Purification of Pfu Polymerase-adapted from Miles Pufall's protocol

Transform BL21DE3(pLysS) with plasmid pET16Pfu (6xHis-Pfu), plate on LB+Chloramphenicol+Ampicillin

Grow an overnight culture, dilute 1:500 in LB with 100 μg/ml ampicillin, 34 μg/ml chloramphenicol. Grow at 37°C to OD_{600}=0.4 and induce at 37°C for 4 hours. Harvest by centrifugation, store drained cell pellet by placing at -20°C.

Resuspend in 10 mL/L original culture using TGI buffer (20 mM Tris-HCl pH 7.5, 10% glycerol, 20 mM imidazole). Lyse thoroughly using an Emulsifex. If you don't have access to one, sonication will suffice. Use 5 M NaCl to adjust sonicate to 250 mM. Also add 0.01% final NP-40 (from a 20% v/v stock). Spin 20 min 12,500 rpm. We used an SS34 rotor.

Heat supernatant in water bath set at 75°C for 30 minutes, cool in ice-water. "Yes, this is a mess."-*Miles Pufall* Add PEI (polyethyleneimine, Polymin P) to 0.1% final from 5% stock (adjusted to pH 8) in increments (e.g., add 1/4th of the total, mix well, repeat). Rotate end-over-end 20 min 4°C. "Yes, this is even more of a mess." -*Miles Pufall* Spin 20 min 12,500 rpm in an SS34 or equivalent rotor. Filter through miracloth Note: (one can skip this step if you use a high speed, 40 k, 40 minute spin. Add another 0.01% NP-40.

Note, I used a NiSeph FF 5 ml column and HPLC. Eluted in a gradient from 20mM-400mM imidazole in 8 column volumes. Pooled peak fractions, concentrated, then ran over Superdex200 gel filtration column. Then dialyzed as described in protocol.
Add NiNTA resin washed into TGI buffer + 250 mM NaCl (beware, no DTT or EDTA). I bound in batch rotating end-over-end 4°C for 2 h. I used 2.5 ml resin/L original culture.

I transferred to column format to wash and elute. Wash well with TGI buffer + 250 mM NaCl + 0.01% NP-40 (TGIN). I eluted in three steps: final concentrations of 45, 220 and 520 mM imidazole (note the 20 mM in TGIN buffer; supplemented using a 5M imidazole stock pHed to 7.5). My second elution was the peak.

Check by SDS-PAGE, should be ~90 kD. Dialyze into autoclaved 2X storage buffer (100 mM Tris-HCl pH 8.0, 0.2 mM EDTA, 2 mM DTT) with 20% glycerol, afterwards add 1 volume autoclaved 80% glycerol and a final concentration of 0.1% Tween-20 and 0.1% NP-40 (using 10% or 20% v/v stock). Or dialyze straight into 1X storage buffer with 50% glycerol if you need additional enzyme concentration.

The prep above was adapted from a protocol that Miles Pufall compiled as a streamlined version of several different published protocols.