

PCSK9 gRNA amplification template PCR Version 2

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Abstract

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Protocol

Preparation

Step 1.

Dilute L2 oligos to 50 uM

Preparation

Step 2.

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

PCR

Step 3.

Prepare the PCR Mix as follows and divide into 4 tubes with 50µL each:

Reagent	Amount
H ₂ O	148 µL
L2	4 µL
50x T7+F+R mix	8 µL
10 mM DNTs	4 µL
GC buffer	40 µL
Phusion	2 µL

PCR

Step 4.

Thermocycler (gRNA Amp protocol):

temp	time
98 °C	30 s
98 °C	10 s
57 °C	10 s
72 °C	10 s
Repeat step 2-4, 30 times	
72 °C	2 min
4 °C	hold

Purify & check

Step 5.

Purify using PCR purification kit

Purify & check

Step 6.

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