PCR

Jiaxin Li[1]

[1]South China University of Technology

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1 Works for me dx.doi.org/10.17504/protocols.io.bniemcbe

Jiaxin Li

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1 Set up a small box with ice, put DNA and 2×high Taq Master Mix into it before going into the Bio-rad S1000TM thermocycler cycler.

2 Add the following reagent to a PCR tube (50 µl) and program the thermocycler as follows:

Q5 polymerase

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component	25ul	50ul
5XQ5 buffer	5ul	10ul
10mM dNTPs	0.5ul	1ul
Forward Primer 10µM	1.25ul	2.5ul
Reverse Primer 10µM	1.25ul	2.5ul
Template (<1000ng)	According to different template	According to different template
Q5 polymerase	0.25ul	0.5ul
ddWater	Add to 25ul	Add to 50ul

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Temperature	time
98°C	03:00
98°C	00:05-00:10
Tm-5°C	00:10-00:30
72°C	20-30s/kb
72°C	02:00
4°C	∞

KOD FX

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component	volumn/ul	Final Concentration
2xPCR buffer	25uL	1X
2mM dNTPs	10uL	400umol/L
10umol/L Forward Primer	1.5uL	0.3umol/L
10umol/L Reverse Primer	1.5uL	0.3umol/L
Template DNA	2uL	Genomic DNA≤200ng/50ul
		Plasmid DNA□10ng/ul□
		cDNA≤200ng
		Crude sample≤0.5-4ul
ddH2O	Add to 50uL	□10ul□
KOD FX	1uL	1unit/50µl
Total	50µl	

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Temperature	time
98°C	T1= 03:00
98°C	T2= 00:10
Tm-5°C	T3=00:30
68°C	T4 : 1min/kb
68°C	T6>T2+T3+T4
4°C	∞

T5 Taq Polymerase

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Component	Volume/ul	Final Concentration
2XT5 Super PCR Mix	5ul	1x
10uM Forward Primer	0.4ul	0.4uM
10uM Reverse Primer	0.4ul	0.4uM
Template DNA	1ul	<1ug
ddH2O	Add to 10ul	
Total	10ul	

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Temperature	time
98°C	03:00
98°C	00:10
Tm-5°C	00:10
72°C	20s/kb
72°C	02:00
4°C	∞

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