

Pyrosequencing

**Genomic DNA extraction
& Purification**



Bisulfite conversion
(methylation sequencing)

Assay design

Qiagen Assay design software



PCR optimization

Genomic or bisulfite-converted DNA template
Annealing temperature
MgCl₂ concentration



Assay validation

- Primer controls pyrosequencing run
- Allele preferential amplification-PCR bias



Pyrosequencing

- Single strand DNA template preparation
- Pyrosequencing run: (24 or 96 well-plates in PyroMark Q24 or PyroMark Q96 MD)



Data compilation



Redesign Assay

(If assay validation fails)