

# Wrapup 2008-06-30 1 of 3

Priority

General

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 – happening now.

[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 [tonight]

[3] Minipreps

[3] Restriction digest

[1] Sequence new Ngn1 – Designed and ordered, (sequence today with existing forward primer)

[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.

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

# Wrapup 2008-06-30 2 of 3

[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

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

[1] Design plasmid

[1] Buy PKMz

pLV-pPKMz-EGFP-Ubc-Bla

[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

# Wrapup 2008-06-30 3 of 3

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

## 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