

## Whole Plasmid PCR

### Materials

1. 1.5mL tubes + PCR tubes
2. PCR mix
3. PCR machine
4. Pipets

### Protocol

1. Prepare PCR mix
2. Add PCR mix to tubes with template and primers
3. Place PCR tubes in PCR machine
4. Set PCR conditions + run

## Whole Plasmid Cloning Recipe

Ingredients	1x	10x*
10x PFX Amp. Buffer	3 uL	30 uL
dNTP (10uM)	0.9 uL	9 uL
MgSO <sub>4</sub> (50uM)	0.6 uL	6 uL
DMSO	1.2 uL	12 uL
Platinum PFX	0.6 uL	6 uL
Plasmid (30ng)	X	10x
Megaprimers (225,450,900 nM)	Y	
dH <sub>2</sub> O	Z	
Total Volume	30 uL	

\* 3 genes x 3 concentrations + 1 (-) = 10 reactions

\* 6.3 + X

\* X = 0.5 uL (in this case)

\* Y = 225/primer concentration 450/[con.], etc.

\* Z = 30 - (X + Y)

\* Mix = 10x PFX Amp. Buffer + dNTP (10uM) + MgSO<sub>4</sub> (50uM) + DMSO + Platinum PFX

### PCR Mix + Primer Concentrations

Megaprimer & concentration	Mix + X (6.3 + X) X = 0.5 uL	Primer (Y) uL	Z = dH <sub>2</sub> O
M 225	6.8	4.33	18.87
M 450	6.8	8.65	14.55
M 900	6.8	17.3	5.9
G 225	6.8	3.74	19.46
G450	6.8	7.48	15.72
G 900	6.8	14.95	8.25
M 225	6.8	4.47	18.73
M450	6.8	8.95	14.25
M 900	6.8	17.9	5.3
Control	6.8	none	23.8

### WP PCR Conditions

94°C	1:00 min
95°C	0:30 min
55°C	0:30 min
68°C	7:00 min
68°C	10:00 min
10°C	HOLD