Protocol 1-5: PCR

General Protocol

1. Standard PCR

1.1 PCR with Pyrobest DNA polymerase

PCR System			
Reagent	Concentration/Activity	Volume (50uL System)	Volume (100uL System)
10x Pyrobest buffer II	10x	5	10
Pyrobest		0.3	0.5
dNTPmix	10mM each	1	2
Primer 1	10uM	1	2
Primer 2	10um	1	2
Template DNA	changeable	0.5	1
MgCl2(Deletable)	0.2M	0.5	1
ddH2O		40.5	81

(Pyrobest DNA polymerase from Takara Co.Ltd.)

PCR Program		
Step	Condition	Time
1	95℃	5min
2	95℃	30sec
3	[Tm(fu)-4]℃	30sec
4	72℃	DNA length/kb/min
5	RETURN TO STEP 2	30-35 cycles
6	72℃	10min
7	4℃	HOLD

1.2 PCR with Hifi Taq SuperMix

PCR System			
Reagent	Concentration/Activity	Volume (50uL System)	Volume (20uL System)
Super Mix	2x	25	10
Primer 1	10uM	1	0.5
Primer 2	10um	1	0.5
Template DNA	changeable	0.5	0.5
ddH2O		22.5	8

PCR Program		
Step	Condition	Time
1	94℃	5min
2	94℃	30sec
3	[Tm(fu)-4]℃	30sec
4	72℃	DNA length/kb/min
5	RETURN TO STEP 2	30-35 cycles
6	72℃	10min
7	4℃	HOLD

2. Fusion PCR

The basic system is similar to common PCR. There are some notes to raise the fusion efficiency:

a. Complementary region length: 15-20bp

b. Raise the annealing temperature in the fusion step.

Fusion PCR Program		
Step	Condition	Time
1	95℃	5min
2	95℃	30-50sec
3	{Tm(fu)+[(-2)~5]}℃	40-80sec

4	72℃	DNA length/kb/min
5	RETURN TO STEP 2	10-15 cycles
6	72℃	5min
7	Add amplification Primers	
8	95℃	2-5min
9	95℃	30sec
10	[Tm(fu)-4]℃	30sec
11	72℃	DNA length/kb/min
12	RETURN TO STEP 2	25-30 cycles
13	72℃	10min
14	4℃	HOLD