

PCSK9 gRNA amplification template PCR

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Abstract

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Protocol

Step 1.

Pre-steps

Dilute L2 oligos to 50 uM

Create 50x T7+F+R mix by combining 50 uM L2 complement oligo, 1mM T7 fwd primer, and 1 mM T7 rev primer in a 2:1:1 ratio

Step 2.

PCR mix

148 uL H₂O

4 uL L2

8 uL 50x T7+F+R mix

4 uL 10 mM DNTPs

40 uL GC buffer

2 uL Phusion

Divide into 4 50 uL tubes.

Step 3.

Thermocycler

gRNA Amp protocol:

98* 30 s

98* 10 s

57* 10 s

72* 10 s

Go to step 2 30 times

72* 2 min

4* hold

Step 4.

Finishing up

Purify using PCR purification kit

NanoDrop to confirm concentration (should be 150 ng/uL)