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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)

[2] O.D. – 4 – 108ng/ul; mostly 20-60ng/ul

[2] Restriction digest with AscI and BsmBI – first 17 coming out at 6pm.

[2] Run restriction digest on gel [David will do that tonight]

[1] Sequence new Ngn1 – Designed and ordered

[1] Set up sequencing reaction for beginning and end

pLV-TRE-Sox17-Ubc-Bla

[1] Sequencing (use existing primers)

pLV-Ubc-rtTA-2A-Bla

[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

[1] Find a single-cut enzyme - PacI

[1] Digest

[1] restriction map [David will run gel tonight]

pFUGW

[3] Make more pFUGW – transformed

[3] Maxiprep – O.D. = 250ng/ul

p148

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

p149

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[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] Redesign reverse primer (David while waiting for gel tonight)

[1] Gradient 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

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