

RAPD analysis with *P. infestans*

Reaction conditions: 25 µl per reaction

Component (add in this order)	1 X	55 X	110 X
sterile di water	16 µl	880	1760
10X buffer 100 mM KCl 200 mM Tris-HCl (pH 8.8) 100 mM (NH ₄) ₂ SO ₄ 20 mM MgSO ₄ 1% Triton X-100	2.5 µl	137.5	275
100X bovine serum albumin (10 mg/ml, nonacylated)	0.25 µl	13.75	27.5
1.25 mM dNTP 1.25 mM each of dATP, dCTP, dGTP, and dTTP	2.0 µl	110	220
5 units/µl Taq DNA polymerase	0.2 µl	11	22
**2 ng/µl DNA stock (in 10 mM Tris 7.5, 0.1 mM EDTA)	2.0 µl	(110)	(220)
**10 µM primer (in 10 mM Tris 7.5, 0.1 mM EDTA)	1.0 µl	(55)	(110)

**usually one or more of these components are first added to the well, and then 25 µl of a "master mix" is added.

Cycling conditions

94°C, 15 sec. 1X
94°C, 30 sec; 35°C, 30 sec; 72°C, 60 sec. 36X
72°C, 2.5 min. 1X
cool to 10°C, then shut off

Electrophoresis

Run on 1.6% TBE gel at ~5 V/cm (4.8 g per 300 ml)
Load 6-12 µl depending on well size
Run until bromophenol blue tracking dye is 1 cm from end of gel
Stain in 0.5 µg/ml ethidium bromide (in water), 30-60 minutes.
Destain in water, 10 to 30 minutes
Photograph