

## PCR WITH DEGENERATE PRIMERS

The PCR is pretty standard, with the main difference being that a higher concentration of primer is used. The concentration of  $MgCl_2$  and the annealing temperature may be varied to optimise the reaction. The extension time may need to be increased for larger (>1 kb fragments). It also sometimes helps to do a hot-start reaction (reduces non-specific annealing of primers) - i.e. the Taq is not added until the tubes have been heated to  $94^\circ C$  (pause the run during the  $94^\circ C$  for 2 minutes step, add the Taq, then proceed). Note that  $1.25 \mu l$  DMSO or  $5 \mu l$  of 5 M betaine should be added if the reaction is carried out on maize.

### 1. Set up PCR, on ice:

genomic DNA	50-100 ng
10 x PCR buffer	2.5 $\mu l$
$MgCl_2$ (25 mM)	1-2.5 $\mu l$
dNTPs (10 mM)	0.5 $\mu l$
each primer (100 $\mu M$ )	0.5 $\mu l$
dH <sub>2</sub> O	to 24.5 $\mu l$
Taq	0.25 $\mu l$

### 2. Run PCR:

$94^\circ C$	2 minutes (add Taq at this stage, for hot-start)
then 35 rounds of:	
$94^\circ C$	30 secs
$45-55^\circ C$	30 secs
$72^\circ C$	1 minute
followed by:	
$72^\circ C$	10 minutes