LONG RANGE PCR

from Cheng et al., Effective amplification of long targets from cloned inserts and human genomic DNA. PNAS 91:5695-5699, 1994.

mmm....these guys convincingly amplify fragments that range up to 42 kb! This paper is a must read for anybody wanting to PCR fragments larger than 700-1000 bp or who have not had good luck with smaller, difficult fragments to amplify (such as high GC content).

COMPONENTS OF 100 UL REACTION

NOTE: The components should be varied depending upon your target sequence, primers, etc. Read the paper to decide what is best for your goals. This is likely quite empirical.

concentration/stock	amount	final concentration
Q-H2O	balance	varies
10X LRPCR buffer I*	10 ul	1X
1.25 mM dNTP's	16 ul	0.2 mM
50% glycerol*‡	0-20 ul	0-20%
10% DMSO ‡	0-10 ul	0-1%
20 uM Primer A	2.5 ul	0.5 uM
20 uM Primer B	2.5 ul	0.5 uM
Template	1.0 ul	varies
5 U/ul BRL TAQ poly'ase	0.3 ul	1.5 U/100 ul
2 U/ul Vent poly'ase	0.15 ul	0.003 U/100 ul

10X LRPCR buffer I: 250 mM Tris-HCl, 500 mM KOAc, 20 mM $MgSO_4$. pH 8.9

*After making these two buffers, destroy contaminating DNA's by placing aliquots in 1.5 ml microfuge tubes and exposing to uv lignt for 10 min in the Stratalinker (the tubes are placed on the Stratalinker tube rack and thus closer to the light source)

‡How much DMSO and glycerol to add is empirical. A good starting point that has worked frequently is to add 10ul each.