SLC library sequencing **GSF lab**

PCR amplification

- For a 1000X coverage, 40 pg of pooled library are required.
- Primers are available in a separate Excel file (CRISPR_Libraries_NGS_primers_3-17.xslx)

PCR reaction:

gDNA	x µL to 40 pg
Q5	0.75 µL
5x GC Enhancer Buffer	10 uL
5x Reaction Buffer	10 uL
Nucleotides 10 mM	1 uL
10µM Primer F	2 µL
10µM Primer R	2 µL
H2O	x µL (to 50 ul tot)

PCR program:

	Temp	Time	Cycles
INITIAL DENATURATION	98°C	1min	1
DENATURATION	98°C	20s	24
ANNEALING	60°C	30s	
EXTENSION	72°C	30s	
FINAL EXTENSION	72°C	5min	1
HOLD	4°C	forever	

- If multiplexing, load 10µL of the (PCR purified) samples in a 1.5-2% gel for the quantification and the normalization of the amount of DNA present in the band by ImageJ (band at 280bp)
- Pool together all the samples, normalizing the amount of DNA added so that each sample has a similar amount of the ~280bp band
- Load on a 2% agarose. Cut the bands at 280bp of the gel and purify with the Qiagen kit:
    - we do not heat up the sample to melt the gel but use 100µL more of the required Buffer and leave it shaking, vortexing and pipetting from time to time until you get a liquid but still dense consistency
    - load the volume in 2 columns and elute with 30µL of H_2O/column.
- Submit 10 ul of the purified sample (at least 0.5 ng/ul) for sequencing. A minimum of 64bp + 2x 8bp are required for sequencing.
- Store the remaining sample at -20C for future reference