Purification of Taq polymerase
Plasmid and protocol from R. Tjian, passed on my Miles Pufall

Transform DH5α with plasmid or streak out frozen cell stock on AMP plate.

Grow 100 ml overnight in LB + 100 μg/ml ampicillin at 37ºC. Dilute at least 1:100, grow at 37ºC to OD600 at least 0.2 and induce with 0.5 mM IPTG at 37ºC overnight. Harvest by centrifugation, drain and store at –20ºC

Resuspend in 10 ml/L original culture in 2X lysis buffer (100 mM Tris-HCl pH 7.5, 2 mM EDTA) with protease inhibitors (PMSF, leupeptin). Lyse thoroughly using an Emulsifex. If you don't have access to one, sonication will suffice. For sonication, split in two or more aliquots at microtip setting 4 for four times one minute each tube (ice in between). Add an equal volume of 0.4 M ammonium sulfate, mix 10 min, spin 12,500 rpm for 20 min in an SS34 or equivalent rotor at 4ºC.

Heat supernatant in water bath set at 75ºC for 30 minutes then cool in ice-water bath with mixing. Add PEI (polyethyleneimine, Polymin P) to 0.6% final from 5% stock (adjusted to pH 8) gradually in increments over 30 minutes. Stir for 2 h at 4°C. Spin 20 min 12,500 rpm in an SS34 or equivalent rotor at 4ºC. . Filter through miracloth.

Load on Phenyl Sepharose column, pre-equilibrated in load buffer (1X lysis buffer + 0.2 M ammonium sulfate). I used 1-2 ml resin/L original culture.
-Wash in same buffer at least 10 column volumes (c.v.).
-Wash in 1X lysis buffer no salt (5 c.v.)
-Wash in 1X lysis buffer + 20% ethylene glycol (5 c.v.)
-Elute with gradient from 0 to 4 M urea in 1X lysis buffer. Taq comes off mid-early, junk comes off late. I have used 5-6 c.v. as the total gradient (more would also work, but don't use less).
-Collect end of gradient with a rinse of 4M urea in 1X lysis buffer.

Check fractions by SDS PAGE. Full-length protein runs ~95 kD, the Stoffel fragment lacking the 5'-3' exonuclease domain runs ~61 kD (this fragment works fine for PCR applications). Contaminating heat-stable junk proteins run small, so make sure to use a high percentage or gradient acrylamide gel.

Several options at this point: just dialyze, precipitate with 60% ammonium sulfate to concentrate (note: at high urea concentration the 'pellet' can float), or run an FPLC or SMART system Poros Heparin column. From Miles Pufall: "The first time I tried all three, but in the end pooled all three because they were all the same. So if your protein looks clean, just dialyze; you can concentrate by dialyzing against higher % glycerol below".

Dialyze fractions of interest into autoclaved storage buffer straight (20 mM Tris-HCl pH 7.5, 100 mM KCl, 0.1 mM EDTA, 0.2% Tween-20, 1 mM DTT, 50% glycerol) or into 2X buffer with 20% glycerol, then add an equal volume of 80% glycerol afterwards.

Optional heparin column: use a gradient of 0.1 to 1 M KCl in 1X lysis buffer with 0.2% Tween-20, 1 mM DTT.