

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°C	5min
2	95°C	30sec
3	[Tm(fu)-4]°C	30sec
4	72°C	DNA length/ kb/min
5	RETURN TO STEP 2	30-35 cycles
6	72°C	10min
7	4°C	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°C	5min
2	94°C	30sec
3	[Tm(fu)-4]°C	30sec
4	72°C	DNA length/kb/min
5	RETURN TO STEP 2	30-35 cycles
6	72°C	10min
7	4°C	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°C	5min
2	95°C	30-50sec
3	{Tm(fu)+[(-2)~5]}°C	40-80sec

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

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