RAPD Protocol

Protocol for Promega and Stoffel brand Taq enzymes (1 reaction):	Protocol for Gibco brand Taq enzyme (1 reaction):	Final Conc.
4 µl dNTP (5mM) mix 2.5 µl 10X PCR buffer 8.33 µl H_20	4 µl dNTP (5mM) mix 2.5 µl 10X PCR buffer 10.83 µl H_20	0.8 mM dNTP mix (0.2 mM each dNTP)
5 µl (25 mM) $MgCl_2$ 0.22 µl (5 U/µl) Taq	2.5 µl (50 mM) $MgCl_2$ 0.22 µl (5 U/µl) Taq	5 mM $MgCl_2$ 0.042 U/µl Taq
3 µl primer (10 ng/µl) 3 µl template DNA (10 ng/µl)	3 µl primer (10 ng/µl) 3 µl template DNA (10 ng/µl)	1.15 ng/µl primer 1.15 ng/µl DNA
Total vol = 26.05 µL 3 µl loading dye	Total vol = 26.05 µL 3 µl loading dye	

dNTP mix (5mM):

12.5 µl of 100mM dATP
12.5 µl of 100mM dGTP
12.5 µl of 100mM dCTP
12.5 µl of 100mM dTTP

Add water up to 1000 µl

Ladder mix:

100 bp ladder 50 µg; 1 µg/µl
166 µl Loading Buffer
784 µl H_2O

total of 100 µl

Loading buffer (6X):

30% Glycerol (make 25ml with H_2)
0.25% Bromophenol blue
0.25% Xylene cyanole

In a cylinder, cover with parafilm and mix by inverting.

PCR profile:

94°C 1 min
35°C 1 min — 3 cycles
72°C 2 min
94°C 10s
40°C 20s — 34 cycles
72°C 2 min
72°C 5 min
4°C soak