

Cpf1 PCR protocol

1. Cpf1 PCR

Before starting:

- Thaw DMSO, HF/GC buffer (GC buffer for templates with high GC content), dNTPs.
- Dilute template to 5-10 ng/uL → LentiCas9-Blast
- Primer stock at 100uM and working solution at 10uM
 - o Example: 88 nmol of primer dilute in 880 uL MiliQ → 100uM
88 nmol = 0,088 ug
uM = ug/L
L = 10⁶ uL
88 nmol/880 ul = 0,088 ug/880 x 10⁻⁶ L = 100 uM

PCR master mix:

H ₂ O	118μL
5x HF/GC buffer	40μL
Template	10μL
Fw	10μL
Rv	10μL
DMSO	6μL
dNTP mix	5μL
phusion polymerase	3μL

mix well (vortex before adding polymerase and after adding phusion polymerase only flick the tubs, **no vortexing**), briefly spin down.

Divide into 8 PCR wells (**about 23μL/well**)

PCR cycles:

98°C		12s	
	98°C	10s	
42x	63°C	25s	
	72°C	58s	(15s/kb; minimum 15s)
72°C		5min	
4°C		---	

For gel extraction make 1% gel, using **thicker and wider** combs.

Collect all reactions in one tube, add 40μL of 6x loading dye.

Load 60μL sample to each slot on the gel.

Go through the gel extraction procedures.

NOTE! Use one column to bind and elute in 20μL