PCR of mouse PCSK9 from cDNA (cleavage template)

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Abstract

Citation: Noreen Wauford PCR of mouse PCSK9 from cDNA (cleavage template). **protocols.io** dx.doi.org/10.17504/protocols.io.e8xbhxn
Published: 28 Jun 2016

Protocol

Step 1.

PCR mix

50 uL H2O

16 uL GC buffer

1.6 uL DNTPs

4 uL 10 uM M13 fwd (-41) primer

4 uL 10 uM M13 rev (-48) primer

1 uL cDNA

.8 uL Phusion

Divide into PCR tubes with 20 or 40 uL each

Step 2.

Thermocycler protocol

98* 30 s

98* 10 s

61* 10 s

72* 10 s

repeat steps 2-4 23 times

72* 2 min

4* hold

Step 3.

Agarose gel

Add 10 uL 6x loading buffer per 40 uL PCR reaction and vortex

Run on agarose gel with largest comb, loading 25 uL per well

Run @ 110 V for 30 min

Step 4.

Cut gel

Use a razor to carefully excise the bands (2 kb) under UV light.

Step 5.

Extract from gel

Load gel slices onto the Ultrafree DA columns and spin at 5,000 g for 10 min

Step 6.

Lyophilize overnight

Step 7.

Add water

Start with 20 uL, dilute (1.5 uL : 8.5 uL H2O), and run on PAGE to estimate concentration