gRNA seed sequence insertion via PCR

All reagents must be kept **on ice** at all times. Assemble and mix **on ice** as well.
We are using the X7 DNA Polymerase (Common stock) and Phusion buffer 5X HF (Sotirios' lab)
Primers are:

- fw.gRNA1 CC**ACTAGT**GCACTGCCCTGTGGATAACAGTTTTAGAGCTAGAAATAGCAAG 51-mer / 45% GC; 23 annealed bp / Tm = 53 °C; 49 annealed bp when fully amplified / Tm = 69 °C
- fw.gRNA2 CC**ACTAGT**TTGAGAAAGACCTGGGATCCGTTTTAGAGCTAGAAATAGCAAG 51-mer / 43% CG; 23 annealed bp / Tm = 52 °C; 49 annealed bp when fully amplified / Tm = 69 °C
- fw.gRNA3 CC**ACTAGT**GATCATTAACTGTGAATGATGTTTTAGAGCTAGAAATAGCAAG 51-mer / 35% CG; 24 annealed bp / Tm = 53 °C; 49 annealed bp when fully amplified / Tm = 64 °C
- rv.gRNA GG**ACTAGT**ATTATACCTAGGACTGAG 26-mer / 42% CG; 24 annealed bp / Tm = 53 °C

Reagents:

- nuclease free mqH_2O
- 5X Phusion HF buffer
- dNTPs
- 10X forward primers
- 10X reverse primers
- template DNA
- X7 DNA polymerase (X7 DNAP)

Procedure:

Thermocycler settings

1. **Initialization (hot start PCR):** 60 seconds at 98 °C for X7 DNAP activation
2. **25 cycles:**
   i. **Denaturation:** 10 sec. at 98 °C for dsDNA denaturation;
   ii. **Annealing:** 30 sec at 55 °C for DNA-primers annealing;
   iii. **Elongation:** 90 sec. at 72 °C for dNTPs polymerization.
3. **Final elongation:** 10 minutes at 72 °C to make sure that all single strands are fully elongated.
4. **Hold:** 10 °C for short-term storage 10°C.

PCR reaction

- ❑ Dilute the primers to 1X working dilution using nuclease free H_2O.
- ❑ Prepare master mix as follow
  Final volume of 440 µL
  - ❑ 325 µL nuclease free mqH_2O
  - ❑ 100 µL 5x Phusion HF buffer
  - ❑ 10 µL dNTPs
  - ❑ 5 µL X7 DNAP

- ❑ Aliquot 44 µL of master mix in nine properly labeled 0.2 mL PCR tube. **Mix before aliquoting**
- ❑ Add 2.5 µL of proper forward primer. **Mix primers before adding.**
- ❑ Add 2.5 µL of proper reverse primer. **Mix primers before adding.**
- ❑ Add 1.0 µL of DNA template. **Mix DNA template before adding.**