Poly Chain Reaction with HotStar Taq

- Purpose

  Warant the quality of DNA amplification with HotStar Enzyme

- Scope

Reagents	Manufacturer
10 x PCR Buffer	
25 mM $MgCl_2$	
dNTP mix (10mM of each)	
hotStar Taq DNA Polymerase 0,5 uL 2,5 units/reaction	Qiagen
Distilled water	
Q	

- Equipment :
  1. Gilson pipettor (2-20 uL ; 20-200 uL;100-1000 uL)
  2. Vortex
  3. Centrifuge
  4. DNA Thermal cycler engine
  5. Disposable gloves
- Procedure
  1. Thawing buffer, dNTP mix, primer solution and 25 mM $MgCl_2$, Q solution. Vortex for dissolve the concentrated salt, and pull spin for decreasing liquid in the wall and close it
  2. Prepare reaction mix like this table

Reagent	Reaction Amount										
	1	2	3	4	5	6	7	8	9	10	
Volume Total	13	23	34	45	55	65	75	85	95	105	
10x Taq Buffer	1,3	2,3	3,4	4,5	5,5	6,5	7,5	8,5	9,5	10,5	1X
5x Qsol	2,6	4,6	6,8	9	11	13	15	17	19	21	1X
dNTP mix 10mM each	0,26	0,46	0,68	0,9	1,1	1,3	1,5	1,7	1,9	2,1	200 uM each
Primer mix (10uM each)	0,65	1,15	1,7	2,25	2,75	3,25	3,75	4,25	4,75	5,25	0,5 uM
Taq Hot Star	0,07	0,12	0,17	0,23	0,28	0,33	0,38	0,43	0,48	0,53	2,5u/100uL
Template	0,65	1,15	1,7	2,25	2,75	3,25	3,75	4,25	4,75	5,25	
-	15,53	9,78	14,45	19,13	23,28	27,63	31,88	36,13	40,38	44,63	
DW	7,48	13,23	19,55	25,88	31,63	37,83	43,13	48,88	54,63	60,38	

  3. Contain MgCl 15 mM
     Template : $<$100ng/10uL reaksi
  4. Mix the premix well and aliquote premix PCR 9,5 uL into PCR tube 200 uL
  5. Add 0,5 uL sample (with DNA concentration $\pm$ 100ng/10uL reaction) into PCR tube number 3. Vortex it. Full spin. Template volume is not more that 1/10 total reaction
  6. Put in to PCR machine
  7. Run the program with cycle

Temperature	Time	Cycle
95°C	15 min	1x
Denaturation (94°C)	0,5-1 min	35-40 x
Annealing (50-68 ^{O}C)*	0,5-1 min	

Elongation (72 ^{O}C)**	1 min	
Final elongation (72°C)	10 min	1 x

*Usually 5 ^{O}C under TM Primer

** elongation DNA 1kb/min

8. Analyze amplicon (electrophoresis), save the amplicon excess in 20°C for long time savings