Genotyping Technologies

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Summary

• PCR
• Sanger sequencing
• RFLP
• Microarrays
Genotyping With PCR
Genotyping With PCR (cont’d)

- Restriction enzymes
- Electrophoresis
Genotyping With PCR (cont’d)

- Sanger DNA sequencing
Genotyping: Taqman Assay

Perfect match TaqMan® probe

Hybridization

Extension

Completed

Probe cleavage: signal

Single mismatch TaqMan® probe

Hybridization

Extension

Completed

Probe displacement: no signal

D1: Dye 1  D2: Dye 2  Q: Quencher  DNA polymerase  Forward primer
Figure 1 | The principle of Pyrosequencing and the output Pyrogram™. Double peak heights indicate incorporations of two nucleotides in a row.
Restriction Fragment Length Polymorphism (RFLP)

Inheritance of RFLP markers

Genotypes

AA  aa  Aa

Parents

Siblings

Aa  Aa  aa  Aa  AA
Restriction Fragment Length Polymorphism (RFLP)

- Used for paternity testing
Restriction Fragment Length Polymorphism (RFLP)

- Use at a crime scene
Microsatellite Markers
High Throughput Genotyping

• Illumina assays
  – GoldenGate & VeraCode: 48-1,536 SNPs
  – Infinium: 200K to 5M SNPs

• Affymetrix
High Throughput Genotyping

- Genomic DNA
  - Attach gDNA to solid support
  - Oligo annealing
  - Allele-specific extension and ligation

- PCR with universal primers
  - Array hybridization and imaging

- Allele Types: A/A, A/G, G/G
High Throughput Genotyping