Plasmid Making (After trial and error)

PCR Purification
Purify 4 tubes of pcr product (450-500ng/uL) using Thermo kit-> yields 138ng/ul.

RE digest and purification
Run RE digest of plasmid, and PCR product for 2 hrs.

Run gel with cut plasmid and gfp. Cast gel with big wells (use comb with orange tape). 100V for 45min.

Gel extraction with Promega kit
Combine all digested product into one tube -> yield of about 30ng/uL

Ligation
Do a 6X reaction according to the kit instructions. Remember to adjust vector and insert concentrations according to NEB online calculator.
ligate O/N in pcr machine at 16deg hold

Transformation and selection
Transform plasmid into competent *E coli* according to standard kit (DH5a).
Plate 20, 40 and 100ul of transformed cells on LB+antibiotic for selection.
Plate 20ul on LB as a control.

Perform colony PCR on colonies grown
Select 2 positive clones for growth (one colony in 3mL LB+Antibiotic, min 16hours) and perform miniprep, send plasmid samples for sequencing
If clone is correct, keep glycerol stocks (30-50% glycerol with stationary culture of bacteria. Update in online registry)