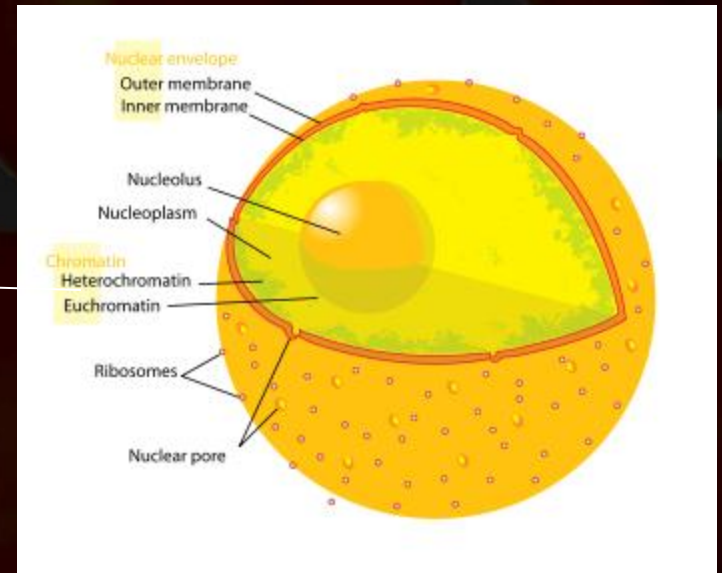
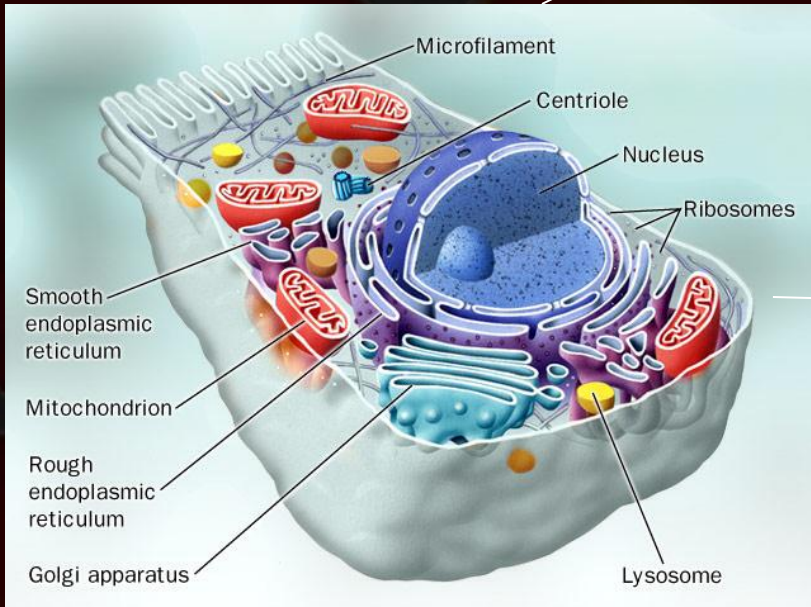
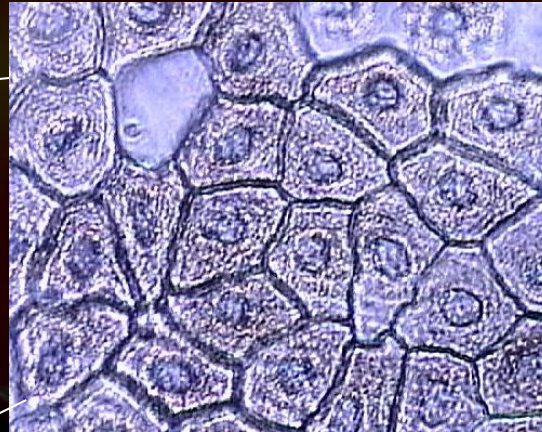


DNA Extraction

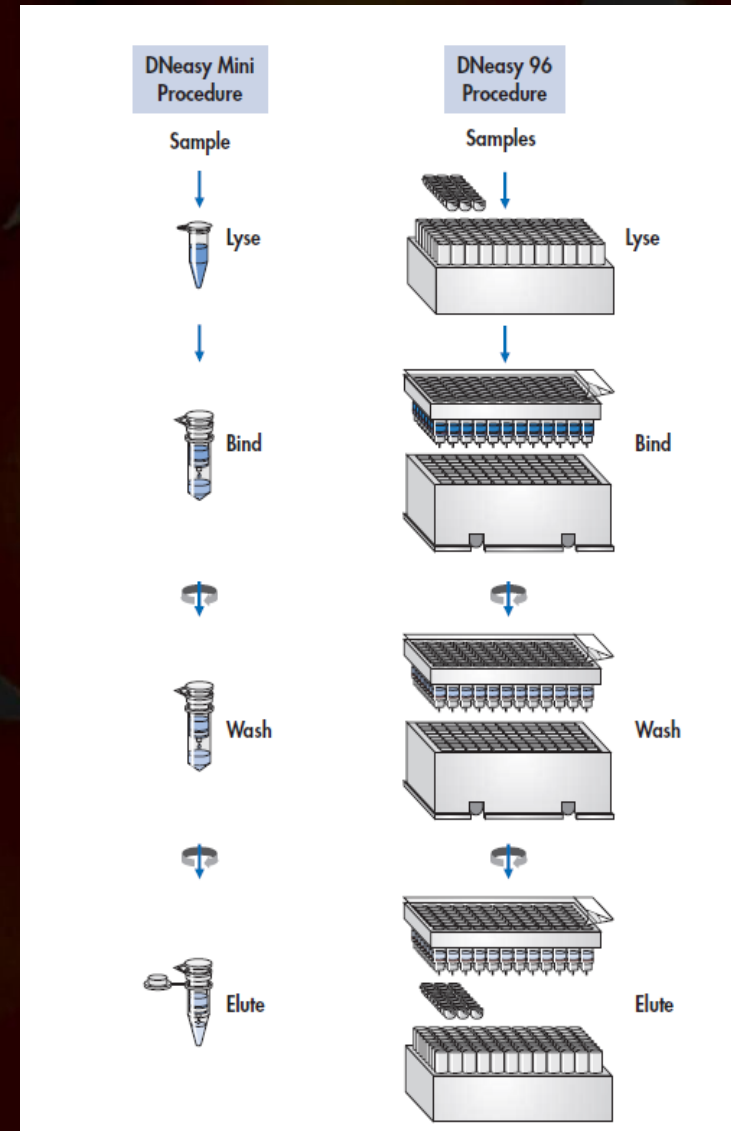


DNA Extraction

- Lot's of techniques for extracting DNA
- Basic Rundown
 - Detergent: Disrupts cell membranes and denatures proteins.
 - Proteases: Enzyme that cuts amino acid chains (usually Proteinase K)
 - Alcohol: Precipitates DNA

Qiagen Dneasy kits

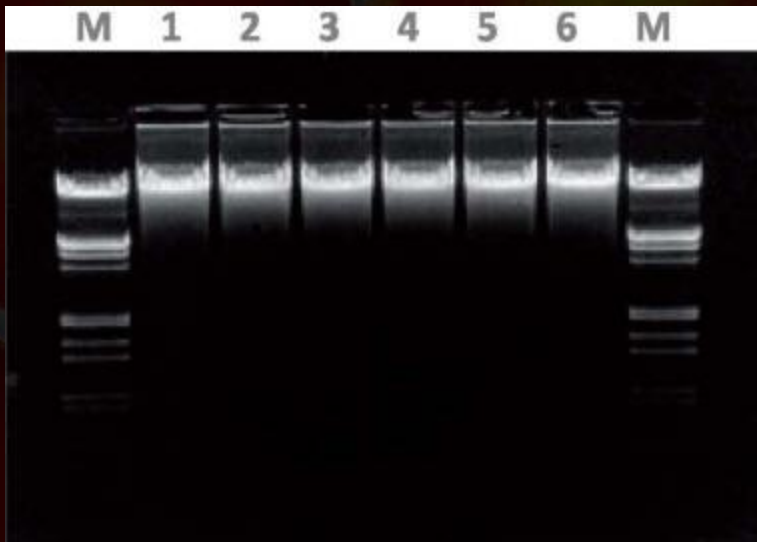
- Small piece of fin clip is added to lysis buffer
- Tissue is digested
- Solution containing alcohol is added
- Precipitated DNA is caught in filter columns
- 2 wash steps to remove salts
- DNA is released from filter by dissolving in weak buffer



Qiagen Dneasy kits

- Quality extracted DNA has very low levels of proteins, cell debris, and salts
- DNA should be in high molecular weight strands

High quality DNA extracts on agarose gel



Low quality DNA extract on agarose gel



PCR: Polymerase Chain Reaction

PCR ingredients:

- Primers – short single stranded DNA
- Taq polymerase: Thermal stable DNA polymerase
- dNTPs: deoxy nucleotide triphosphates (ATCGs)
- Buffer with magnesium (magnesium is an essential cofactor for Taq)
- Thermal cycler
 - 95°C - 10 min. } 35 cycles
 - 95°C - 1 min.
 - 60°C - 30 sec.
 - 72°C - 1 min.
 - 4°C - Hold

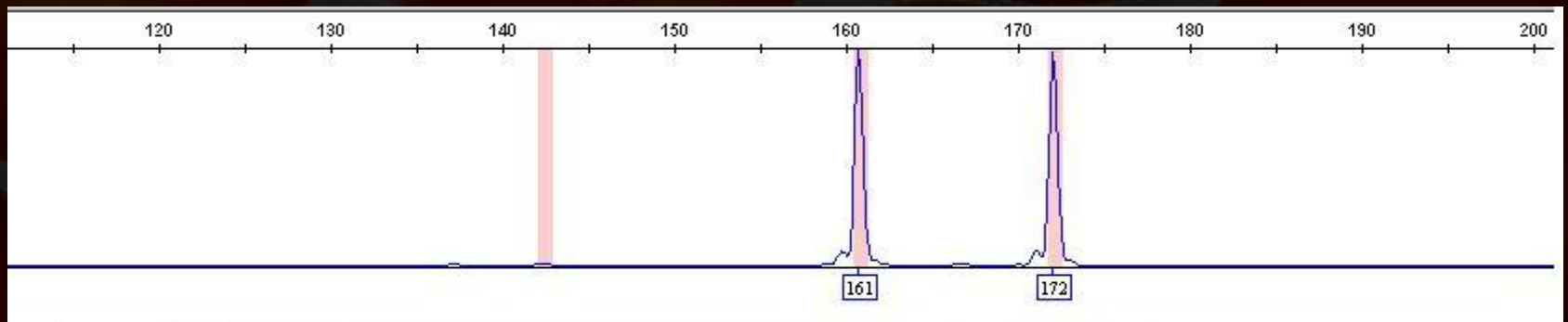
PCR movie:

**Polymerase Chain
Reaction**



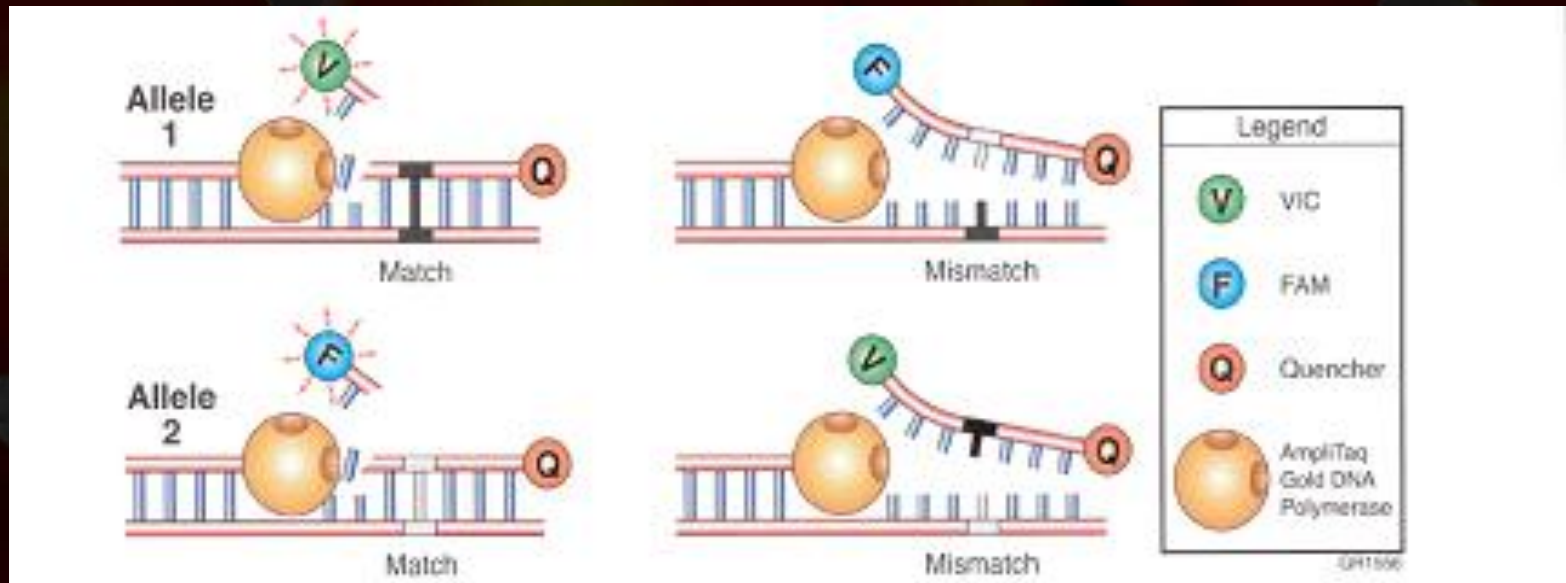
PCR: Polymerase Chain Reaction

- Microsatellite PCR
 - Primers flank microsatellite repeat region
 - Uses forward primer with a 5' fluorescent tag
 - Example: 6FAM – AGGCCTAGAGAAATTACGCA
 - Sizes of PCR products are then determined on the 3730 DNA analyzer



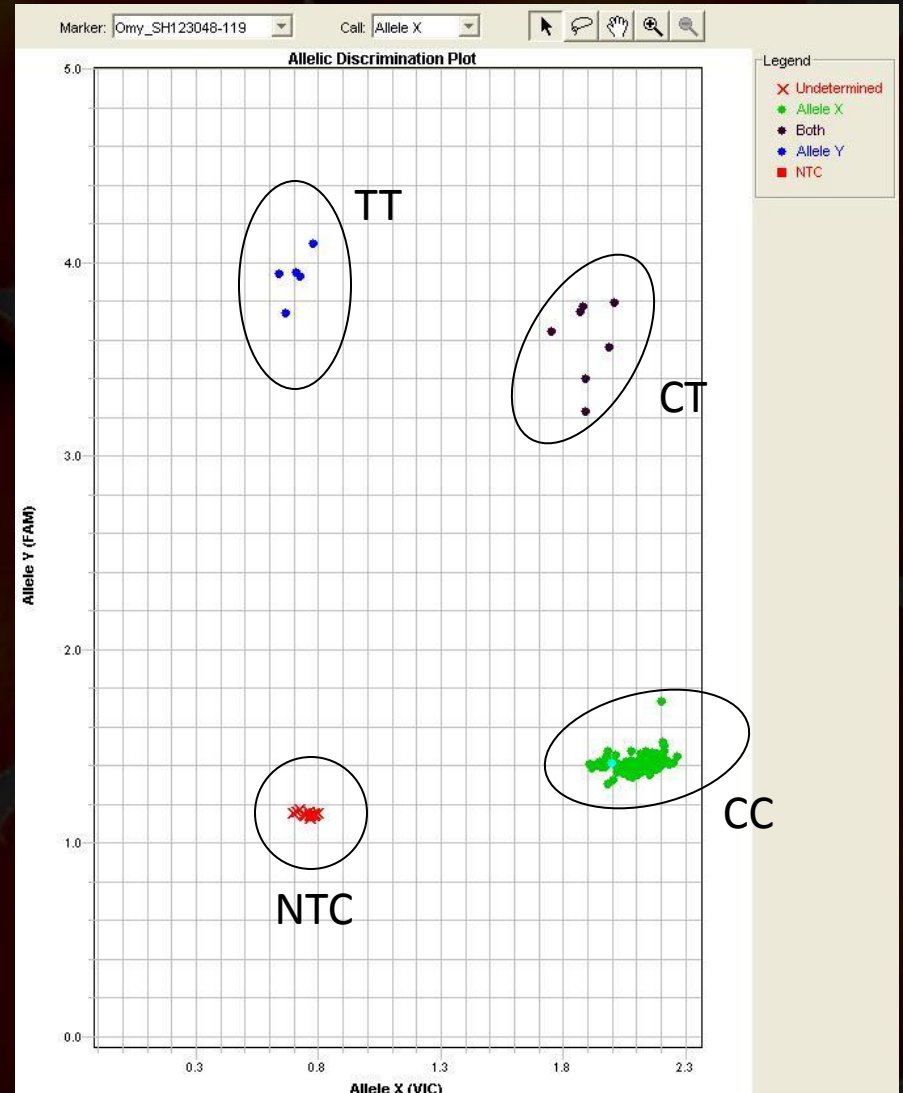
PCR: Polymerase Chain Reaction

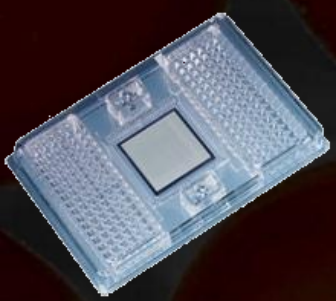
- SNP (Single Nucleotide Polymorphism) PCR
 - SNP site is determined using Fluorescent labeled probes
 - 6FAM – AGGCTAGGCTAGCATAAC – Quencher



Basic SNP genotyping

Output of a SNP genotyping reaction:

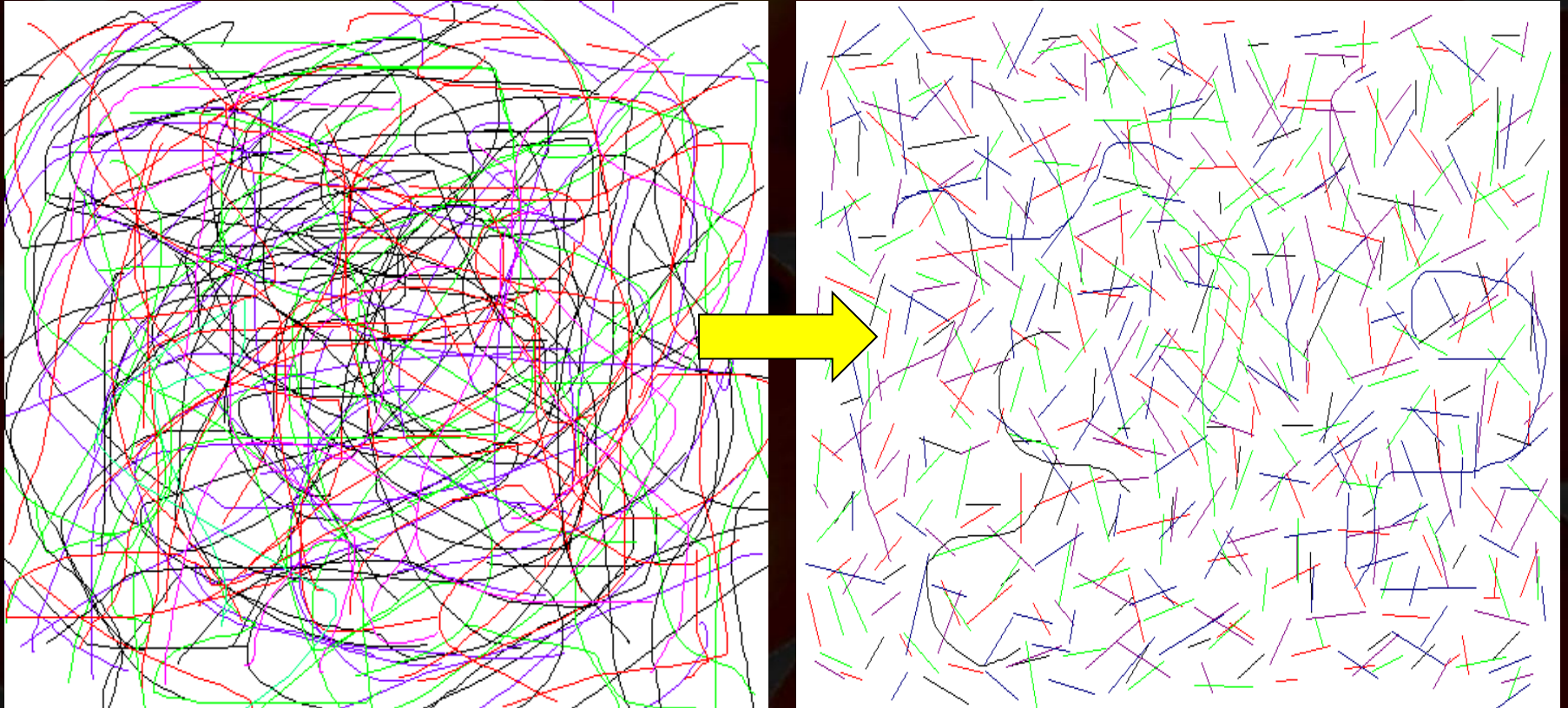




Fluidigm SNP genotyping

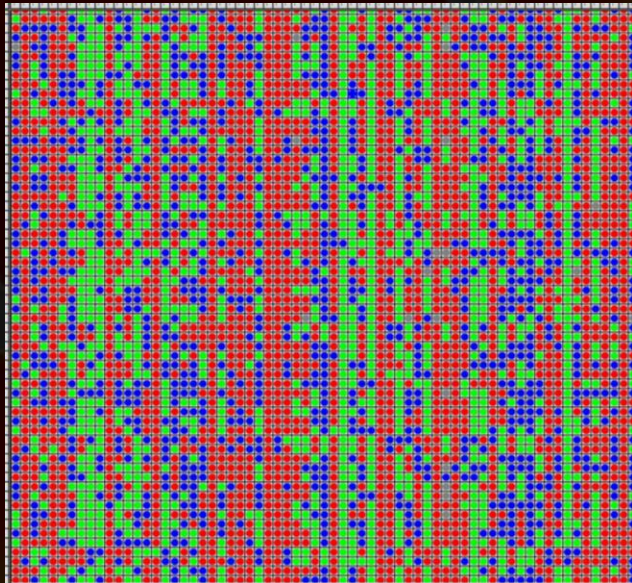
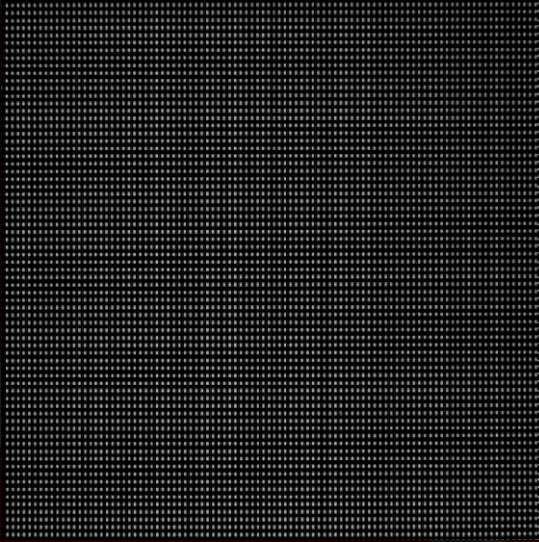
- Same basic chemistry
- Performs 96 SNP assays on 96 samples in 1 Chip (9,216 genotypes)
- 7nL reaction volume (~700 fold less volume than our typical ABI-7900 reaction)
- Our typical high quality DNA extract has ~20,000 copies/uL of any given 100 bp nuclear DNA fragment
- In the Fluidigm chip only ~60 copies will be present in the reaction chamber

Pre-Amplification



Boosts the starting copy number of each target by about 100 fold (Target DNA = $\sim 0.0003\%$)

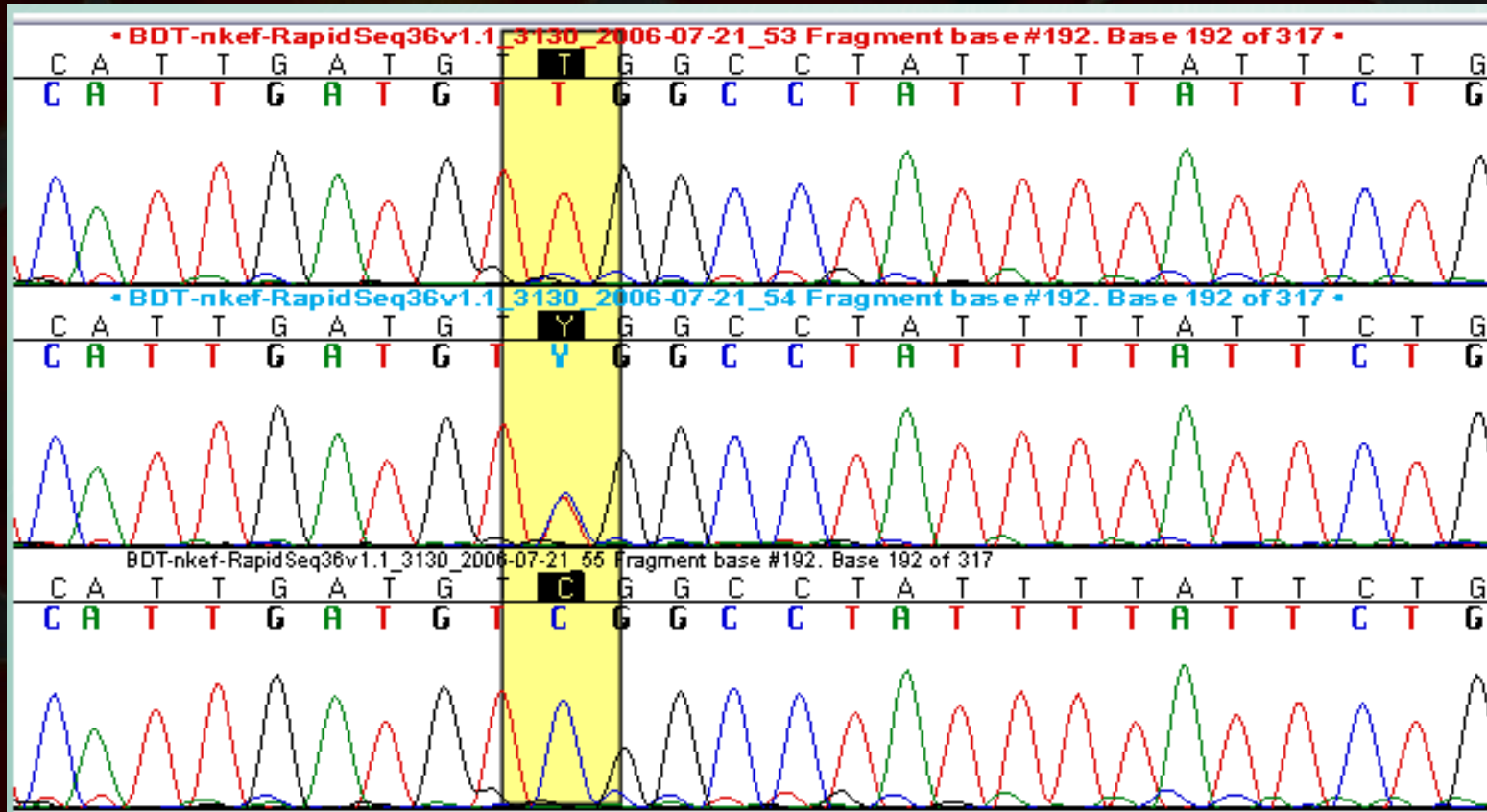
Fluidigm 96.96 Array



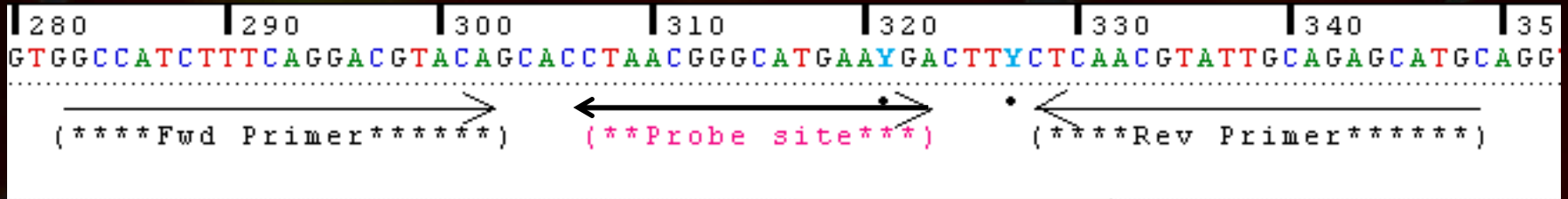
- The array is loaded with PCR reagents and the pre-amplified samples
- PCR resumes inside the chip for 50 cycles in the FC-1 thermal cycler
- Chip is then read using the camera inside the EP-1 instrument.

How to design a SNP assay

Sequencing data is aligned and edited
-SNP sites are identified and cataloged



How to design a SNP assay



- Design MGB probes with an annealing temp of about 45°C (MGB will raise this by about 20 °C)
 - Ideally the SNP should be in the middle
- Design forward and reverse primers flanking the probe site making sure that no nucleotide variations are incorporated into the primer sites
 - The probe should start within 10 bases of the extending primer

SNP assay Development

- Chinook salmon
 - About 300 SNP assays available
 - Currently using two 96 SNP panels for parentage and GSI applications
- Steelhead
 - About 500 SNP assays available
 - Currently using two 96 SNP panels for parentage and GSI applications
- Sockeye
 - About 150 SNP assays available
 - One 96 SNP panel in use
- Coho
 - About 250 SNP assays available
- Cutthroat trout
 - About 200 SNP assays available
- Lamprey
 - RAD sequencing project for SNP discovery and assay development