Lox WT gene PCR: amplification from genomic DNA

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Abstract

This protocol is a standard polymerase chain reaction, optimized to amplify a () bp gene flanked by two loxP sites. (?)

Refer to page 32, April 5, 2017 entry in Miguel's laboratory notebook for the original document.

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Materials

Phusion high-fidelity PCR kit F553S by Thermo Scientific

Protocol

PCR reaction

Step 1.

Into a 2.5 mL Eppendorf® tube add:

50 uL H2O

16 uL GC buffer

1.6 uL dNTPs solution

4 uL 10 uM Forward primer

4 uL 20 uM Reverse primer

1.5 uL DNA to amplify (lox)

0.8 uL Phusion polymerase

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Total=80 uL

Add 20 uL into four 0.2 mL tubes

--Ready for thermal cycler (vid. step 2).

Thermal cycler protocol

Step 2.

95*C, 5 min

95*C, 30 seconds

60*C 30 seconds

72*C, 30 seconds

30 X

72*C, 5 min

4*C infinite hold

Result example

Step 3.

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