Shew-E-poration

Experiment Date:

Source: (Myers & Myers, 1997; Gralnick Lab; Romine lab; Pacific Northwest National Labs, contact david.culley@pnl.gov)

Experimenter:

Assembled: 6/27/2012

Reagent	Details	Quantity
Electroporator	Lab	
0.1 cm electroporation cuvette		1 per rxn
Electrocompetent Shewanella frozen stock	See: Making Electrocompetent Shewy	50 µL
De-salted Plasmid DNA	(name)→	0.1-0.5 µg
Recovery media (SOC)		250 µL
LB agar plate	With antibiotic, if necessary	1 per rxn

Procedure:

Critical Steps:

- Pre-chill containers on ice:
  - 0.1 cm cuvette (keep in bag to minimize water from ice getting on it)

☐ **Thaw Plasmid on ice**

☐ **Thaw Electrocompetent Shewy on ice**

- Minimize time thawed

☐ **Add Plasmid mixture to thawed Shewy cells, mix GENTLY by stirring with pipette**

- 5- 50ng/transformation for plasmid isolated from E. coli and 5-50pg for plasmid isolated from MR-1

☐ **Incubate on ice for 2 minutes**

☐ **Transfer cells and DNA mixture to pre-chilled 0.1 cm electroporation cuvette**

☐ **Zap cells**

- Myers and Myers: Resistance, 200 ohms; capacitance, 25 µFD ; voltage, 0.55 kV

- Pacific Northwest Labs: 750V, 400ohms, 25µF

☐ **IMMEDIATELY pipette 250 µL of recovery media into the cuvette and mix GENTTLY by *microtriturating**

- Getting the cells into SOC and out of the cuvette as quickly as possible is very important for cell survival

☐ **Recover 1-2 hours at 30 °C (or room temp.) with gentle agitation**

- Longer is OK for plasmid transfers where replication isn't a problem. For determining transformation efficiency, knockouts or transposon mutagenesis, use the 1 hour recovery

☐ **Plate 35-150µl on selective media.**

- Use the minimal concentration of antibiotic needed to kill untransformed MR1- particularly important for low copy number plasmids

Antibiotic resistance in Shewella oneidensis MR-1 (determined with aerobic cultures):

Antibiotic:	concentration (ug/ml):	Growth at 18 hrs:
Control	0	Lawn
Kanamycin	50	0
	40	0
	30	0
	20	0
	10	~50 cfu
	5	Thin lawn
	2.5	Lawn
Gentamycin	15	0
	7.5	0
	3.75	~120 cfu
	1.875	Thin Lawn
Ampicillin**	500	Lawn
	200	Lawn
	100	Lawn
	50	Lawn
Chloramphenicol	40	0
	20	0
	10	Thin Lawn
Tetracycln	25	0
	12.5	0
	6.25	0
	3.125	0
	1.6	Lawn

*microtriturating: mixing by pipetting up and down 5-10 times, works best if pipetting >10% total volume

** Note on Ampicillin Resistance in Shewanella oneidensis

Although there are several candidate beta-lactamase genes present in the Shewanella oneidensis genome, MR-1 appears to be fairly susceptible to Ampicillin and Carbenicillin when plated at low cell densities. Little or no growth is observed for the first few days when the number of viable cells plated is kept below 6^4 cells/cm2 at Amp 100 or 1000/cm2 at Amp 50. Above these densities, increasing numbers of colonies were observed with increasing plating density.

From these results it appears that the resistance expressed by each cell is fairly weak and many cells must work together to inactivate enough Amp to allow growth. Therefore, the use of bla selection vectors in MR-1 appears to be possible for certain applications, (although titration of Amp concentration and plating density may be required).

Myers, C. R., & Myers, J. M. (1997). Replication of plasmids with the p15A origin in Shewanella putrefaciens MR-1. *Letters in applied microbiology*, *24*(3), 221-5. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9080705