

Wrapup 2008-06-23 1 of 3

Priority

General

Plasmids

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

- [1] Sequence pLV-TRE-Ngn1-IRES2-EGFP. [Katia took them over – results will arrive by Friday.]
- [Cil] Look for another template (another tube of pLV-TRE-Ngn1-IRES2-EGFP).
- [1] If another tube of pLV-TRE-Ngn1-IRES2-EGFP is found, do another restriction digest with PacI, ClaI. [Molly will put digest in -20 at 6:20.]
- [1] Run the pLV-TRE-Ngn1-IRES2-EGFP digest with PacI and ClaI. (Accurate bands)
- [1] Gel extract (Bad OD)
- [3] Transform pLV-TRE-Ngn1-IRES2-EGFP; plate.
- [3] Midiprep one colony of pLV-TRE-Ngn1-IRES2-EGFP [No colonies, 8 hours was too few.]
- [1] Clarify the massive confusion with respect to Patrick's Ngn1 primers.
- [1] Use Patrick's primers and template. Run another PCR and use his PCR program. (The band should be around 870bp.)
- [1] If the PCR did work, gel extract, measure OD and then SOE. (David and Navin will tonight)
- [1] Set up Ngn1-EYFP-mKate PCR
- [1] Set up Ngn1-EYFP PCR (in case the Ngn1-EYFP-mKate construct does not work)
- [1] Run gel of SOEings
- [1] Gradient PCR [Molly will pick up at 9pm]
- [1] Run gel on gradient PCR products [come in at 10am tomorrow]
- [1] Extract both products (but only use the long one now).
- [1] O.D. 192 ng/ul for Ngn1-EYFP-2A-mKate; ~300ng/ul for Ngn1-EYFP
- [2] Insert digest with SfiI [comes out in 5min]
- [2] PCR purify insert digest.
- [2] Measure O.D.
- [1] Digest pLV-TRE-Sox17-Ubc-Bla with SfiI [double-check that]
- [1] Run vector digest on gel.
- [1] Redo digest – 10ug (or 20ug if we have it). [David will re-digest tonight].
- [1] Run on 1% agarose gel at a higher voltage (110V) for 2 hours on a longer gel (no middle combs). [try to get rid of u-shaped bands – ask VectorNTI]
- [1] Gel extract.
- [1] Measure O.D.
- [2] CIP vector digest
- [2] PCR purify vector digest CIP
- [2] Measure O.D.
- [2] Ligate
- [3] Transform

pLV-TRE-Sox17-Ubc-Bla

- [3] Maxiprep colony 2-1. [Negligible OD]
- [3] Grow two colonies overnight [Evan and Navin]
- [3] Midiprep.

pLV-Ubc-rtTA-2A-Bla

- [2] Digest pFUGW with EcoRI and AgeI

Wrapup 2008-06-23 2 of 3

[2] Run pFUGW-EcoRI-AgeI digest on gel.

[2] Gel Extraction did not work; had to be redone.

[2] Gel extract try 2; see what the OD is (10.17ng/ul).

[2] CIP pFUGW-AgeI-EcoRI

[2] PCR purify (8.x ng/ul)

[2] OD

[2] Ligate pFUGW-AgeI-EcoRI-CIP and rTA-2A-Bla-BspEI-EcoRI (take out tomorrow morning). 35ul of Ligation reactions (pFUGW-AgeI-EcoRI-CIP) thrown out by mistake, but still enough left over

[2] Run the pFUGW-AgeI-EcoRI digest again (3rd time) at least 20ug.

[2] CIP vector digest

[2] PCR purify vector digest CIP

[2] Measure O.D. – 90.17ng/ul

[2] Ligate – Cil will take out in the morning.

[3] Transform ligation into ultra-competent cells Signal-to-noise ratio 1:2 and 3:1 hundreds of colonies

[3] Saturday – Navin will transform today's pLV-Ubc-rTA-2A-Bla. Signal to noise ratio 1:2 ~55 colonies

[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.

[3] Restriction map – cut with MluI. Do as many as you can as soon as you can.

p148

[3] Transform p148. Grow overnight on plates (Evan). Got ~500 colonies.

[3] Setup for maxiprep – grow overnight in 400ml LB Amp.

[3] Maxiprep

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

[3] Trust in God that the sequence is correct (Pray?) (Donations?)

p149

[3] Transformed

[3] Pick colony

[3] Grow colony for maxiprep. Take out into +4C Wed. @ 8:30am (Navin)

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

[3] Trust in God that the sequence is correct (Pray?) (Donations?)

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-TRE-Mash1-EYFP-2A-mKate-Ubc-Bla (in same plasmid as Ngn1?)

Wrapup 2008-06-23 3 of 3

[1] Design plasmid [do this one first]

pLV-NeuronalPromoter-Neuronal specific CFR

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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