TITLE PROJECT

Continued from page

9/19 Repeat PCR dTAG 9/15 → Success

	1X
2x Phire	12.5
10μM Primer Mix	1
released DNA	1
dw	10.5
	25

9/20 Colony PCR pBA904 SOX9 g1, g2, g3, g4, NTCR3

	1X	25X
2x Phire MM	12.5	312.5
10μM each primer	1	25
OP05 OV268 Bacteria	1	~~25~~ -
dw	11	275
	25	

9/21 SOX9_g1 2nd RNP for DTAG: i) mCherry Cassette 1, 2, 3 } BFP+ cells; 4) BFP Cassette 1, 2, 3, 5), 6) } mCherry+ cells

Step 1: Prepare guide in MM → distribute to 8-strip

FOR#		1X	6X
50128934	crRNA (100μM) SOX9_g1	2	~~12~~
10731,91	tracRNA (100μM)	2	~~12~~

Incubate 95°C for 5min → Cool to 4°C

Step2: Prepare Cas9 RNP in 1.5-mL Eppendorf

	1X	6X
Into Cas9	2	12
PBS	3	18

- Add 5μL Cas9 into 4μL guide RNA per rxn
- Incubate Room Temp for 30 min

Continued to page

READ AND UNDERSTOOD BY:

SIGNATURE DATE SIGNATURE DATE

TITLE PROJECT

Continued from page

Step 3 Homology Cassette (bead purified PCR product)
1000 ng needed

	1X	3X	
mCherry Cassette (113ng/uL)	8.84	26.5	add to RNP mix
BFP cassette (149ng/uL)	6.71	20.1	divided into 2 tubes

Lonza Master Mix	1X	6.5X
P3 Solution	16.4 uL	106.6
Supplement SI solution	3.6 uL	23.4

Step 4 : Prepare Cells

- Add 2mL 0.25% Trypsin-EDTA to 10-cm HT29 (mCherry +/ BFP+)
- Quench in 6mL media
- Conc :
- Spin down 2min 400g _____ mL of cell suspension. Decant
  - ↳ need 0.5 mil cells / sample
    - 3 samples = 1.5 million cells BFP+
    - 3 samples = 1.5 million cells mCherry
- Resuspend in Lonza Master Mix (20uL / sample)
- Distribute into 8-strip containing RNP mix (3 each)
- Transfer to electrode container

Step 5: Lonza
- FF 139 program for HT-29 cell line on X-Unit

Step 6: Plate in 96-well plate
- Add 100uL media to well of electrode
- Transfer to 96-well
- Top off to 200uL per well

Continued to page

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SIGNATURE DATE SIGNATURE DATE