Look What They've Done
To My Shoes!

SCU_China Project 2016

Protocol
AarC Team

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AarC

Overlap extension PCR Cecropin XJ Expression Team

1.Product two fragment

Primers

(1) Previous fragment:

gctctagatagctactagagtcacacaggaaagtactagatgaccgaacgtatc Xba-I-AarC-F

gcacgatcgaaatagtcttgcagcatcggacggtaatccgg Repair-Pst-I-R

(2) Rearward fragment:

ccggattaccgtccgatgctgcaagactatttcgatcgtgc Repair-Pst-I-F

tgcactgcagcggccgctactagtaaaaaaccccctcaagacccgtttagagg T7T-Pst-I-R

Diluted to 10 pmol/ul

Reaction system

(1) 5ul of I-5™ 2X High-Fidelity Master Mix

0.2ul of primer Xba-I-AarC-F

0.2ul of primer Repair-Pst-I-R

0.2ul of template DNA at 0.1ng

up tp 10ul

(2) 5ul of I-5™ 2X High-Fidelity Master Mix

0.4ul of primer Repair-Pst-I-F

0.4ul of primer T7T-Pst-I-R

0.2ul of template DNA at 0.1ng

up tp 10ul

PCR program

(1) 98C/2min

(2) 98C/10s

(3) 62C/10s

(4) 72C/20s

(5) Repeat cycle (steps 2 to 4, 34 times)

(6) 72C/2min

2.Splicing two fragment

Primers

gctctagatagctactagagtcacacaggaaagtactagatgaccgaacgtatc Xba-I-AarC-F

tgcactgcagcggccgctactagtaaaaaaccccctcaagacccgtttagagg T7T-Pst-I-R

Diluted to 10 pmol/ul

Reaction system

(1) 25ul of I-5™ 2X High-Fidelity Master Mix

2ul of primer Xba-I-AarC-F

2ul of primer T7T-Pst-I-R

1ul of template DNA at 0.1ng (two fragments are equimolar)

up tp 50ul

PCR program
(1) 98C/2min
(2) 98C/10s
(3) 55C/10s
(4) 72C/20s
(5) Repeat cycle (steps 2 to 4, 34 times)
(6) 72C/2min

HPLC
Sample
The bacterial cultures were centrifuged at 3000g for 10min.
Take the supernatant and centrifuged at 25000g for 10min.
Take the supernatant and filtered by a 0.22um filter.

Mobile phase
Methanol- 0.01mol/l K_2HPO_4(3:97)
Phosphoric acid is used to regulate pH to 2.0

Chromatographic condition
C18 column(4.6×250mm, 5um)
Flow rate is 1ml/min
Injection volume is 60 ul
Detection wavelength is 210nm
Column temperature is 25C

Standard
Accurately weighed 1000mg acetic acid and use mobile phase adjust volume to 10ml
Dilut to 0.01mg/ml, 0.1mg/ml, 1mg/ml, 10mg/ml, 100mg/ml.
Use different concentration to draw standard curve.