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°C (pause the run during the 94°C for 2 minutes step, add the Taq, then proceed). Note that 1.25 μl DMSO or 5 μl 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 μl
$MgCl_2$ (25 mM)	1-2.5 μl
dNTPs (10 mM)	0.5 μl
each primer (100 μM)	0.5 μl
dH_2O	to 24.5 μl
Taq	0.25 μl

2. Run PCR:

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