Wrapup 2008-07-01

Priority

General

Debugging

Plasmids

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

- [2] Measure O.D. 48.94ng/ul
- [2] Ligate – coming out at 6pm and 9am
- [3] Transform – signal to noise 1:1 (tabletop) and 1:2 (16 hour)
- [3] Miniprep – O.D.'s 20-30ng/ul
- [2] Design restriction digest (pick AscI and BsmBI) – expect bands at 8315 and 3553.
- [3] Restriction digest with AscI and BsmBI
- [3] Run on gel.
- [1] PCR Ngn1-EYFP-2A-mKate (did not trust)
- [1] PCR SOEings of Ngn1 with EYFP-2A-mKate, Ngn1-EYFP with EYFP-2A-mKate, Ngn1-EYFP with mKate
- [1] Run gel - good bands!
- [1] Gel extract
- [1] O.D. - 117.2470ng/ul; 260/280 2.4508
- [2] Ligate Ngn1-EYFP-2A-mKate into pLV-TRE- - Ubc-Bla
- [3] Transform
- [3] Minipreps – 25 from each plate (50 total)
- [3] Restriction digest with AscI and BsmBI
- [1] Sequence new Ngn1 – Designed and ordered
- [1] Set up sequencing reaction for beginning and end

pLV-TRE-Sox17-Ubc-Bla

- [3] Grow two colonies overnight [Evan and Navin]
- [3] Midiprep. – O.D. 250 and 270ng/ul
- [3] Restriction map with BamHI and MluI; expect bands at 7498 and 3461.
- [3] Digest with BamHI and MluI
- [3] Run on gel – make sure you have the right ladder and supercoiled ladder and undigested.
- [1] Give some to Cil; sequence some.
- [1] Design sequencing primers.
- [1] Order primers.
- [1] Sequencing (use existing primers)

pLV-Ubc-rtTA-2A-Bla

- [3] Sunday Evan and Lena will pick 40 minipreps (10 from each plate) for growth.
- [3] Extract DNA
- [3] O.D. 30-40ng/ul
- [2] Design restriction map.
- [2 and 3] Restriction map – cut with MluI. Do as many as you can as soon as you can.
- [3] If you find a good one – transform today to maxiprep tomorrow
- [3] On the three decent-looking samples [7-2, 7-8, 7-6], transform now.

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[3] Set up for midiprep tonight 11pm [Navin] – comes out at noon

[3] Midiprep – O.D.'s 266 and 415ng/ul

[2] Design sequencing primers - ordered

[1] Sequence (wait for primers)

[3] Restriction map with MluI – expect bands at 6386 and 3933 – gel is running.

[3] Restriction digest and map again with remaining minipreps

[3] Find a single-cut enzyme and digest, restriction map

pFUGW

[3] Make more pFUGW – transformed

[3] Maxiprep – O.D. = 250ng/ul

p148

[3] O.D. [~500 ng/uL]

p149

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

pLV-TRE-PKMz-Ubc-Hyg

[Cil] Request gene from lab X

[1] Design plasmid

[1] Buy PKMz

pLV-pPKMz-EGFP-Ubc-Bla

[Cil and Team #1] PCR PKMz gene and promoter out of chromosome

[1] Design Plasmid

[1] Buy the promoter (PKMz)

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

[1] PCR SOEing to make the plasmid – add Ubc-Hyg to PCR SOEing

Design SOEing primers for Mash1-Cerulean-Ubc-Hyg

[1] The backbone's going to be Hef1a/LacO

pLV-TRE-Ngn1-EYFP-Ubc-Bla

[1] PCR Ngn1-EYFP (from Ngn1-EYFP and Ngn1-EYFP-mKate)

[1] Run gel

[1] Extract

pLV-NeuronalPromoter-Neuronal specific CFR

??

Vector NTI Stuff

What we have in the database / load database

Look over primer design for Ngn1-EYFP

Put primer design manual in public folder

Design PCR SOEing primers for pLV-Hef1a-LacO-Mash1-Cerulean-Ubc-Hyg

Lentivirus

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

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Experiments

Experiment: Ngn1-EYFP-2A-mKate

- Infect cells
- Add Dox
- Observe differentiation
- Make Yellow/Red artificial brains
- 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
- Start planning microfluidics and also the optical twizzlers
- Bio nanoforce