PCR gBlocks Cas9

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

PCR master mix:

H_2O	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	|*°C	25s	
	|72°C	15s	(15s/kb; minimum 15s)
72°C		5min	
4°C		---	

* 62ºC for Cas9

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