PCR protocol

1. assemble all reaction components.

component	50μl reaction
Buffer(2×)	25μl
DNA polymerase	1μl
Forward primer(20μM)	1μl
Reverse primer(20μM)	1μl
dNTP	1μl
Template DNA	1μl
ddH_2O	20μl

Note:

(1) stick pipette into liquid while adding components.

(2) get the liquid on the tube wall down by centrifuge to decrease the loss of fluid.

(3) have total volume 1~2μl lesser than 50μl in final components assembly because you will lose some fluid while transferring.

2. PCR reaction.

step	temperature	time
Initial denaturation	95℃	5min
Cycle reaction(30 times)	95℃	30s
	56℃	30s
	72℃	1kb/min
Final extension	72℃	5min
hold	4℃	/