

# Wrapup 2008-06-12 1 of 2

Priority

General

Journal Club tomorrow: Molly and Evan chairing.

Caroline Jr. and Hamza will be presenting on Monday, when Ron and Cil are here.-

Plasmids

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

[1] PCR SOEing: Ngn1-EYFP-2A-mKate – Started PCR amplify for Ngn1, EYFP, mKate

[1] Redo PCR for Ngn1 [Eric will pick it up.]

[1] Run gel of PCR products

[1] Gel Stain / Gel Picture

[1] Gel Extract

[1] O.D.

[1] Extract PCR products from gel  
-- cut out gel for EYFP (12.6 ng/ul) and mKate (31.4 ng/ul)

[1] PCR together EYFP and mKate (store @-20C, David, 6:30pm)

[1] Run PCR product on gel [Analytical side and Sample side]

[1] Gel Picture/ Gel Stain

[1] Gel Extract

[1] O.D. (72.7 ng/ul)

pLV-TRE-Sox17-Ubc-Bla

[2] Pick colonies of pLV-TRE-Sox17-Ubc-Bla (tried 3 plates – 2 worked. 10 total, 5 from each of two plates)

[2] Grow 10 minipreps – Prepare growth cultures; 12

[2] Miniprep 10 cultures of pLV-TRE-Sox17-Ubc-Bla (5 surviving, 100 – 300 ng/ul)

[2] Restriction map pLV-TRE-Sox17-Ubc-Bla with PacI, NheI/BsiWI

[2] Run on gel

[2] Gel Picture [Add to lab notebook!]

[3] pLV-TRE-Sox17-Ubc-Bla Colony 6 – set up growth culture for [Ask Cil].

pLV-Ubc-rtTA-2A-Bla

[2] Ligate pFUGW-AgeI-EcoRI-CIP and rtTA-2A-Bla-BspEI-EcoRI

[2] Transformation of ligation. Grow overnight on plates (Andrew). Got ~50 colonies.

[3] Grow 12 minipreps of ligation plate. (Evan to 37C shaker)

[2] 11 Minipreps [1 casualty]

[2] O.D. [14.6-105.9 ng/uL] [3 more need to be taken tomorrow.]

[2] Restriction Map [Andrew will come and take it out.]

[2] Run on gel

[2] Gel picture

p148

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

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

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[3] Maxiprep

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

[3] Restriction Map

[3] Run on gel

[3] Gel picture

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] Design Restriction map

[3] Restriction Map

[3] Run on gel

[3] Gel picture

## 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