PCR WITH GENOMIC DNA

Reactions are carried out in 25 μl. The annealing temperature may need to be varied depending on the primers used. The extension time is roughly proportional to the size of fragment being amplified – e.g. up to 1 kb of DNA needs about 1 minute extension, up to 2 kb needs about 2 minutes. If the reaction is carried out on maize then 1.25 μl of DMSO or 5 μl of 5 M betaine should be added to the reaction.

1. Mix together the following, on ice:

genomic DNA	50-100ng
10x Taq buffer	2.5 μl
$MgCl_2$ (25 mM)	1.5 μl
dNTPs (10 mM)	0.5 μl
each primer (10 μM)	0.5 μl
dH_2O	to 24.75 μl

2. Add 0.25 μl Taq (still on ice).

3. Place tubes in PCR machine and start run:

Step 1	94°C	2 minutes
Step 2	94°C	30 secs
Step 3	55°C	30 secs
Step 4	72°C	1-2 minutes
Step 5	repeat steps 2-4, 34 times	
Step 6	72°C	10 minutes
Step 7	4°C	