Prepare Gibson reaction

1) **pMel-10 + pAQR1**

	pMel-10 PCR	gRNA pAQR1	Mastermix	Sterile MQ water
Conc fragment (ng/µL)	31,9	2,5	X	X
Volume (µL)	3	4	10	3

2) **Positive control**
   Prepare positive control: 10 µL of control + 10 µL of Gibson mastermix

Gibson reaction
2 hours 50 degrees

Transform to *E. coli* (5 tubes of E. coli)

- Transforms 2 µL of Gibson assembly to E. coli: construct and positive control.
- Also transform 2 µL of 4X diluted Gibson assembly: construct and positive control (4X dilution: mix 5 µL of Gibson assembly mix with 15 µL of sterile MQ water).
- Do a negative control: transform with 2 µL of 6X diluted pMel-10 PCR (6X dilution: 1 µL pMel-10 PCR + 5 µL sterile MQ water). This will transform 10 ng of linear empty plasmid.
- Plate on total five LB + amp plates (2x construct + 2x positive control + 1x negative control)
- 37 degrees' overnight (max 16h)