

## General PCR SOP

Chemical	Volume ( $\mu\text{L}$ )
LaTaq Master mix	12.5
p1 (100 mM)	<del>0.5</del> 1 <i>uL</i>
p2 (100 mM)	<del>0.5</del> 1 <i>uL</i>
Template*	2 (for colony), 100 ng for PCR from plasmid/DNA only
Water	<del>0.5</del> water until total = 25 <i>uL</i>
Total	25

- If the template is plasmid, the concentration should be 10-15 ng/ $\mu\text{L}$ .

Step #	# of cycles	Temperature	Time
Step 1	(1 cycle)	94°C	5 min for colony PCR, 2 min for DNA
Step 2.1	(30 cycle)	$T_d = 94^\circ\text{C}$	30 sec
Step 2.2	(30 cycle)	$T_a = \text{Construct Dependent}$	30 sec (30 sec per kilobase)
Step 2.2	(30 cycle)	$T_{\text{ext}} = 72^\circ\text{C}$	2 min
Step 3	(1 cycle)	72°C	7 min