PCR using PfuUltra II Fusion HS DNA Polymerase

Revised on July 8, 2011

1. Add the following components to a microcentrifuge tube:

Components	**Final Concentration**	**Volume**
H_2O	Not applicable	42.25 µl
10X PfuUltra II Reaction Buffer	1X	5 µl
10 mM dNTP mixture	0.25 mM each	1.25 µl
Forward primer (40 pmol/µl, 40 µM)	0.2 µM	0.25 µl
Reverse primer (40 pmol/µl, 40 µM)	0.2 µM	0.25 µl
PfuUltra II Fusion HS DNA Polymerase	N/A	1 µl
Total reaction volume	N/A	50 µl

   If desired, a master mix can be prepared for multiple reactions, to minimize reagent loss and to enable accurate pipetting.
2. Add templates (5~100 ng) and mix contents of the tubes.
3. Cap the tubes and centrifuge briefly to collect the contents.
4. Incubate tubes in a thermal cycler at 95°C for 2 min to completely denature the template.
5. Perform 12-30 cycles of PCR amplification as follows:

Denature	95°C for 20 sec
Anneal	Tm-5°C for 20 sec
Extend	72°C for 15 sec per kb
6. Followed by a final incubation of 72°C for 3 min.
7. Maintain the reaction at 4°C after cycling. The PCR products can be stored at -20°C until use.
8. Analyze 2 µl of the products by agarose gel electrophoresis and visualize by ethidium bromide staining. Use appropriate molecular weight standards.

Touchdown PCR

1. Carry out PCR using the following reaction conditions (program HMTDOWN):

Cycle	Temp.	Time	
1:	95°C	2 min	
2:	95°C	20 sec	
3:	Tm*[1] + 3°C	20 sec	-0.5°C per cycle
4:	72°C	15 sec/kb	Go to 2, 19 times
5:	95°C	20 sec	
6:	Tm*[2] - 5°C	20 sec	
7:	72°C	15 sec/kb	Go to 5, 19 times
8:	72°C	3 min	
9:	4°C	forever	

   *[1] Tm of the primer, which has higher Tm than another

   *[2] Tm of the primer, which has lower Tm than another