Desaturase genes PCR Protocol:

(a). Using TaKaRa Ex Taq™ kit

1. Prepare 50 µl reaction in 0.5 ml PCR tube on ice

	Volume	Final concentration
TaKaRa Ex Taq (5 units/µl)	0.25µl	0.025 units
10X Ex Taq Buffer	5µl	1X
dNTP Mixture (2.5 mM each)	4µl	200µM
DNA template	Depend on concentration	±300ng
Forward Primer (10x)	1µl	1X
Reverse Primer (10x)	1µl	1X
Sterilized distilled water	Up to 50µl	

2. Mix gently by vortex and briefly centrifuge to collect all components to the bottom of the tube.
3. Transfer PCR tubes from ice to a PCR machine and then run with the following PCR profile

	Temperature	Time
Denaturation	98℃	3 minutes
30 cycles	98℃	30 seconds
	Depend on type of primer*	30 seconds
	72℃	2.5 mins
Final extension	72℃	10 minutes
Storage	15℃	∞

* For iGEM VF2 and VR primers, annealing temperature is 56 ℃;
for edited VF2 and VR primers (with more annealing sequences), the temperature is 61 ℃; for newly designed primers (with more annealing and RBS sequences), the temperature is 62 ℃.