Pfu purification

- Grow 400-500ml of cells to OD_{600}=0.6 in LB and antibiotics
- Induce with 1 mM IPTG for 4 hours.
- Pellet cells, wash with PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 1.8 mM KH_2PO_4) and resuspend in 20 ml PBS300 (PBS + extra 150mM NaCl) and lysozyme.
- Lyse by sonication, spin out and chuck the pellet.
- Heat supernatant to 75˚C for 20-30 min., spin out and chuck denatured stuff.
- Prepare a 1 ml column of Ni-NTA agarose and equilibrate with 10 ml PBS300.
- Apply heat-treated supernatant to column.
- Wash with 20 ml PBS300 + 15 mM imidazole.
- Wash with 3ml PBS300 + 25 mM imidazole and collect 1ml fractions.
- Elute with 5 ml PBS300 + 500 mM imidazole and collect 1ml fractions.
- Run an aliquot of every fraction on a 7.5% gel.
- Combine peak fractions and add an equal volume of storage buffer (50 mM Tris pH 8, 0.1 mM EDTA, 1 mM DTT, 0.1% NP40, 0.1% Tween 20, 50% Glycerol).
- Dialyse against 2 changes of 1L of storage buffer at 4˚C.
- Aliquot and store at –80˚C.
- Make serial dilutions of the enzyme in storage buffer and compare to the commercial one in a PCR reaction to determine the good dilution factor.

The pET-Pfu plasmid is Kan^R.