1] Digest pLV-Ubc-Puro to get Puro out

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

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

[1] SOE

May be a primer issue

[3] RMap pLV-Ubc-Puro to ensure correct sequence. [May require sequencing]

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 – OD 26

[2] Digest vector

[2] Gel Extract vector

[2] Cip vector

[2] PCR Purify vector

[2] Digest insert MCUH

[2] PCR Purify MCUH insert

[2] Ligate

[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

[3] midiprep – 647ng/ul

[3] Restriction digest and map

[3] Midiprep DRD5; OD: ~130

[D] Primers ordered

[1] PCR GLRA1 and DRD5

[1] Gel extract

Lbx1 is here!

[3] Transform Lbx1

[3] Grow Lbx1

[3] Midiprep Lbx1 – 59 ng/ul

[1] PCR Lbx1 out of plasmid

[1] Gel extract

[1] SOE

pLV-TRE-Lbx1-Ubc-Bla

[David] order primers by noon

[1] PCR Lbx1

pLV-TRE-GLRA1-Ubc-Neo

[D] Design and order

[1] PCR GLRA1, Ubc, Neo

[1] Run GLRA1 on gel

[1] Extract

[1] ReSOE 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

[2] Digest vector and insert

[2] Ligate

[3] Transform

[3] Minipreps

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

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[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?

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

Experiment 3: AINV cell type, pLV-TRE-Ngn1-EYFP-Ubc-Bla

[MDK] Infect cells

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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.