#Cell-Cell attack and Gene Transfer

##Testing Transformation in M9 with Controls

###Sep 21 2016

###Cell cultures

Attacker: CP2204 Rif-r Nov-s Sp-s. Stock 9/??/16. OD 0.2 BoxMorrison2016#1-20.

Victim: Cp2215 Nov-r Sp-r. Stock 9/??/16 OD 0.2 BoxMorrison2016#21-40

Thaw Stock cells @ 0C. Dilute for growth in CDM + 1%CAT 12mL/tube.

Media batch CAT: COMB, CDM+CHO+G:9/12/16

To CAT from stock shelf add phosphate and glucose. For 300 mL CAT add 10 mL 0.5 M K2HPO4 (sterile filtered) and 3 mL 20% glucose (sterile filtered).

cells	Cp2204	Cp2204
vol	250 uL	150 uL
tube	A	B
10:15	37C	37C
11:00	0.001	0.005
11:48	0.008	0.008
1:00	0.24	0.052
		RT
2:30	0.101	0.119

###Attack Reaction

Cell Prep:

Volume required per RX =	500
Inducer volume =	95
OD required =	2
Volume started =	12000
Grow to OD of:	0.1028806584
Resuspend in:	500
pippette into inducer:	405

Take 2 x 12 mL tubes of cell suspension and spin down at 8k rcf for 8 min in chilled incubator.

Resuspend and add to inducer

Load each strain in syringe

Reaction Scheme: When cells meet in droplet device each will be at OD = 1

Droplet generation was done with an ice pack on the syringes. The heated stage was used to monitor temperature which was about 28C The outlet tubing fed into a reaction tube in a heat block on ice. Droplets were generated for about 2.5 hours, and then the reaction tube was placed in a heat block at 37 C for 30 minutes.

reaction started at:

Inducer Prep:

# reactions	2		total volume in reaction	500		
Component	Stock	unit	Inducer	Dilute by	Working Stock	incu
CSP	250	ug/mL	0.2*	50	5	20
BSA	4	%	0.04	10	0.4	50
CaCl2	1	M	0.005	10	0.1	25
					total volume inducer:	95

**typically 0.1 ug/mL of CSP is used, it was doubled in this case to encourage transformation*

Preparing Working Stocks:

Working Stocks	Stock	M9	dH2O
CSP	25 uL	1 mL	-
BSA	100 uL	1 mL	-
CaCl2	100 uL	-	1 mL

Emlusion Breaking

Starting with 200 uL cells in emlusion add 500 uL CAT

Spin down for 30 seconds at 100 x g (in simple centrifuge at heidi's bench)

Pipette oil out from bottom

add two times volume of pico break and gently rock tube

spin again for 1 min at 100-1000 x g

pipette out top(cells) ~ 700 uL

Transfer to another tube and bring up to 2 mL with CAT

Incubate for 1 hour at 37 C in a heat block

Dilution Scheme

200 uL cell emulsion was transfered in to 2 mL CAT previously and incubated for 1 hour

150 uL pipetted into 1.5 mL CAT to make 10^-2, 3xRNS, 3xRS, 3xRN, one for further dilution

150 uL pipetted into 1.5 mL CAT to make 10^-3

150 uL pipetted into 1.5 mL CAT to make 10^-4

150 uL pipettd into 1.5 mL CAT to make 10^-5, 3xR, 3xS

Plates are filled with:

1. 3 mL CAT agar
2. 1.5 mL cells + 1.5 mL agar
3. 3 mL CAT agar
4. 3 mL Drug agar

Drug Assay Prep

Drug	overlay	Stock
R	40 ug/mL	20 mg/mL
N	10 ug/mL	10 mg/mL
S	160	100 mg/mL

Drug	overlay ug/mL	Stock mg/mL	Volume agar mL	pipette mL
R	0.04	20	15	0.03
N	0.01	10	15	0.015
S	0.16	100	15	0.024

###Results

Cell Counts Cells/mL

Plate	Dilution	Colonies/Squares	Magnification	Colonies
1	1.00E-02			0
1	1.00E-02			0
1	1.00E-02			0
1	1.00E-02			0
1	1.00E-02			0
1	1.00E-02			0
1	1.00E-02			0
1	1.00E-02			0
1	1.00E-02			0

1	1.00E-05			63
1	1.00E-05			53
1	1.00E-05			60
1	1.00E-05			9
1	1.00E-05			8
1	1.00E-05			11

Summary of Results by cell count (cells/mL)

RNS	RN	RS	R	S
0	0	0	3911111.111	622222.2222