

p53 GENOTYPING PCR:

The p53 PCR is a multiplex PCR that recognizes both wild-type and mutant allele. The wild type allele is around **375 nt** and the mutant allele is around **575 nt**

METHOD:

Prepare a mastermix(w/o DNA) to a volume of 19ul/sample:

Primers: X7: 5' - TAT ACT CAG AGC CGG CCT
 X6.5: 5' - ACA GCG TGG TGG TAC CTT AT
 NEO: 5' - TCC TCG TGC TTT ACG GTA TC

DNA	1
10X	2.0
10mM dNTP	0.4
X7 (20 uM)	0.4
X6.5 (20 uM)	0.15
Neo (20 uM)	0.35
taq	0.5
dH2O	15.2 ul

PCR conditions:

94 for 4'	
94 for 30")
62 for 1') 30 cycles
72 for 1')
72 for 5'	
10 for ever	