PCR of mouse PCSK9 from cDNA (cleavage template) Version 2

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Abstract

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Protocol

PCR

Step 1.

Prepare the PCR mix and divide into PCR tubes with 20 or 40 uL each

Reagent	Amount
H2O	50 µL
GC buffer	16 µL
DNTPs	1.6 µL
10 uM M13 fwd (-41) primer	4 µL
10 uM M13 rev (-48) primer	4 µL
cDNA	1 µL
Phusion	.8 µLsdf

PCR

Step 2.

Thermocycler protocol

temp	time
98 °C	30 s
98 °C	10 s
61 °C	10 s
72 °C	10 s
Repeat steps	2-4, 23 times
72 °C	2 min
4 °C	hold

Agarose gel electrophoresis

Step 3.

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

Agarose gel electrophoresis

Step 4.

Prepare an agarose gel with the largest comb and load 25 µL per well

Agarose gel electrophoresis

Step 5.

Run @ 110 V for 30 min

DURATION
00:30:00

Gel extraction

Step 6.

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

Gel extraction

Step 7.

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

DURATION
00:10:00

Step 8.

Lyophilize overnight

DURATION
16:00:00

Step 9.

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