

## General amplification of mitochondrial DNA frequents of *Phytophthora infestans*.

### PCR per reaction

10 x PCRbuffer + MgCl <sub>2</sub>	2.5 µl
dNTP's 5mM	1 µl
Taq DNA polymerase 5U/µl	0.2 µl
MQ	16.8 µl
MgCl <sub>2</sub> 25 mM	2 µl
Primer forward 10mM	0.5 µl
Primer reverse 10 mM	0.5 µl

- Add 23 µl mix per PCR tube.
- Add 2µl DNA to every PCR tube. (2µl MQ instead of the template DNA for the negative control) DNA concentration must be about 20 ng/µl.
- Following PCR 7,5µl PCR-product (plus 2 µl loadingbuffer) on 1% agarose gel.
- Use 10 µl Lambda HindIII/EcoRI DNA as a size marker(500 ng per lane).

### PCR-program

2 minutes	94°C	1 cycle
1minute	94°C	
30 seconds	62°C	35 cycli
1 minute	72°C	
10 minutes	72°C	1 cycle
∞	4°C	1 cycle

### Digestion

- Add per reaction:
  - # 5 µl PCR-product
  - # 1 µl enzyme (5U/µl)
  - # 2 µl restriction buffer (10X)
  - # 13 µl MQ or distilled water
- 4 hours digestion or overnight at 37°C
- Add 4 µl loading buffer to digested DNA suspension and load total digest on 2% agarose gel
- Use 5 µl of a 100 bp DNA ladder (50 µg/ml, Biorad).