

- PrimeSTAR™ HS DNA Polymerase origin
- Super - thermophilic - bacteria
(Blunt - End !)
- 1. (fidelity)
 2. pfu
 3. priming
 4. Library cloning
 5. 가 Hot Start PCR

- cDNA high fidelity PCR
user
- high fidelity
.
- : Human genome 8.5kbp, *E.coli* genome
10kbp, λ DNA 20kbp

PCR

(Total 50µl)

Human Genome DNA
 5 ng 500 ng (< 500 ng)
 λDNA 10 pg 10 ng
 E.coli Genome DNA
 100 pg 100 ng
 Plasmid 10 pg 1 ng

5×PS Buffer	10 µl
2.5mM dNTP Mixture	4 µl
Primer 1	0.2 0.4µM (final conc.)
Primer 2	0.2 0.4µM (final conc.)
Template	< 500ng
PrimeSTAR DNA Polymerase (2.5U/µl)	0.5µl
D.D.W	up to 50µl

PCR

98	10sec	] 30cycles	98	10sec	] 30cycles
55	<u>5,15 sec</u>		68	1Kbp/min	
72	1Kbp/min				

or

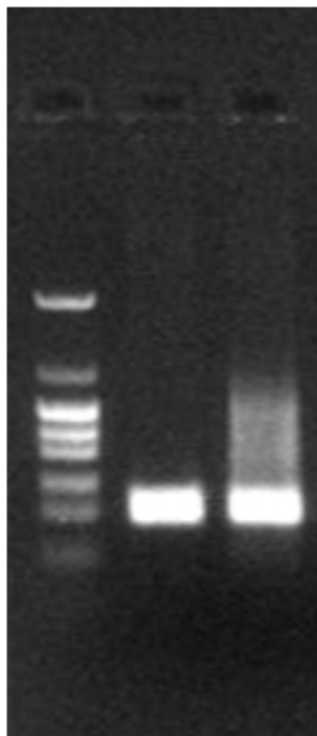
)	98	5	10sec	.
94	15sec	30sec	.	

- : PrimeSTAR™ HS DNA Polymerase Taq 가
 , Taq PCR .
- Annealing temp : 55 .
- Annealing time : (primer 가 25 mer)
- Tm value ≥ 55 : 5 sec
- Tm value < 55 : 15 sec
- : primer 가 25 mer
- - 5 sec
- annealing time smear .
- Tm value (): $2(NA+NT)+4(NC+NG)-5$
- : Tm value 가 70 2 step

Q&A : Annealing Temp & Time

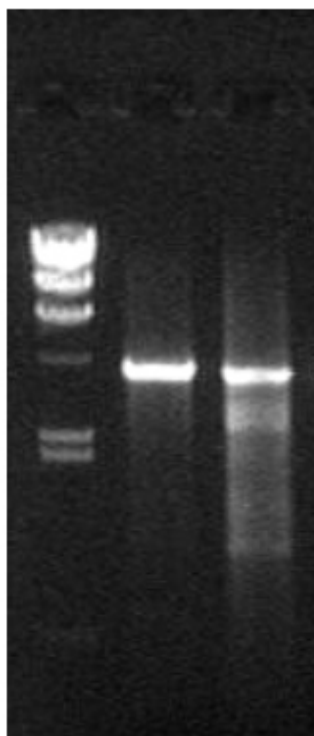
Human genomic:0.5kb

M1 1 2



Human genomic:4 kb

M2 1 2



M1 pHY marker

M2 λ Hind III digest

Lane 1 annealing at 55

for 5 sec

Lane 2 annealing at 55

for 30 sec

- PCR condition
 - annealing time
 - annealing temp : 58~65
 - PCR program : 3 steps → 2 steps

procedure.

- PrimeSTAR™ HS DNA Polymerase high fidelity priming Taq
annealing temp .
- - (1) T_m value annealing time confirm.
 - (2) annealing temp , Gradient PCR .
 - Gradient PCR , 55 (7t)
 - Taq annealing temp 7t .
 - (3) annealing temp smearing pattern
 2 step .