

11/25/14

Amp 166.6 $\mu\text{g}/\mu\text{l}$
Cephalothin 200 $\mu\text{g}/\mu\text{l}$
Ceftazidime 10 $\mu\text{g}/\mu\text{l}$

Only Amp

① 50 ml LB + 250 μl of 100 mM IPTG.

② 6 μl Amp in 3 ml of LB + kan + IPTG.

$$6 \mu\text{l} \times 166.6 \frac{\mu\text{g}}{\mu\text{l}} = 999.6 \mu\text{g} / 3 \text{ml}$$

(lib2, plate 1) *** = 333.2 $\mu\text{g}/\text{ml}$

Plate 1 W, A1, A3, A4, A5, A8, A10, A11

2 W, B2, B4, B7, B9, B10, B11, B12

3 W, C3, C4, C7, C9, C10, C11, C12

4 W, D4, D8, D11, D12, E1, E3, E4.

5 E5, E8, E10, E11, F1, ~~F3, F4, F6~~. B10 \rightarrow A10

6

A11
Amp

$$\frac{\text{IPTG}}{238}$$

$$m = \frac{\text{MW}}{1000}$$

$$= .1 \text{M} \times 238 \times \frac{1}{1000}$$

$$= \frac{23.8 \times 1}{1000}$$

$$= \frac{23.8 \times 6.43}{1000}$$

$$m = \frac{\text{MW}}{1000}$$

$$\frac{1539 \times 1000}{.1 \text{M} \times 238} = \frac{1539}{23.8}$$
$$= \underline{\underline{6.43 \text{ mJ}}}$$

Total 28 unique mutants out of 192 clones screened.

N366R
S338A
Y241W

L139E+Y241G
L139W+Y241W
L139W+Y241G
L139E+S338W
Y241G+S338A
Y241A+S338A
Y241W+N366R
Y241W+S338A

L139W+Y241A+N366R
L139W+Y241A+S338W
L139W+Y241A+N366R
L139W+Y241W+S338A
L139W+Y241G+S338A
L139W+Y241A+S338A
L139Y+S338A+N366R
L139Y+Y241W+S338W
L139Y+Y241A+S338A

L139E+Y241G+S338A+N366R
L139E+Y241W+S338A+N366R
L139W+Y241W+S338A+N366R
L139W+Y241A+S338A+N366R
L139Y+Y241W+S338W+N366R
L139Y+Y241A+S338W+N366Y
L139Y+Y241A+S338W+N366R
L139Y+Y241G+S338A+N366R

Exp date.
11/25/14

Library 2, Plate 1
#1

		Antibiotic: Amp		Date: 11/26/15									
Clone	Lane	1	2	3	4	5	6	7	8	9	10	11	12
WT	A	-	+	+	+	+	+	+	+	+	+	+	+
A1	B	-	-	-	-	-	-	+	+	+	+	+	+
A3	C	-	-	-	-	-	-	-	+	+	+	+	+
A4	D	-	-	-	-	-	-	-	+	+	+	+	+
A5	E	-	-	-	+	+	+	+	+	+	+	+	+
A8	F	-	-	-	-	-	-	-	+	+	+	+	+
A10	G	-	-	-	-	-	-	-	+	+	+	+	+
A11	H	-	-	-	+	+	+	+	+	+	+	+	+

333 166.5 83.2 41.6 20.8 10.4 5.2 2.6 1.3 .6 .3 .16

Exp date
11/25/14

Library 2, Plate 1
#2

		Antibiotic: Amp				Date: 11/26							
Clone	Lane	1	2	3	4	5	6	7	8	9	10	11	12
W	A	-	+	+	+	+	+	+	+	+	+	+	+
B2	B	-	-	-	-	-	-	-	+	+	+	+	+
B4	C	-	-	-	-	-	-	-	+	+	+	+	+
B7	D	-	-	-	-	-	-	-	+	+	+	+	+
B9	E	-	-	-	-	-	-	-	+	+	+	+	+
B10	F	-	-	-	-	+	+	+	+	+	+	+	+
B11	G	-	-	-	-	-	-	-	+	+	+	+	+
B12	H	-	-	-	-	-	-	-	+	+	+	+	+

333

166

83

41

20

10

5

2.5

1.5

0.6

.3

.15

Expp. data
11/25/14

Library 2, Plate 1
3

Antibiotic: Amp		Date: 11/26/15											
Clone	Lane	1	2	3	4	5	6	7	8	9	10	11	12
WT	A	-	+	+	+	+	+	+	+	+	+	+	+
C3	B	-	-	-	-	-	-	-	+	+	+	+	+
C4	C	-	-	-	+	+	+	+	+	+	+	+	+
C7	D	-	-	-	-	-	-	+	+	+	+	+	+
C9	E	-	-	-	-	-	-	-	+	+	+	+	+
C10	F	-	-	-	-	-	-	+	+	+	+	+	+
C11	G	-	-	-	-	-	-	-	+	+	+	+	+
C12	H	-	-	-	-	-	-	-	+	+	+	+	+

333 166 83 41 20 10 5 2.5 1.3 .6 .3 .15

Exp Date
11/25/14

Library 2, Plate 1
#4

		Antibiotic: Amp		Date: 11/26/14									
Clone	Lane	1	2	3	4	5	6	7	8	9	10	11	12
WT	A	-	+	+	+	+	+	+	+	+	+	+	+
D4	B	-	-	-	-	-	-	-	+	+	+	+	+
D8	C	-	-	-	-	-	-	-	+	+	+	+	+
D11	D	-	-	-	-	-	-	-	+	+	+	+	+
D12	E	-	-	-	-	+	+	+	+	+	+	+	+
E1	F	-	-	-	-	-	-	-	+	+	+	+	+
E3	G	-	-	-	-	-	-	-	+	+	+	+	+
E4	H	-	-	-	-	-	+	+	+	+	+	+	+

333 166 83 41 20 10 5 2.5 1.3 .6 .3 .15

EEP Date
11/25/14

Library 2, Plate 1
5.

		Antibiotic: Amp		Date: 11/26/14									
Clone	Lane	1	2	3	4	5	6	7	8	9	10	11	12
E5	A	-	-	-	-	-	-	-	+	+	+	+	+
E8	B	-	-	-	+	+	+	+	+	+	+	+	+
E10	C	-	-	-	-	-	-	-	+	+	+	+	+
E11	D	-	-	-	-	-	-	-	+	+	+	+	+
F1	E	-	-	-	-	-	-	-	+	+	+	+	+
	F												
	G												
	H												