

## Primer phosphorylation (Total volume: 50 $\mu$ L)

### 1、Composition:

-oligo mix (1 $\mu$ M, Each primer 1/36 $\mu$ M) : 30 $\mu$ L

-T4PNK: 1 $\mu$ L

-10\*buffer: 5 $\mu$ L

-ddH<sub>2</sub>O: Add to 50 $\mu$ L

2、Put the samples in 37°C incubator for 1 hour.

### 3、LCR:

Name	Total volume 50 $\mu$ L	Total volume 25 $\mu$ L
Phosphorylated oligo mix	1 $\mu$ L	0.5 $\mu$ L
40U/ $\mu$ L Taq ligase	0.5 $\mu$ L	0.25 $\mu$ L
10*buffer	5 $\mu$ L	2.5 $\mu$ L
ddH <sub>2</sub> O	Add to 50 $\mu$ L	Add to 25 $\mu$ L

### Procedure

94°C, 2min

94°C, 30s

51°C, 4min

51°C, 7min

12°C, max

×40

### 4、PCR of LCR's products

		Total volume 50 $\mu$ L	Total volume 25 $\mu$ L
LCR's products		5 $\mu$ L	2.5 $\mu$ L
2.5 $\mu$ M dNTP		4 $\mu$ L	2 $\mu$ L
5 $\times$ Q5 buffer		10 $\mu$ L	5 $\mu$ L
Primer	F	5 $\mu$ L	2.5 $\mu$ L
	R	5 $\mu$ L	2.5 $\mu$ L
Enzyme Q5		0.25 $\mu$ L	0.125 $\mu$ L
ddH <sub>2</sub> O		Add to 50 $\mu$ L	Add to 25 $\mu$ L

### Procedure

98°C, 30s  
 98°C, 10s }  
 55°C, 20s }  $\times 30$   
 72°C, 30s }  
 72°C, 2min  
 12°C, max