Device flow through Cell count

How many cells make it through the droplet generating device and emulsion breaking

November 15 2016

One volume of cells is made into and emulsion with the device and compared to a control volume.

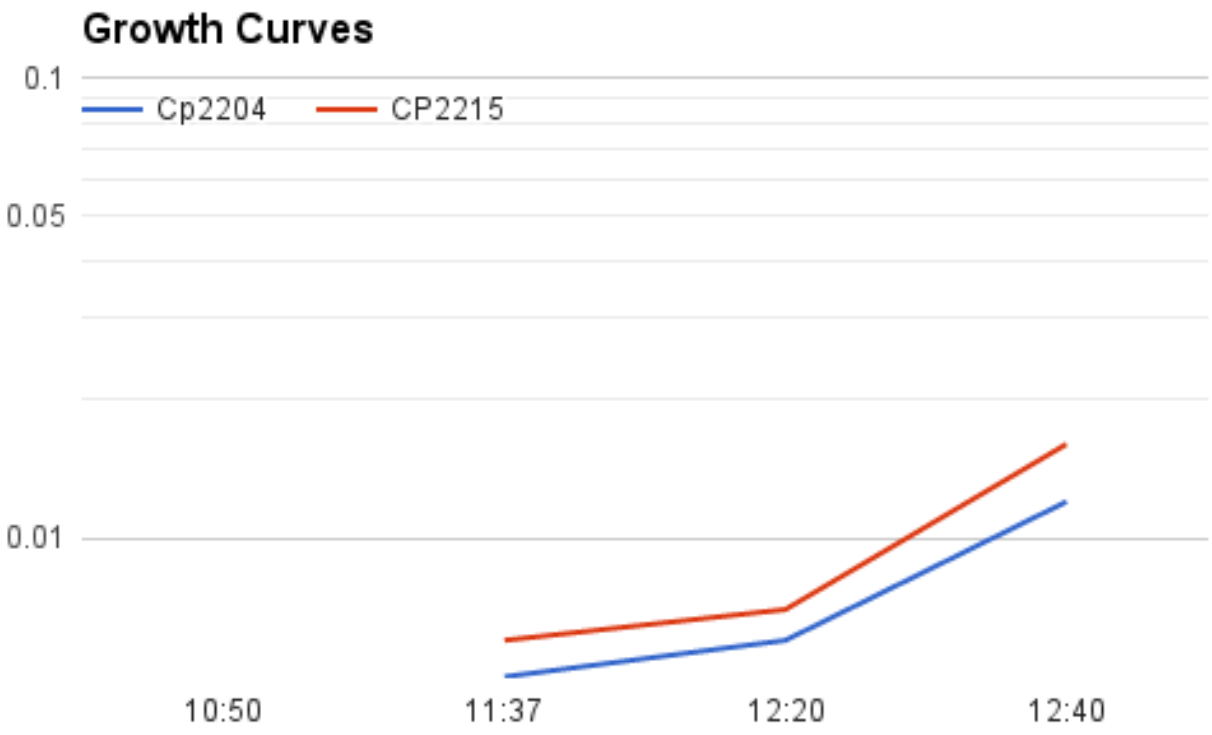
Cell cultures:

Strain	Competence	Rifampin	Spectinomycin	Novobiocin	Label
CP2204	inducible w CSP	Resistant	Sensitive	Sensitive	RFP
CP2215	non-competent	Sensitive	Resistant	Resistant	GFP

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

Grow to OD of 0.015 and chill.

Cp2204	CP2215
200 uL	20 uL
37C	37C
0.005	0.006
0.006	0.007
0.012	0.016
0C	0C



Attack Reaction

Cell Prep:

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

Pour off supernatant and resuspend in 0.45 mL M9 to make OD 0.4.

Combine 300 uL of each strain to make 600 uL cells each at OD 0.2.

Pull 500 uL of cell mix into syringe

Inducer Prep:

Produce 700 uL of double strength inducer in M9 and pull 500 uL into a syringe:

cell suspension volume to match	**700**					
Component	Stock	unit	Inducer	Dilute by	Working Stock	2x inducer
CSP	250	ug/mL	0.1	50	5	28

BSA	4	%	0.004*	10	0.4	14
CaCl2	1	M	0.005	10	0.1	70
M9						588

**typically 0.04 % of BSA is used, it was reduced to prevent fouling of hydrophobic device*

Preparing Working Stocks:

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

Reaction Scheme:

Droplet production was performed in the cold room.
When cells combine with inducer in droplet device they will be diluted to 0.1 each.
The remaining cell mix and inducer was kept in the cold room to be combined as a control.
After 100 uL was pumped through the device (resulting in about 200 uL of droplets in oil)
The emulsion and control reaction are placed in a heat block at 37C for 30 minutes

reaction started at:

Emlusion Breaking

Both reaction tubes were taken out and placed at room temperature while emulsion breaking was formed

1. Starting with 200 uL cells in emlusion add 500 uL CAT
2. Spin down for 30 seconds at 100 x g (in simple centrifuge at heidi's bench)
3. Pipette oil out from bottom
4. add two times volume of pico break and gently rock tube
5. spin again for 1 min at 100-1000 x g
6. pipette out top(cells) ~ 700 uL

Reactions ~200 uL were transfered to another tube tube and brought up to 2 mL with CAT

Reactions were incubated for 60 minutes at 37 C.

Dilution Scheme

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

RX	Dilution	Colonies	squares	squares/plate	colonies/plate	Drug	Cells/ml

1	1.00E-06				0	R	0.00E+00
1	1.00E-06				0	R	0.00E+00
1	1.00E-06				0	R	0.00E+00
1	1.00E-06				1	S	6.67E+05
1	1.00E-06				1	S	6.67E+05
1	1.00E-06				3	S	2.00E+06
1	1.00E-02				0	RS	0.00E+00
1	1.00E-02				0	RS	0.00E+00
1	1.00E-02				0	RS	0.00E+00
1	1.00E-02				0	RN	0.00E+00
1	1.00E-02				0	RN	0.00E+00
1	1.00E-02				0	RN	0.00E+00
1	1.00E-01				0	RNS	0.00E+00
1	1.00E-01				0	RNS	0.00E+00
1	1.00E-01				0	RNS	0.00E+00
2	1.00E-06				2	R	1.33E+06
2	1.00E-06				5	R	3.33E+06
2	1.00E-06				5	R	3.33E+06
2	1.00E-06				12	S	8.00E+06
2	1.00E-06				11	S	7.33E+06
2	1.00E-06				18	S	1.20E+07
2	1.00E-02				0	RS	0.00E+00
2	1.00E-02				0	RS	0.00E+00
2	1.00E-02				0	RS	0.00E+00

2	1.00E-02				0	RN	0.00E+00
2	1.00E-02				0	RN	0.00E+00
2	1.00E-02				0	RN	0.00E+00
2	1.00E-01				0	RNS	0.00E+00
2	1.00E-01				0	RNS	0.00E+00
2	1.00E-01				0	RNS	0.00E+00

Summary of Results by cell count (cells/mL)

rx	R	S	RS	RN	RNS	
emulsion	1	0.00E+00	1.11E+06	0	0	0
control	2	2.67E+06	9.11E+06	0	0	0

Discussion and Conclusion

Cell pellet didn't make it?
Holding at 4C in M9 kills cells?
M9 old?

Should I check droplet formation in newly coated devices with CDM or CAT?

Appendix:

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).