Cloning

- Plan construction
  plasmid according to host organism/selection marker/copy number/etc.
  order primers for insert (if needed)

  (SnapGene Software is nice)
- Prepare insert (PCR) and clean it up
- Restriction Digest of plasmid and insert
  1-2ug DNA
  1ul of each restriction enzyme
  2ul buffer (FastDigest)
  ddW up to 20ul
- Blunting (optional)
  Add 1ul of Klenow(it will fill 5' and remove 3') and 1ul of 2mMdNTP to restriction mix and incubate 15 min at 37C
- To prevent plasmid ligation on itself add 1ul of CIAP/other alkaline phosphatase after restriction of plasmid and incubate 1h 37C (optional)
- Gel purification of fragments (run agarose gel with fresh TAE buffer, cut fragment with clean knife then use DNA purification kit)
- Ligation with Instant Ligase Master Mix (NEB)
  Combine 20-100ng of plasmid and insert (1:1, 1:3 or 3:1 molar ratios)
  Add ddW up to 4ul
  Add 4ul of Blunt or Sticky end Ligation Mix and mix by pipeting
  Keep on ice for 10min (for blunt ends keep 15min at Troom and then place on ice)
- Bacterial Transformation