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 MgCl₂ 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
   - MgCl₂ (25 mM) 1-2.5 µl
   - dNTPs (10 mM) 0.5 µl
   - each primer (100 µM) 0.5 µl
   - dH₂O 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