

XRCC2 Genotyping

1. Prepare DNA by standard isopropanol/ethanol extraction.
2. Bring final volume of each oligo to 100 pmol/ μl (each) using ultrapure H_2O .

wt	KMO99	GGT TCT ATC TTG TGC TTT TGT GTG TTT
	KMO100	TCT GTT TTC CCC GTT CCT TCT G
mut	KMO99	GGT TCT ATC TTG TGC TTT TGT GTG TTT
	KMO101	GCA TGC TCC AGA CTG CCT TGG

3. Set up a master mix (on ice) for each primer as follows:

12.5 μL	Promega GoTaq Mastermix	} X No. of samples
0.2 μL	KMO99	
0.2 μL	KMO100	
0.2 μL	KMO101	
10.9 μL	H2O	

4. Add 1 μl template DNA to corresponding well in PCR plate.
5. Add 24 μl master mix to each well in PCR plate
5. Seal using Microseal film.
6. Keep reactions on ice and load onto PCR block pre-heated to 94°.

PCR Program

94° 1:30 min.	} 25 cycles for WT reaction
94° :35 sec	
58° 1 min.	} 25 cycles for mutant reaction
72° :45	
72° 5 min	
10° forever	

Expected band sizes:

WT: approx. 545bp
Mut: approx. 280bp