**Library Prep, QVEU**

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**Application**

Create deep mutational scanning libraries via molecular cloning. This workflow is for use on plasmids ~10-kb in size and inserts under 800 bp.

**SOPs Used**

WEO 0001

WEO 0002

WEO 0003

WEO 0004

**Reagent Output**

Plasmid

**Data Output**

Plate images

**Other Resources**

**Notes**

**Workflow Overview:**

1. Use Golden Gate cloning **(SOP WEO-0003)** to replace insertion cassette in insertion-ready library plasmid with insert of interest. Use **350 ng** backbone, **~2:1** molar ratio insert:backbone, **2 µL** Golden Gate enzyme mix, 20 µL reaction. Use **60 cycles**.
2. PCR clean-up products from step 1 **(SOP WEO-0005)**. Use **8 µL** **water, 50°C** to elute DNA.
3. Transform insertion library into electrocompetent *E. coli* **(SOP WEO-0002).** 
   1. Plate at least 1 dilution after outgrowth (1 µL in 99 µL) on both Carbenicillin plates and counterselected Chloramphenicol/Ampicillin plates to quantify unligated plasmid. Grow overnight at 37°C. When colonies are big enough for easy counting, photograph them. **Output**: plate images.
   2. Grow 50 mL secondary culture from remaining primary culture for 16 hours overnight.
4. Extract plasmid from secondary culture using Midiprep **(SOP WEO-0001)**. **Output:** Plasmid DNA.