

Illumina SNP Genotyping Technologies BeadArray and BeadChip Platform

SNP Genotyping Assay and SNP Multiplexing Level per Sample

- **GoldenGate Assay**

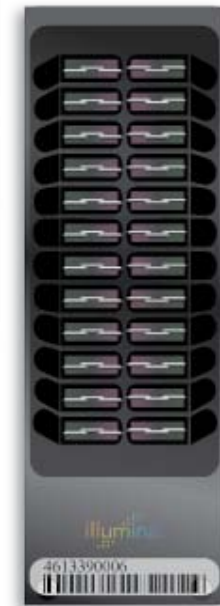
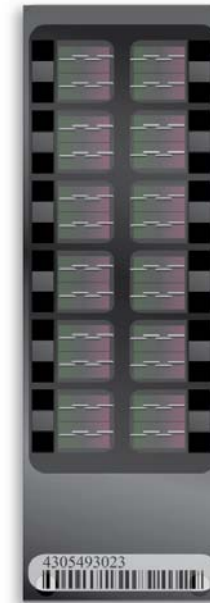
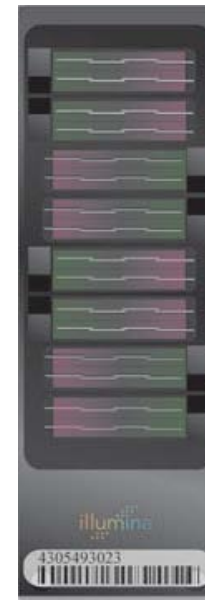
OPA synthesis containing 48, 96, 144, 192, 384, 768, 1536, or 3072-plex SNPs per sample assay

- **Infinium Assay**


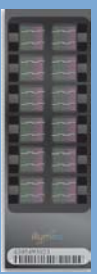

Custom made iSelect chips to assay 3072 up to 1M SNPs per sample.

Infinium products targeted toward Agricultural markets

- ▶ BovineHD BeadChip
 - 8 samples, 777,962 SNPs
- ▶ BovineSNP50 BeadChip
 - 24 samples, 54,609 SNPs
- ▶ CanineHD BeadChip
 - 12 samples, 172,115 SNPs
- ▶ OvineSNP50 BeadChip
 - 12 samples, 54,241 SNPs
- ▶ PorcineSNP60 BeadChip
 - 12-samples, 62,163 SNPs
- ▶ MaizeSNP50 BeadChip
- ▶ iSelect Custom Panels

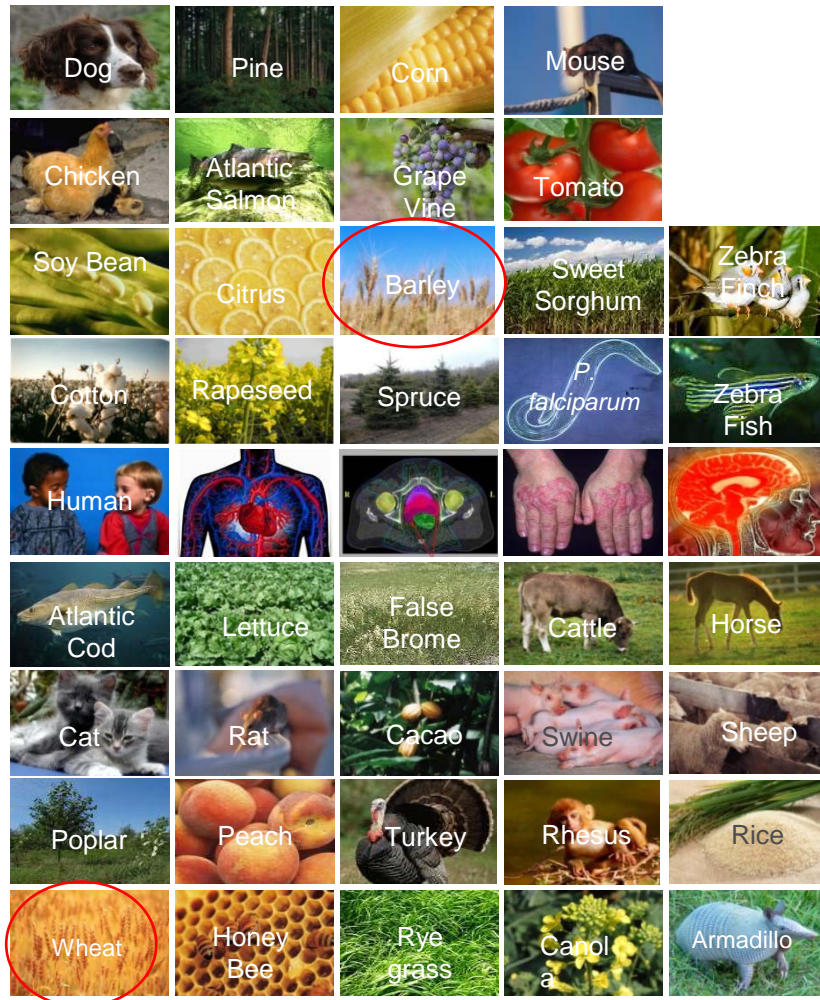


iSelect BeadChip Formats and Supported Marker Densities

			
BeadChip Format	24-sample	12-sample	4-sample
Previous plex range	3,072 – 60,800	60,801 – 200,000	n/a
New plex range	3,072 – 90,000	90,001 – 250,000	250,001 – 1M
Add-on content range*	Up to limit of 90,000	Up to limit of 250,000	Up to limit of 1M

*Add-on content range allowed = (maximum attempted beadtypes on BeadChip) MINUS (existing beadtypes in original pool)

Custom Genotyping on the Infinium Assay



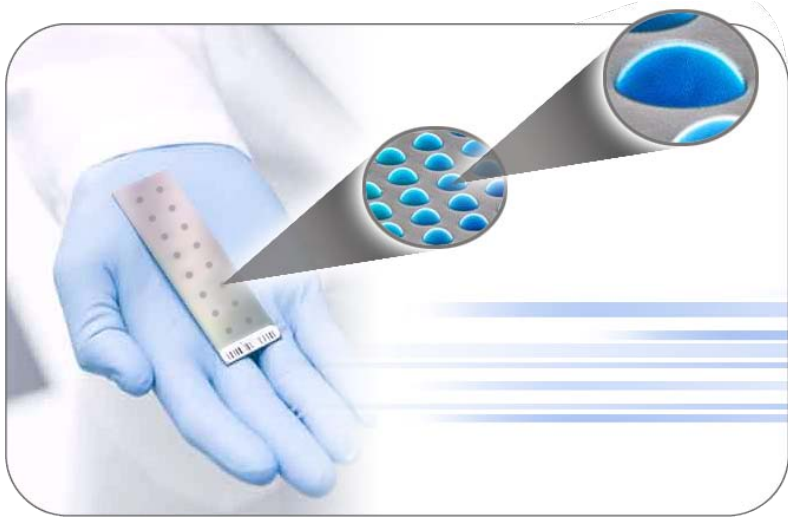
Applications

- ▶ **Genome-wide selection**
- ▶ **Genetic Prediction**
- ▶ **SNP discovery**
- ▶ **Commercial Agriculture screening panels**
- ▶ **Targeted follow-up studies after GWAS**
- ▶ **Targeted disease panels**
- ▶ **Targeted rare variant panels**
- ▶ **Sample QC and tracking panels**
- ▶ **Consumer Genomics**
- ▶ **Forensics**

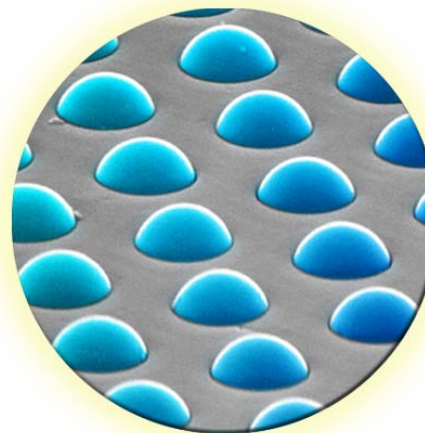
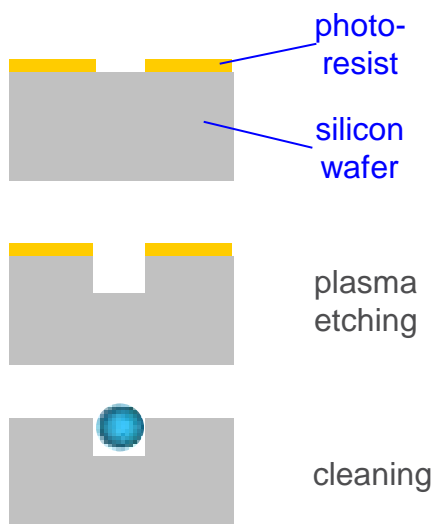
The Right Content Lets You Capture More Biology



BeadArray technology and BeadChip manufacturing

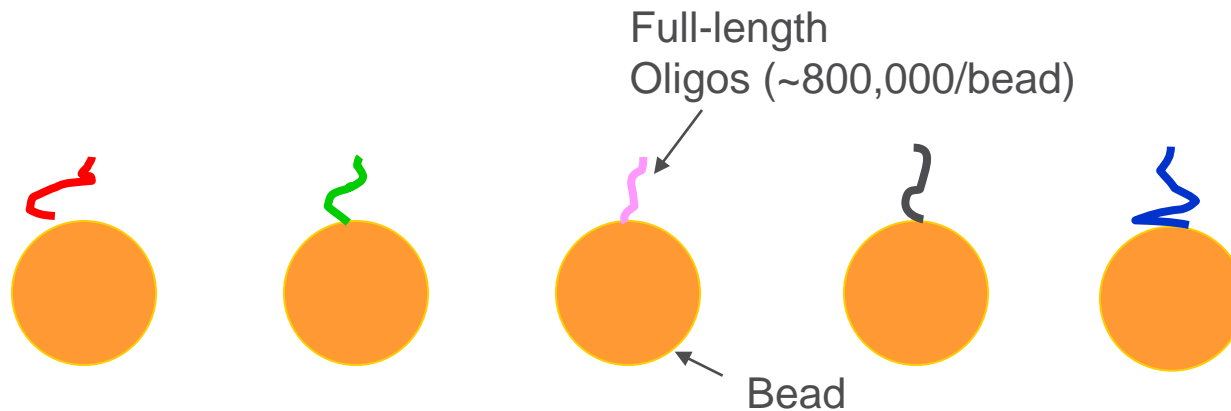


Microfabrication of BeadChip Wells



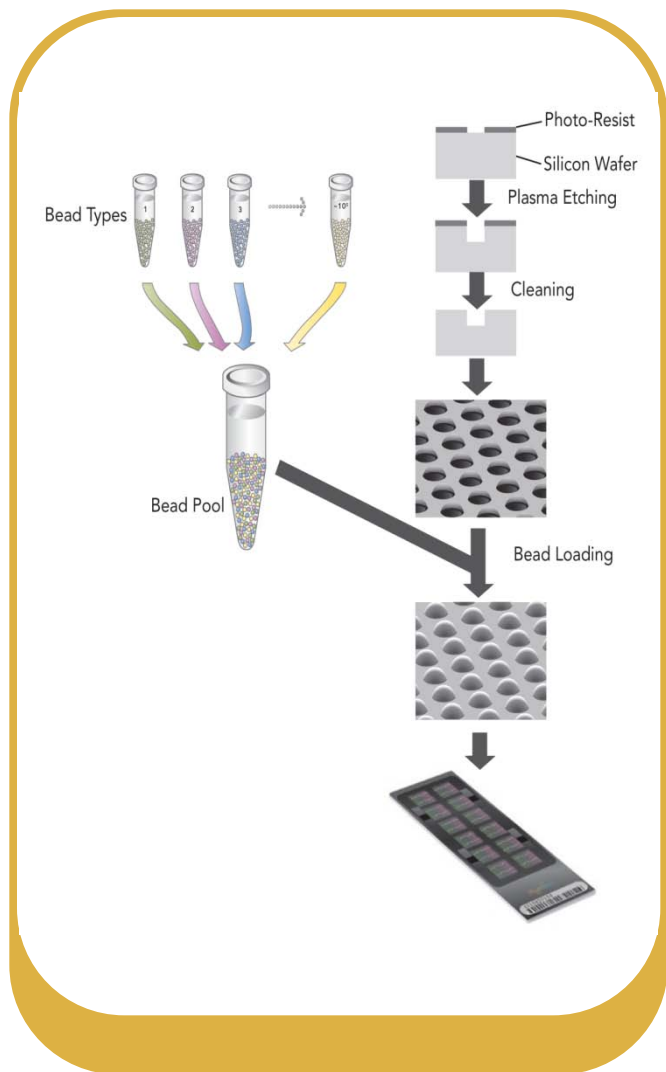
2 μ m beads in wells

Beads and BeadTypes

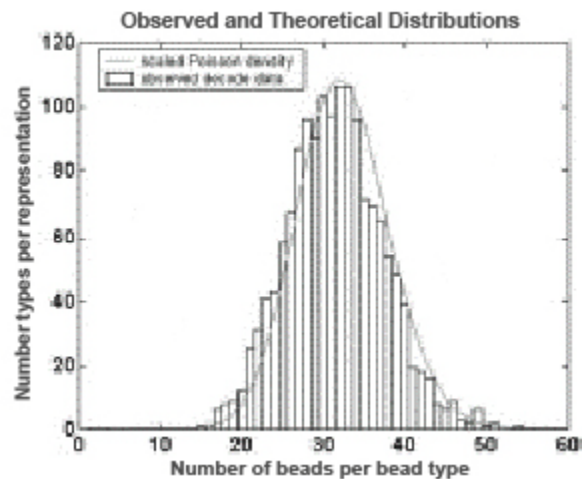


Redundancy: average of ~15 beads per beadtype

Bead Preparation and Array Production



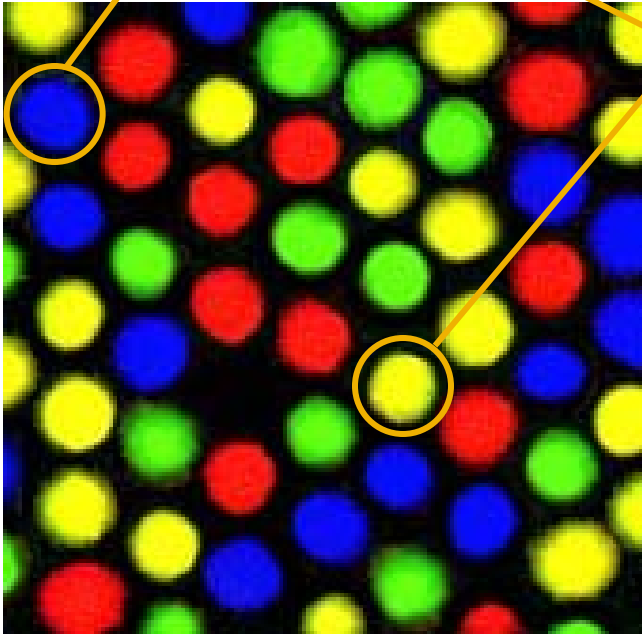
- Unique oligo for each bead type
- Bead Pool can be > 1,000,000 bead types
- Random self-assembly of beads
- Average ~15 beads per beadtype
- Functional validation of array



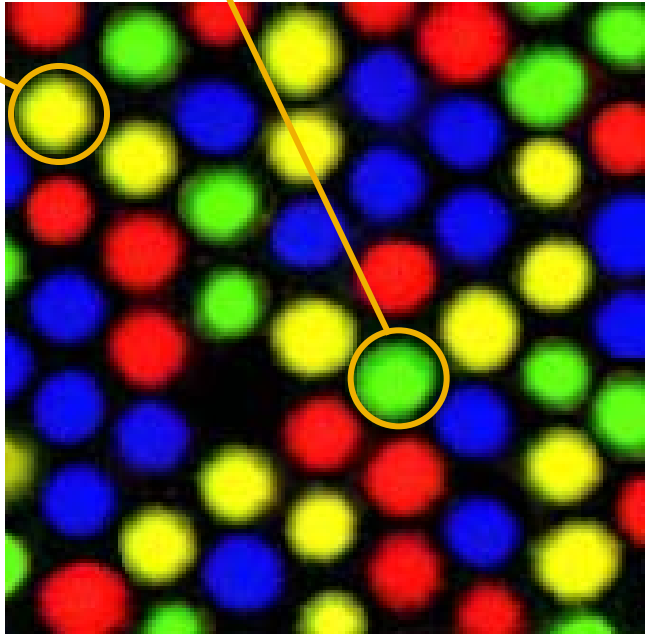
Bead Decoding

Example: 16 Bead Types

Decoder Oligo	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Decode hyb 1	Blue	Blue	Blue	Blue	Green	Green	Green	Green	Yellow	Yellow	Yellow	Yellow	Red	Red	Red	Red



Decoder hybridization 1

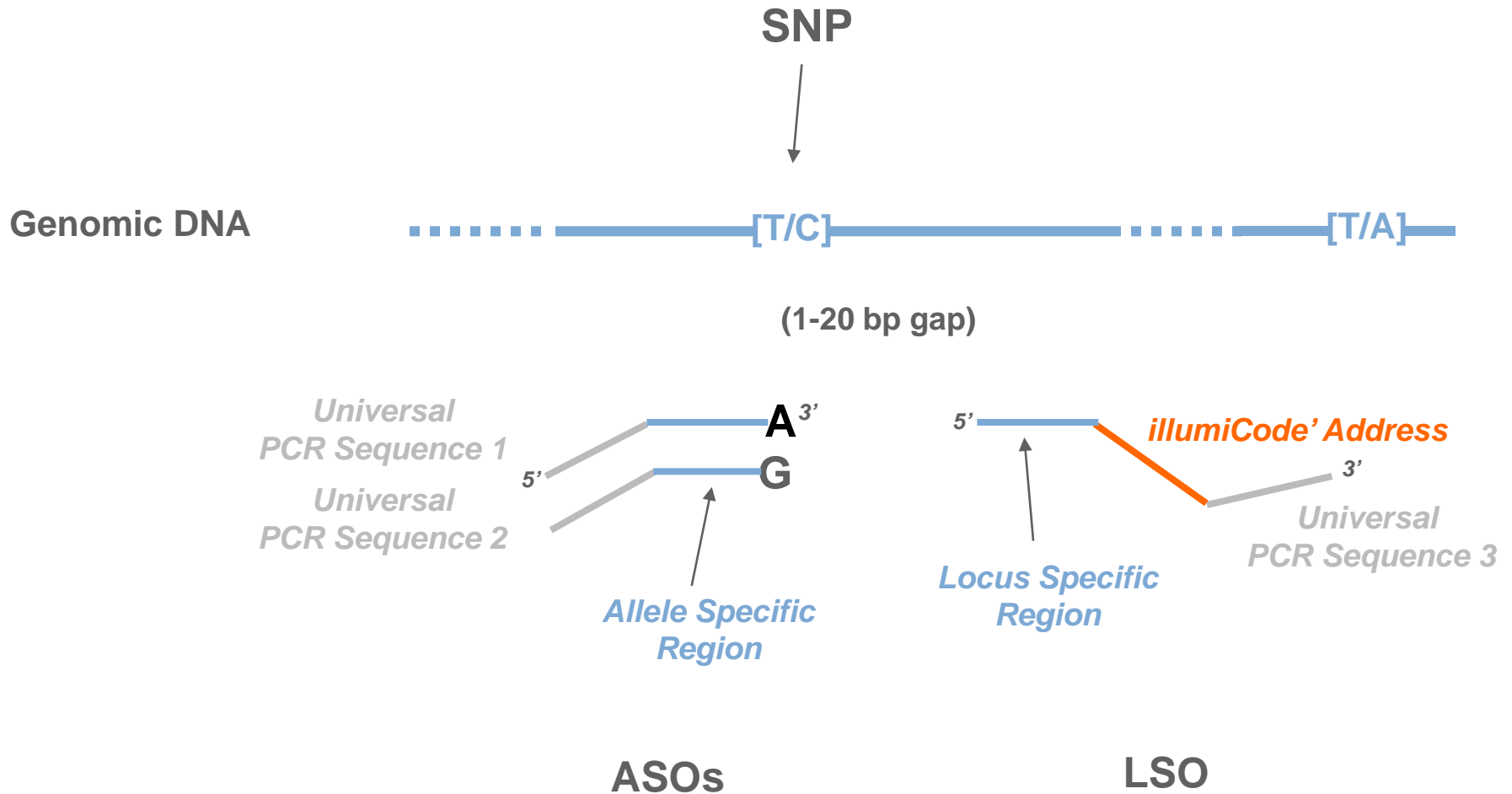


Decoder hybridization 2

Generating a **.dmap** file

GoldenGate Assay Biochemistry

GoldenGate Assay: Primer Design

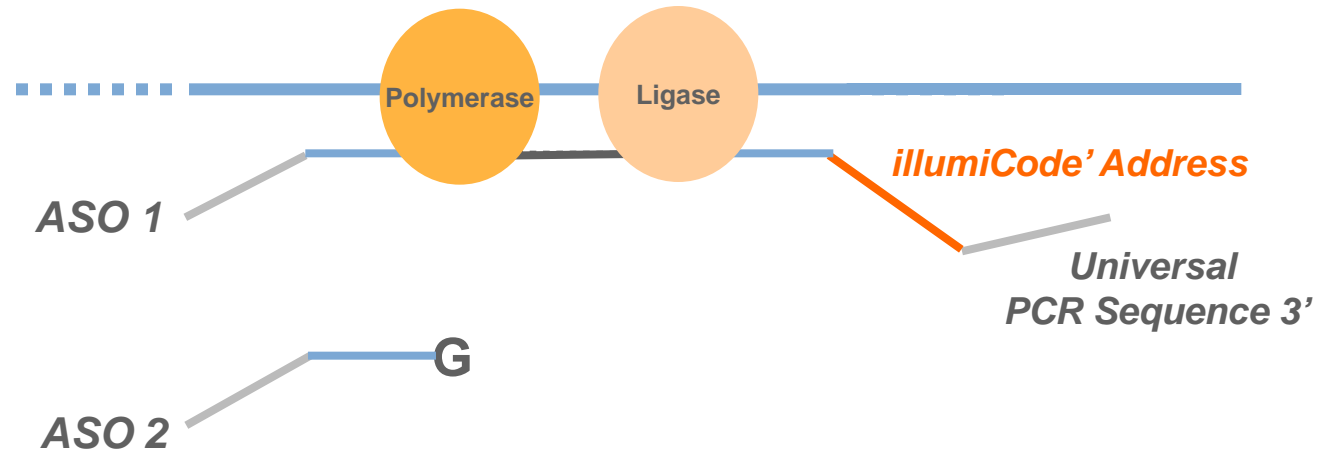


GoldenGate Assay: Biochemistry/Assay Diagram

- Allele-specific extension and ligation

Genomic DNA

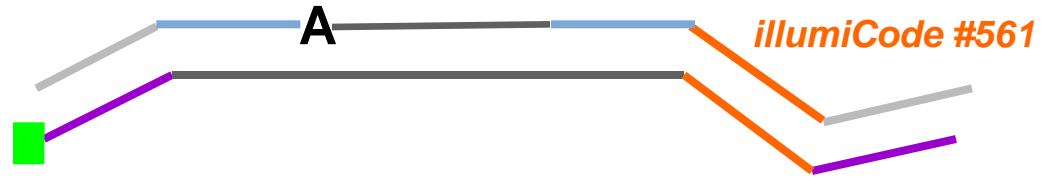
Allele Specific
Extension &
Ligation



GoldenGate Assay: Biochemistry/Assay Diagram

- Amplification

Amplification
Template



PCR with
Common
Primers

*Cy3 Universal
Primer 1*



*Cy5 Universal
Primer 2*

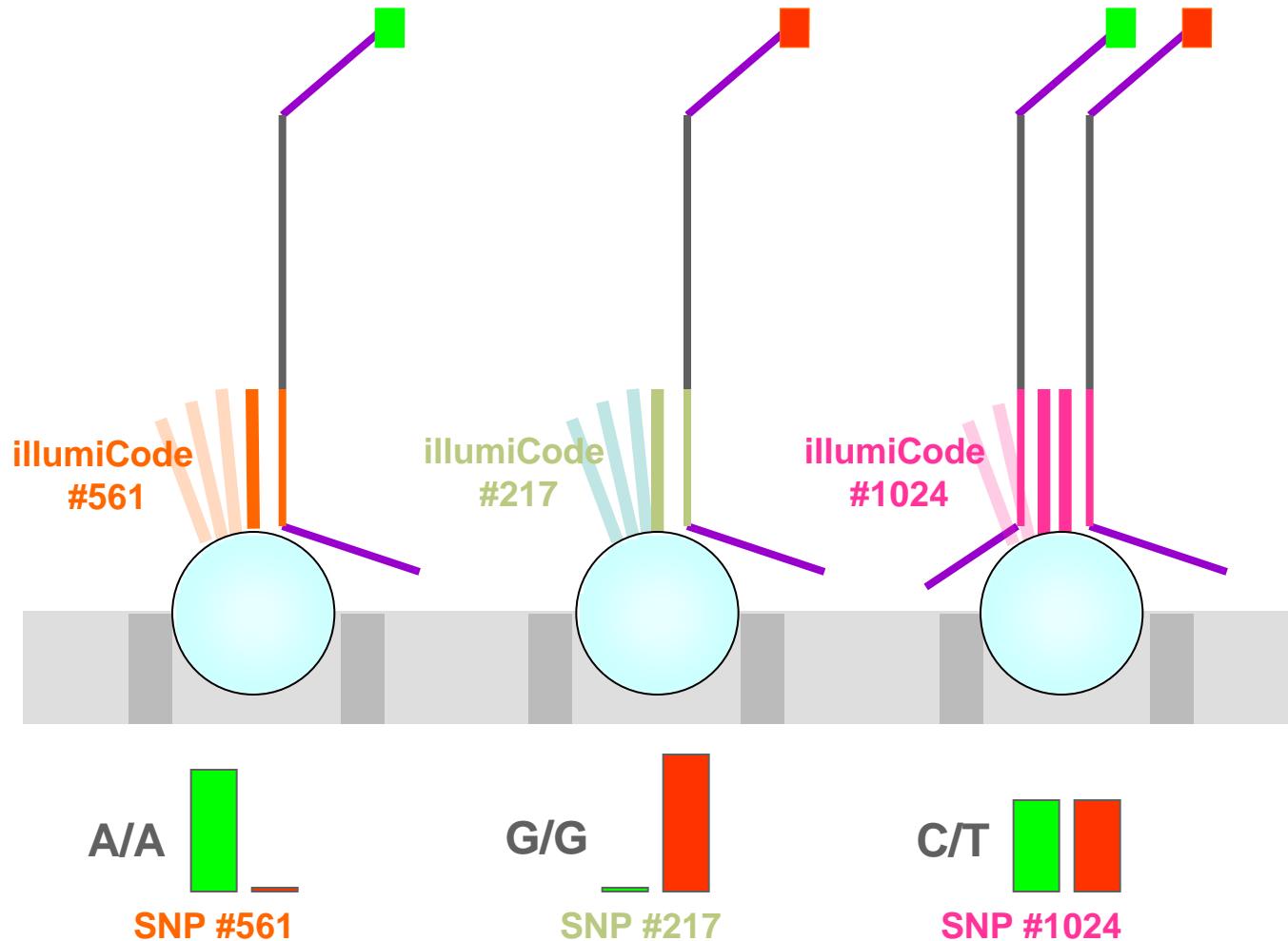


*Universal
Primer P3*



GoldenGate Assay: Biochemistry/Assay Diagram

- Hybridization to VBP



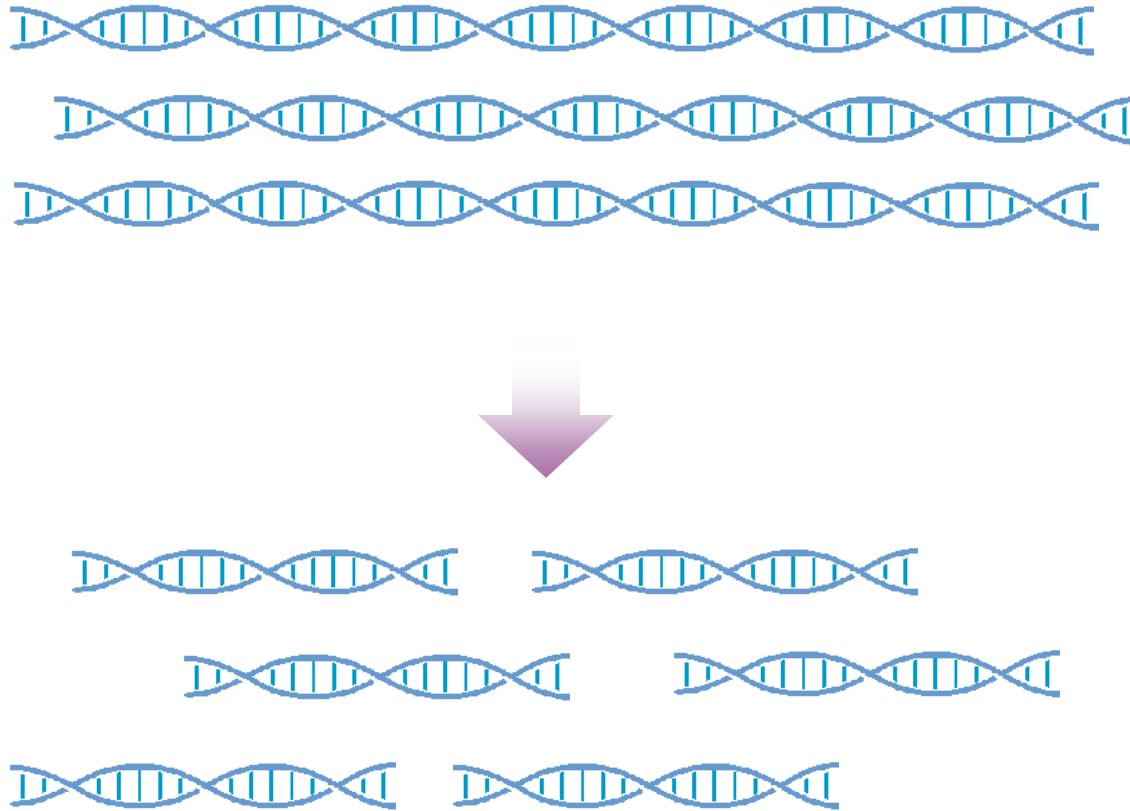
Infinium Assay Biochemistry

Whole-genome amplification



- ▶ **Optimized WGA reaction minimizes GC bias**
- ▶ **Up to 1000-fold amplification**

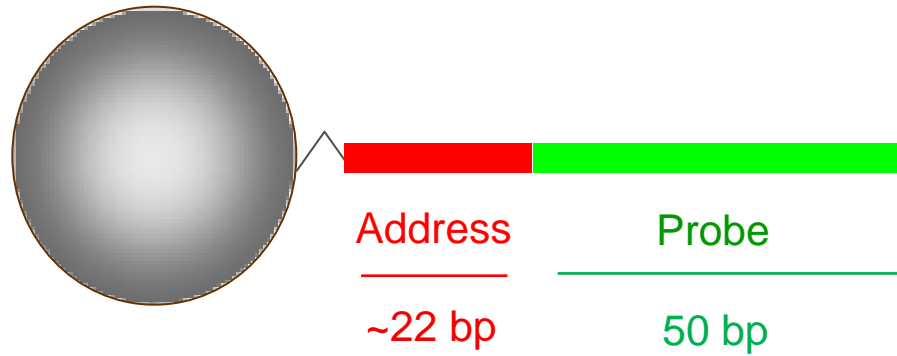
Fragmentation



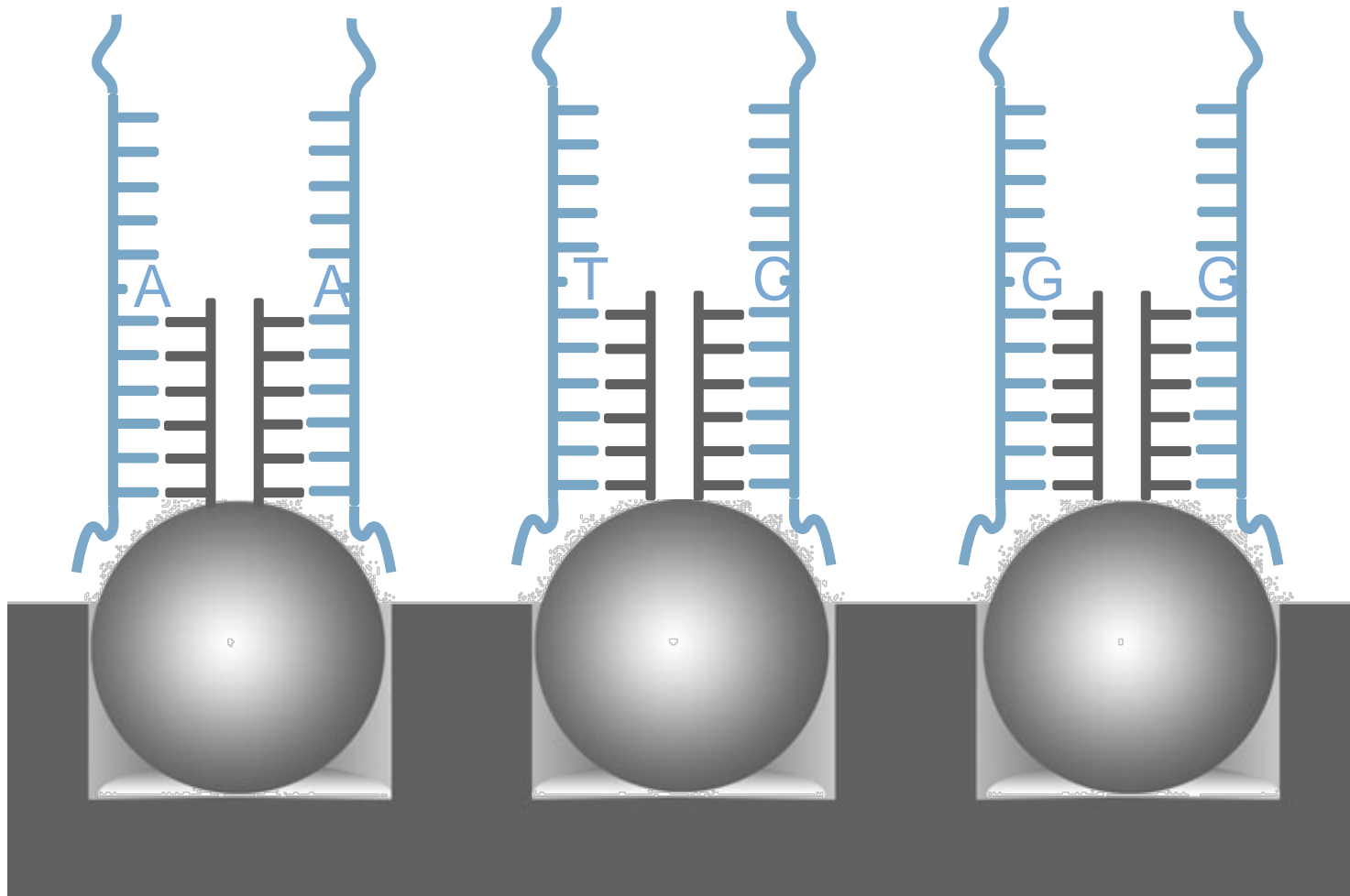
- Robust endpoint fragmentation
- Allows access to vast majority of genome

Infinium Probes and BeadTypes

Locus-Specific

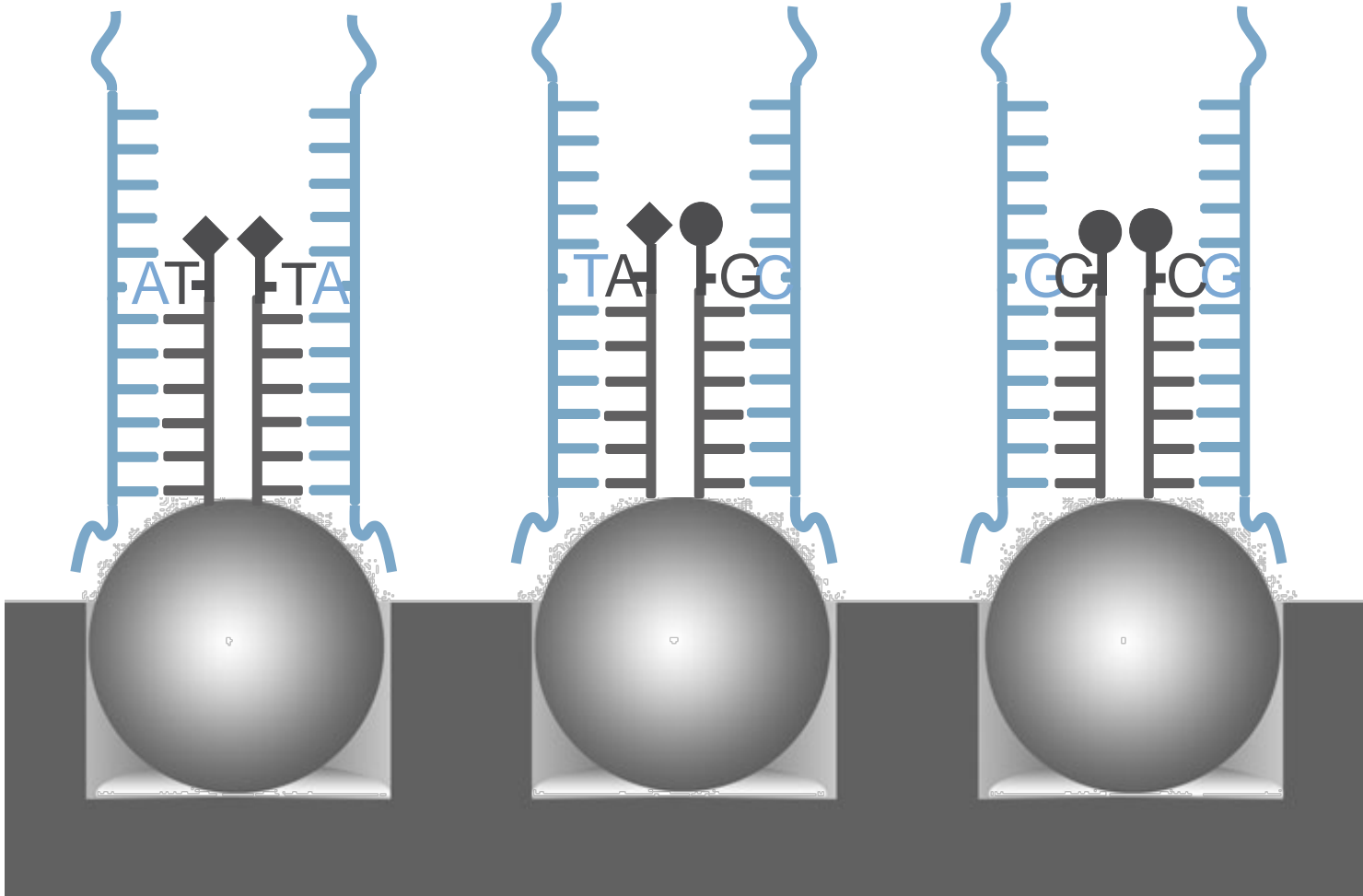


Hybridization

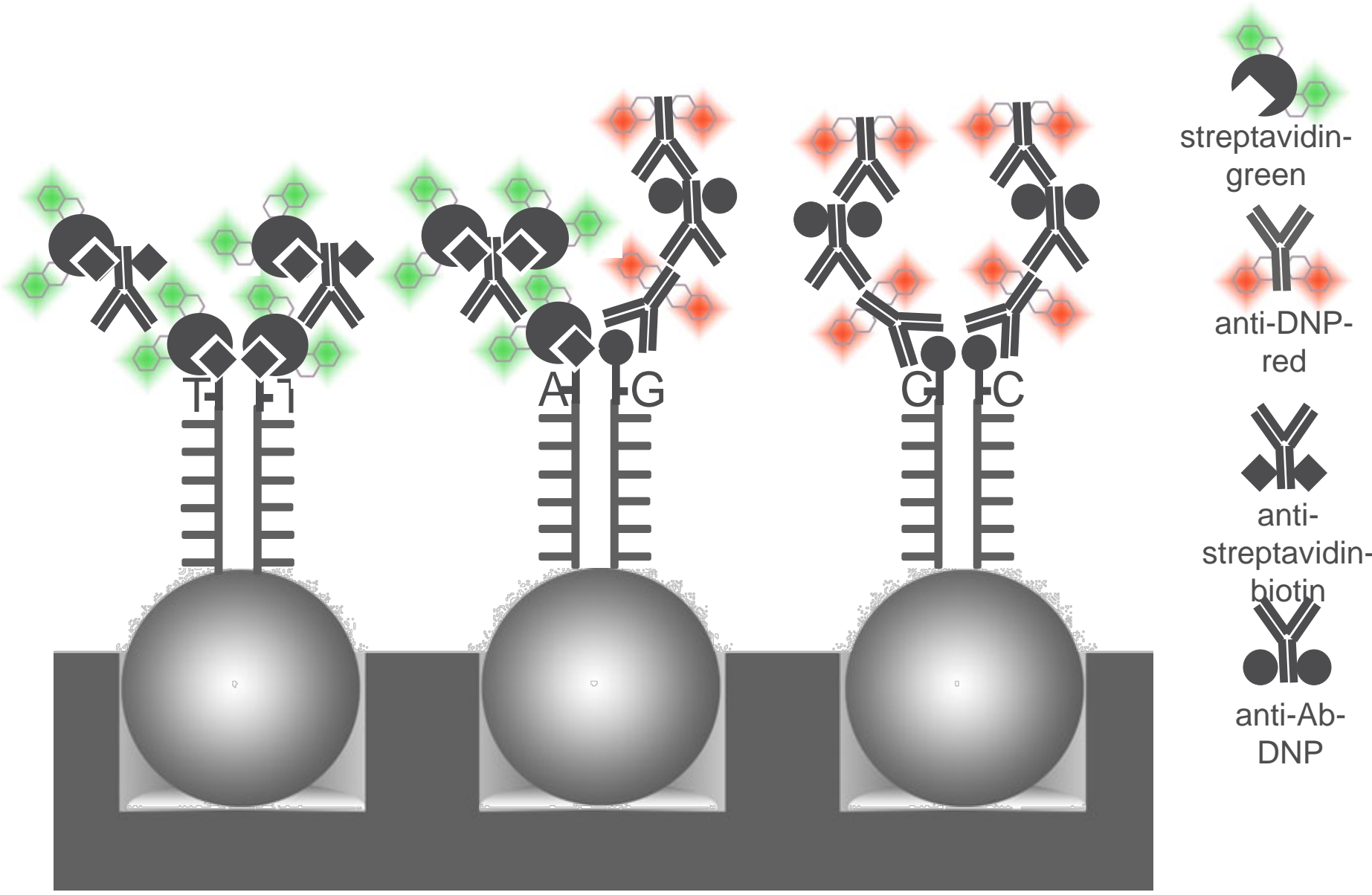


Single Base Extension

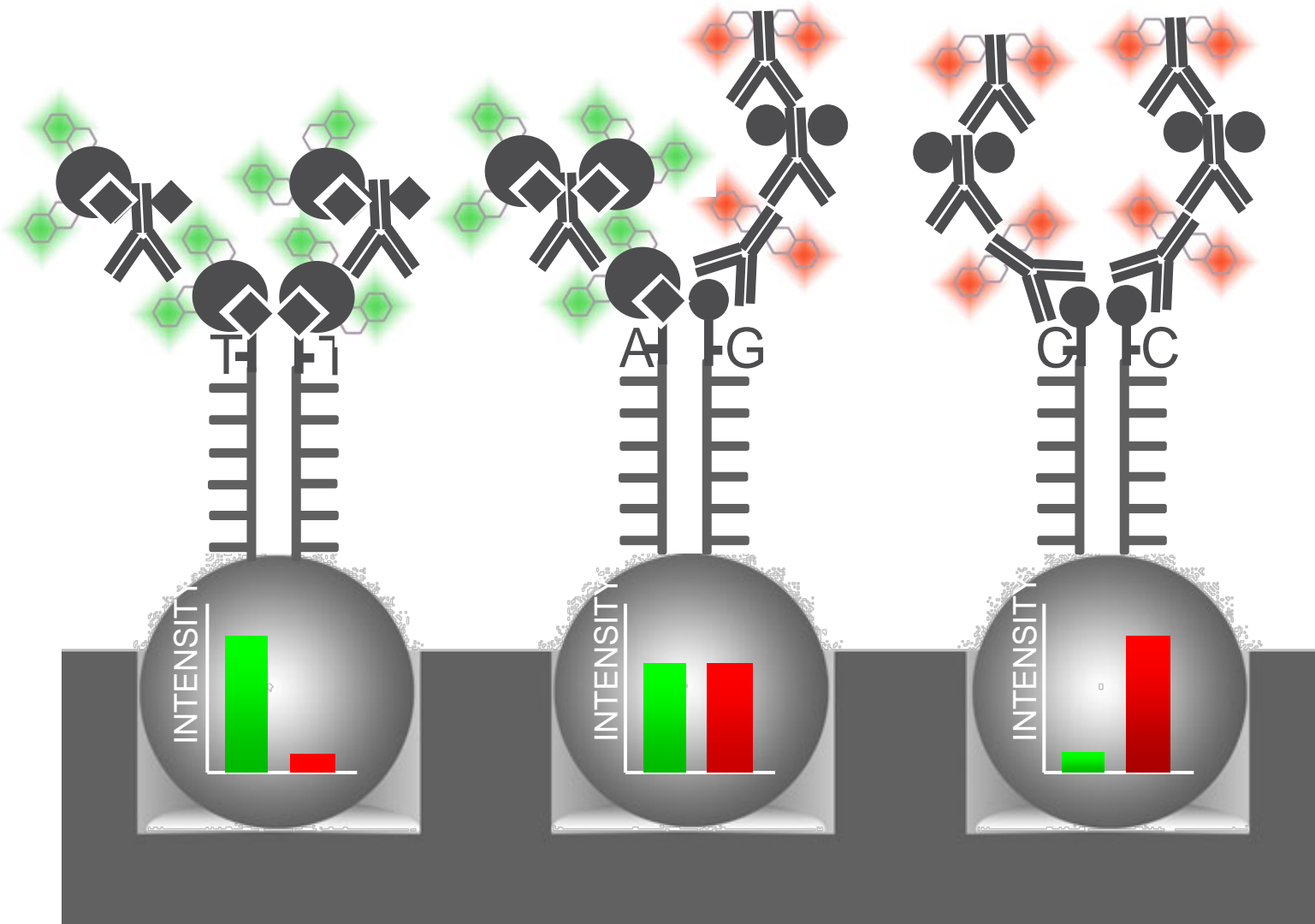
C G dinitrophenol-labeled ddNTPs
A T biotin-labeled ddNTPs



Stain



Image

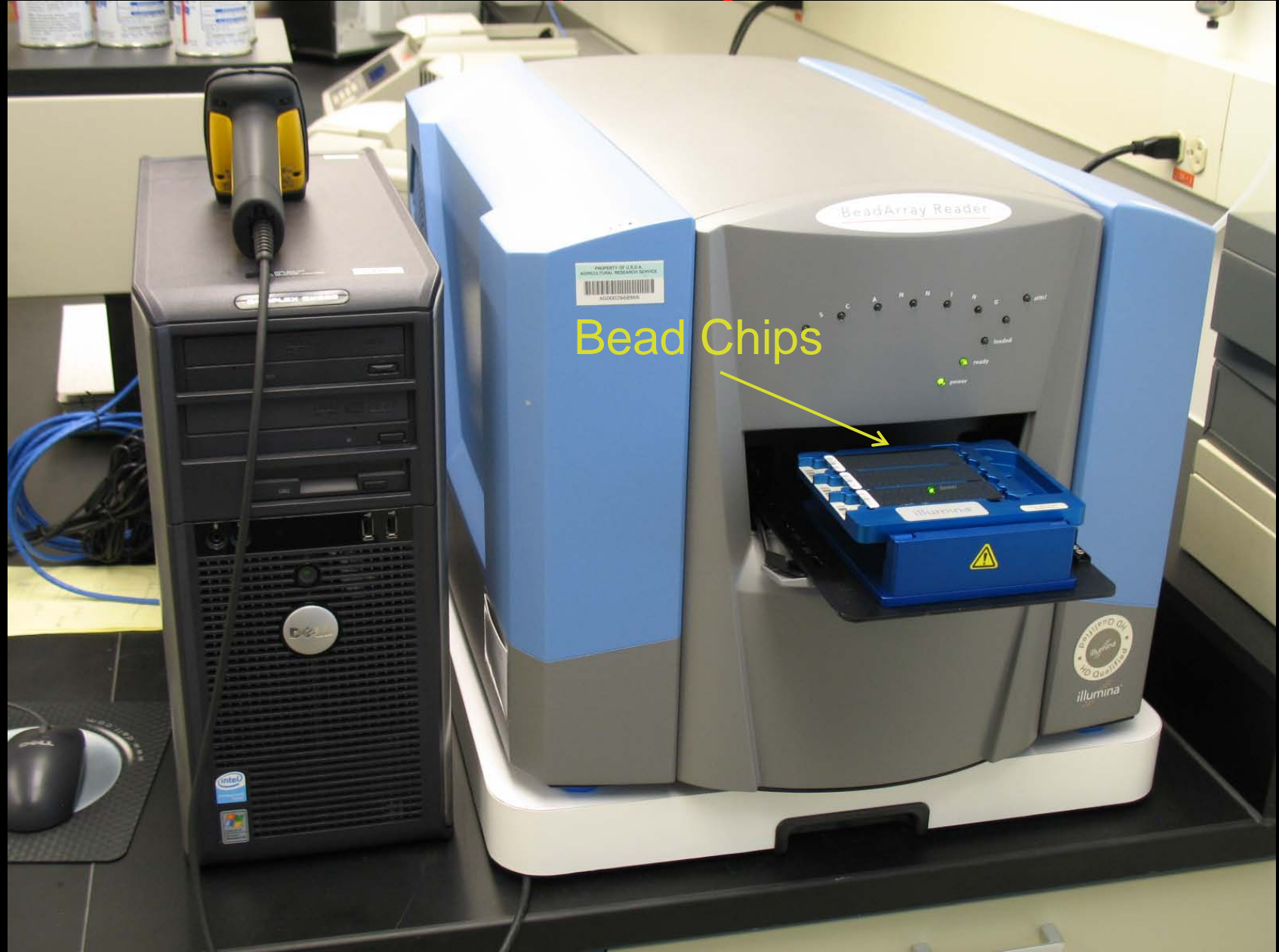


BeadChip Scanning and SNP Assay Detection

- Input file – .dmap files containing bead locations
- Output file – .idat files containing hybridization intensity data

BeadStation

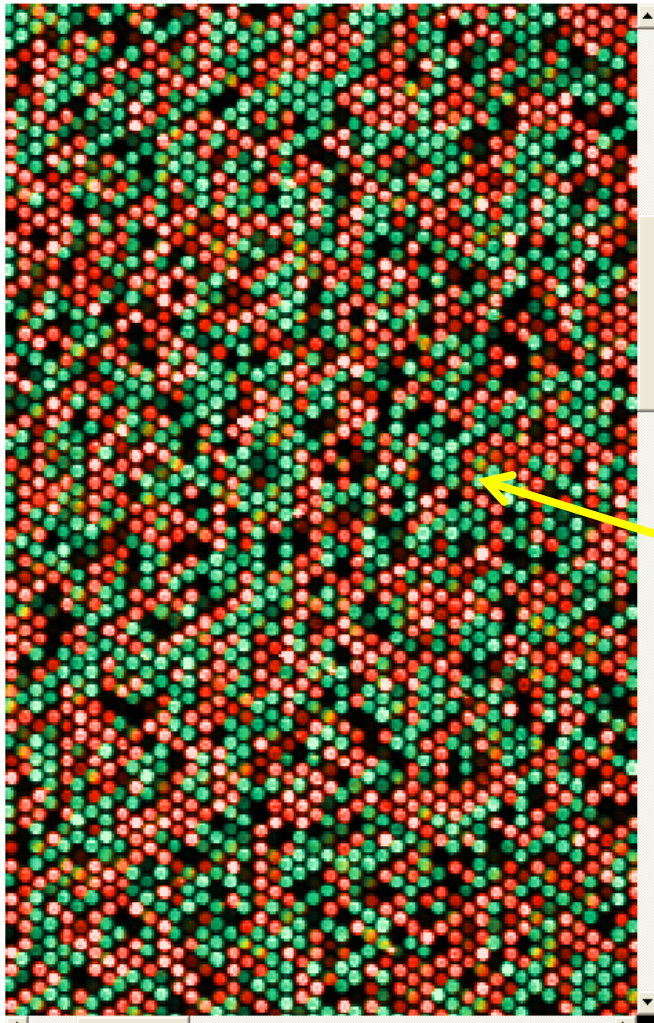
To scan BeadChips and generate raw data



BeadChip scanning in progress

illumina BeadScan

START → SETUP → TILT → ALIGN → **SCAN** → REV



Green

Red

Overlay

X 315
Y 450
Green 763
Red 3624

Section	G Sat	G P95	G P5	R Sat	R P95	R P5
R01C01	0	23460.09	00372.09	0	29560.55	00372.09
R01C02	0	21406.06	00362.73	0	28440.80	00362.73
R02C01	0	23240.21	00370.82	0	30050.16	00370.82
R02C02	0	24325.56	00349.02	0	31640.67	00349.02

5067458007

5067458013

5067458021

<< Stop

Pause

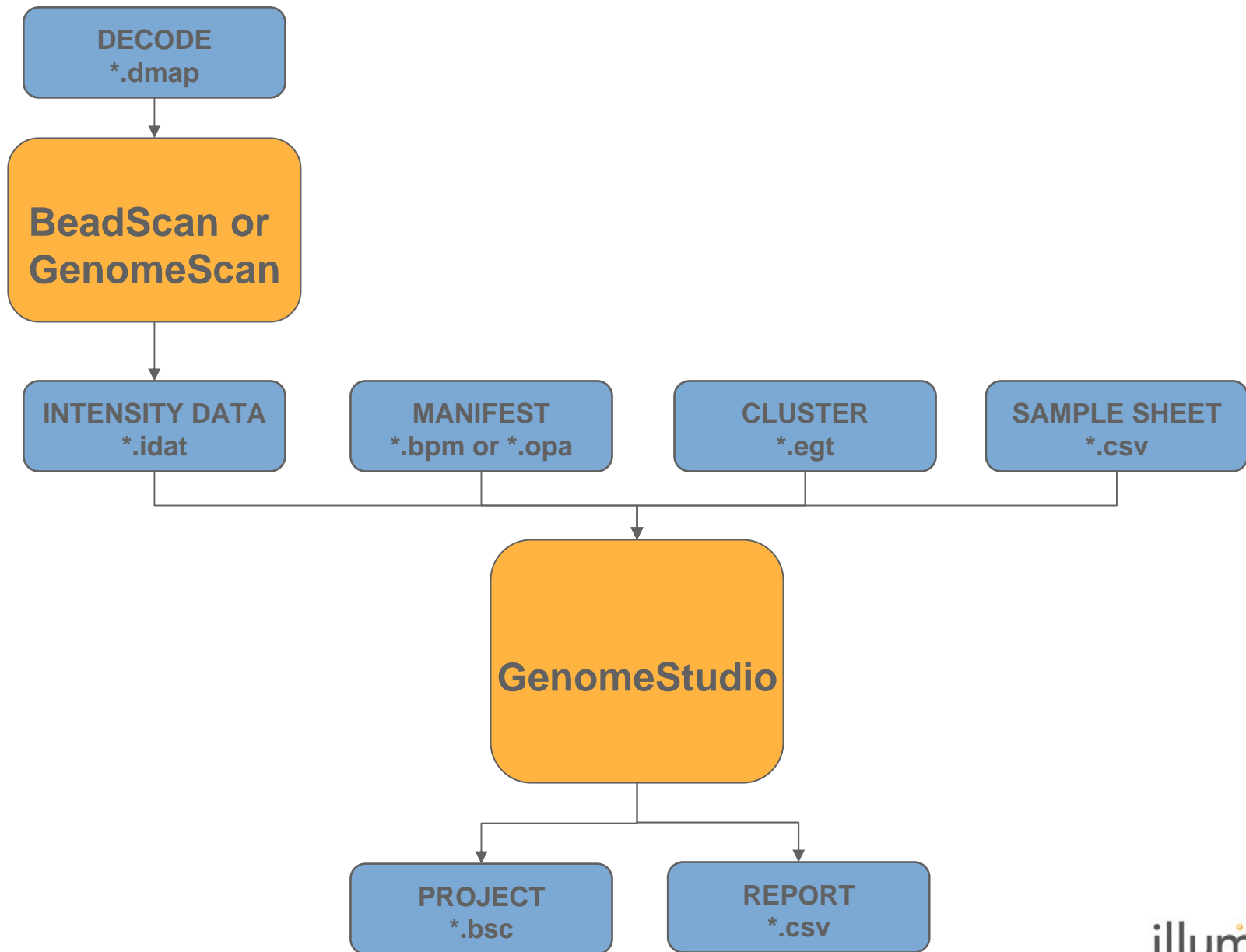
Each bead translates to raw data for one SNP

Data Analysis and Genotype Calling using GenomeStudio Software



Loading Data

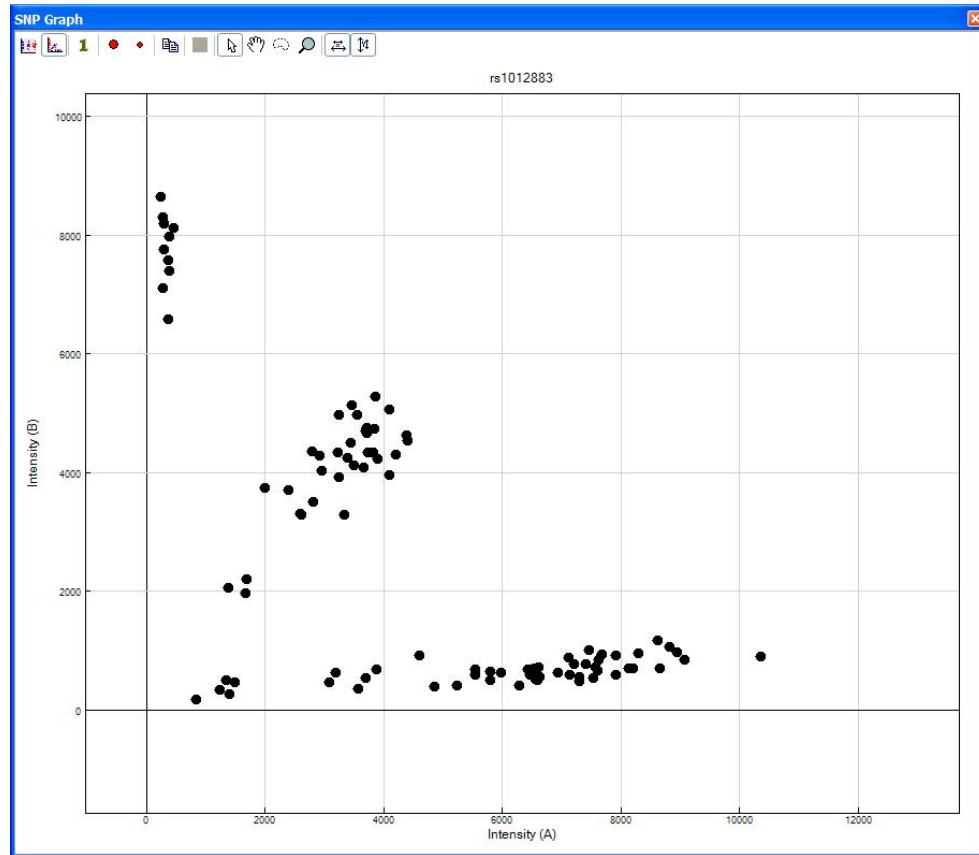
File Types



Load Data

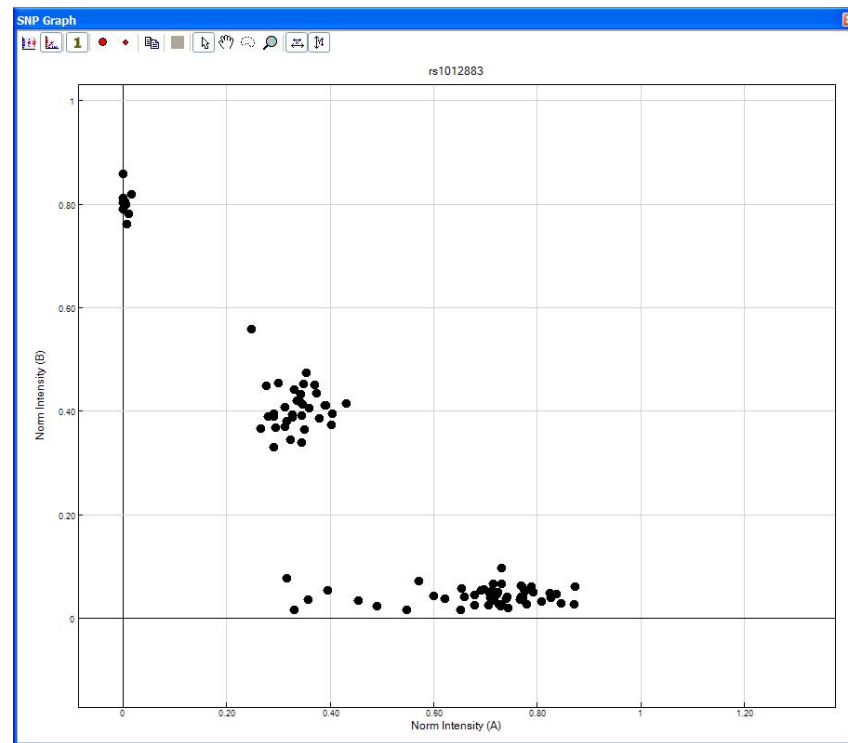
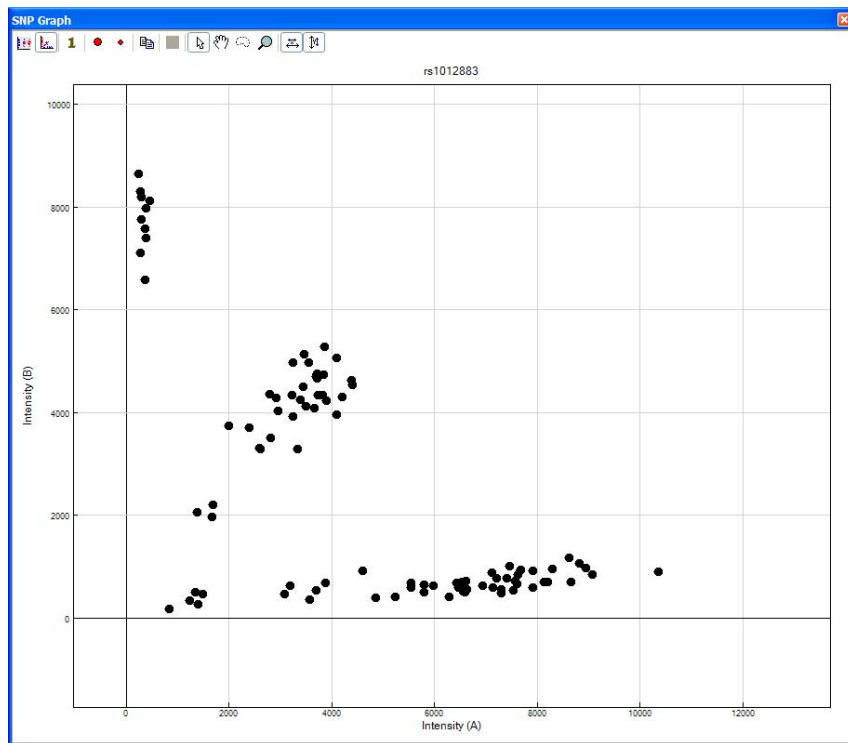
<u>File</u>	<u>Extension</u>	<u>Description</u>	<u>Source</u>	<u>Required?</u>
Data	*.idat	Contains the mean red and green signal intensities for each bead type	GenomeScan	Yes
Manifest	*.bpm *.opa	Lists the SNP ID and annotation for each bead type	iCom	Yes
Cluster	*.egt	For each locus, defines the allowable signal intensity ranges for AA, AB, and BB genotypes	iCom / User-created	No
Sample Sheet	*.csv	For each sample, lists the microtiter plate locations, replicate and family relationship information	User-created	No

Plotting intensities in Cartesian space



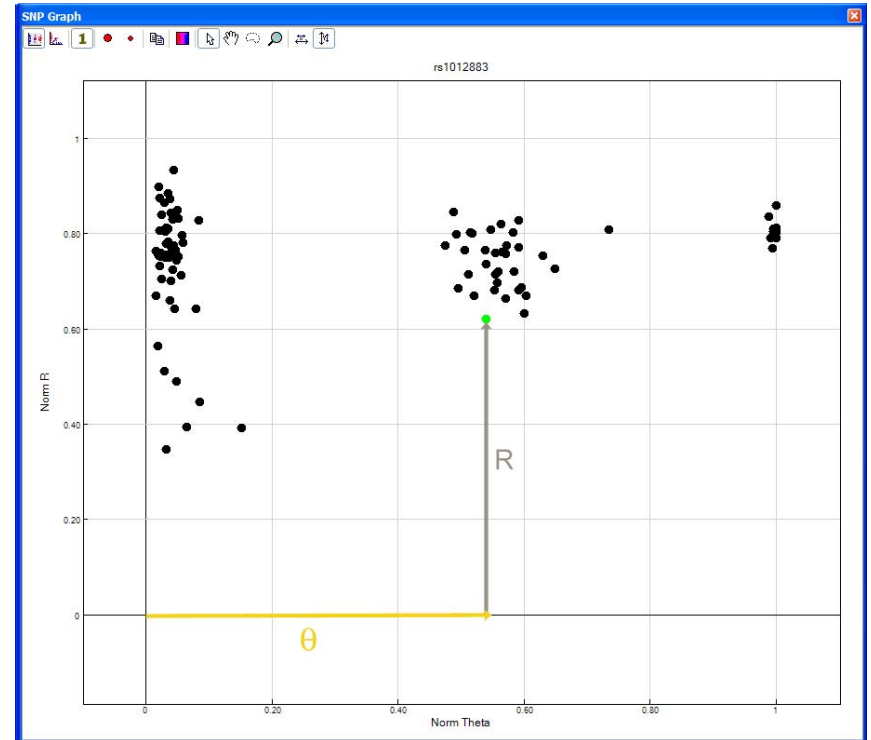
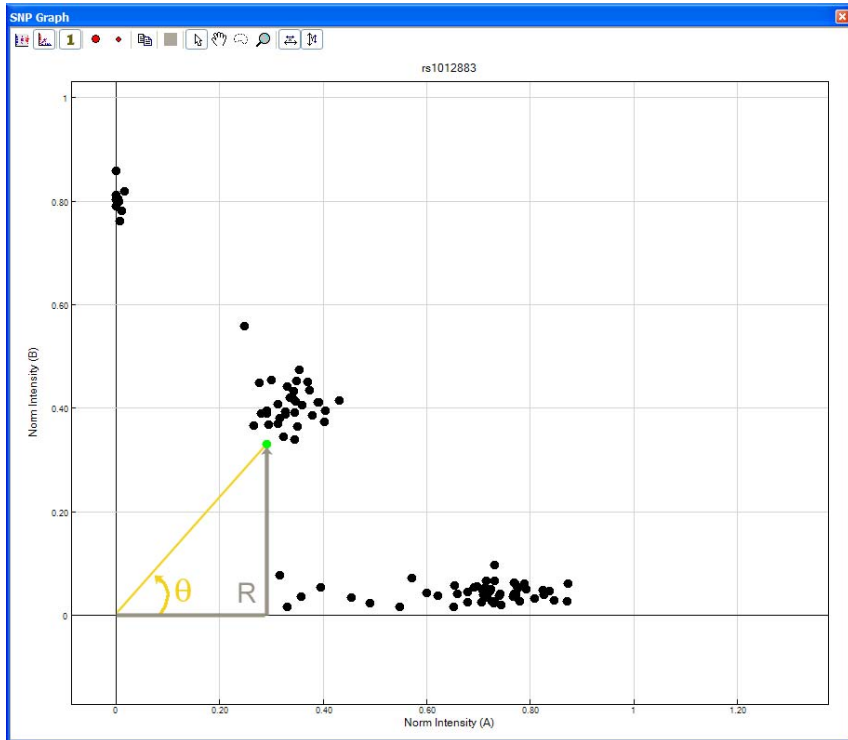
Signal intensities for 96 samples at one locus
Y axis is green signal intensity
X axis is red signal intensity

Normalization



Proprietary normalization algorithm

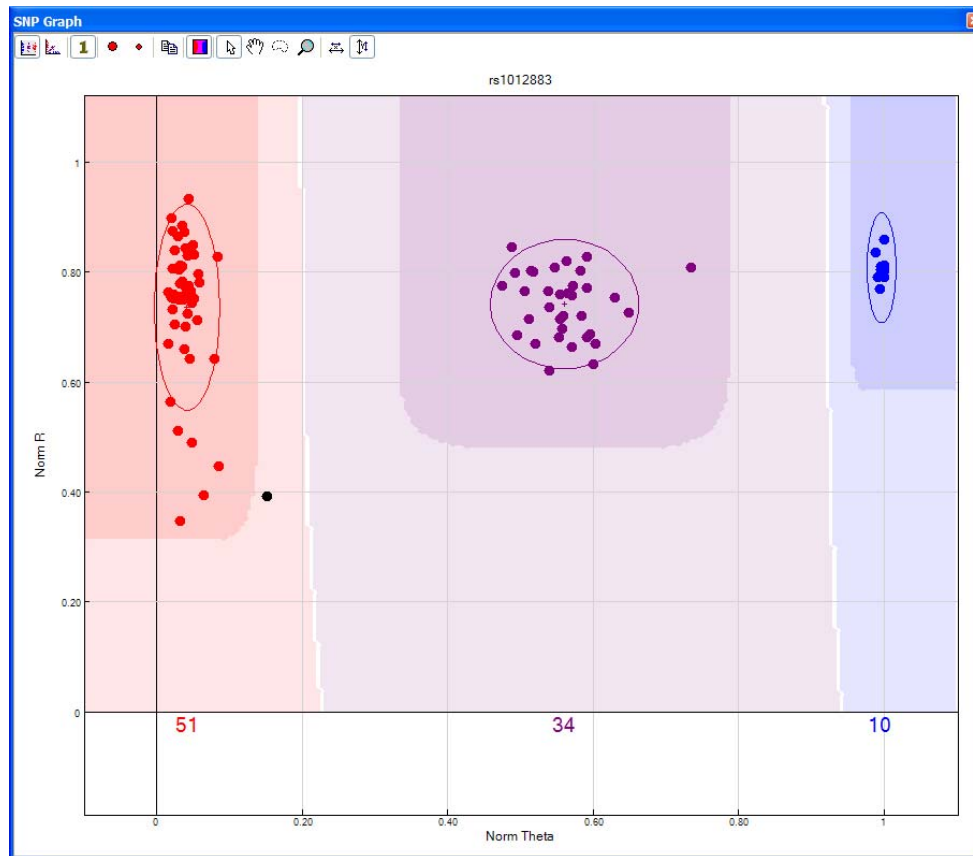
SNP Graph - Polar Coordinates



θ = angle from X axis

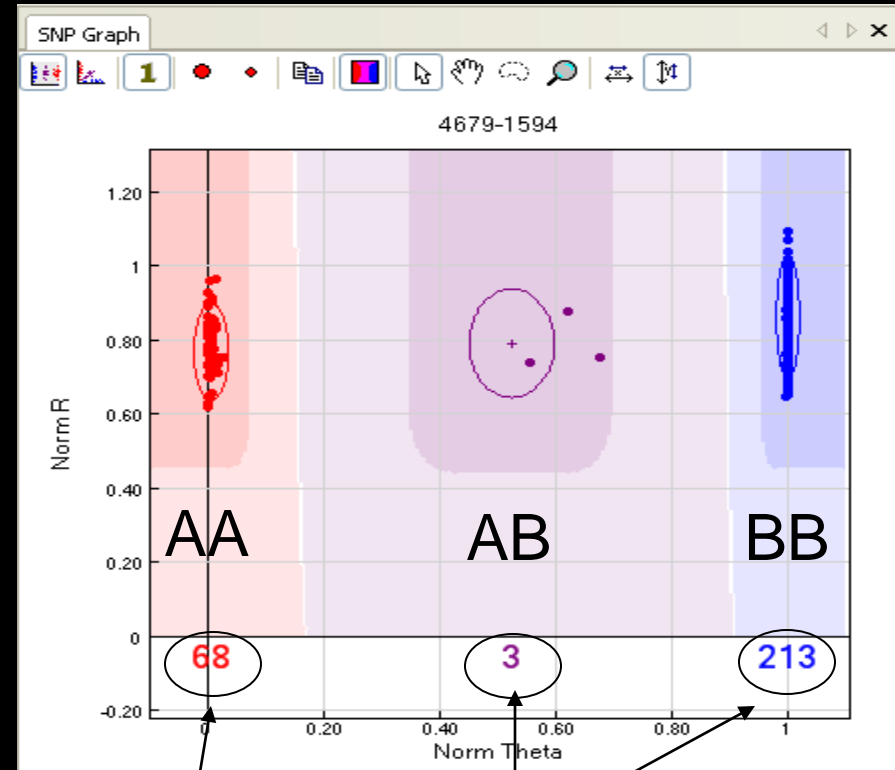
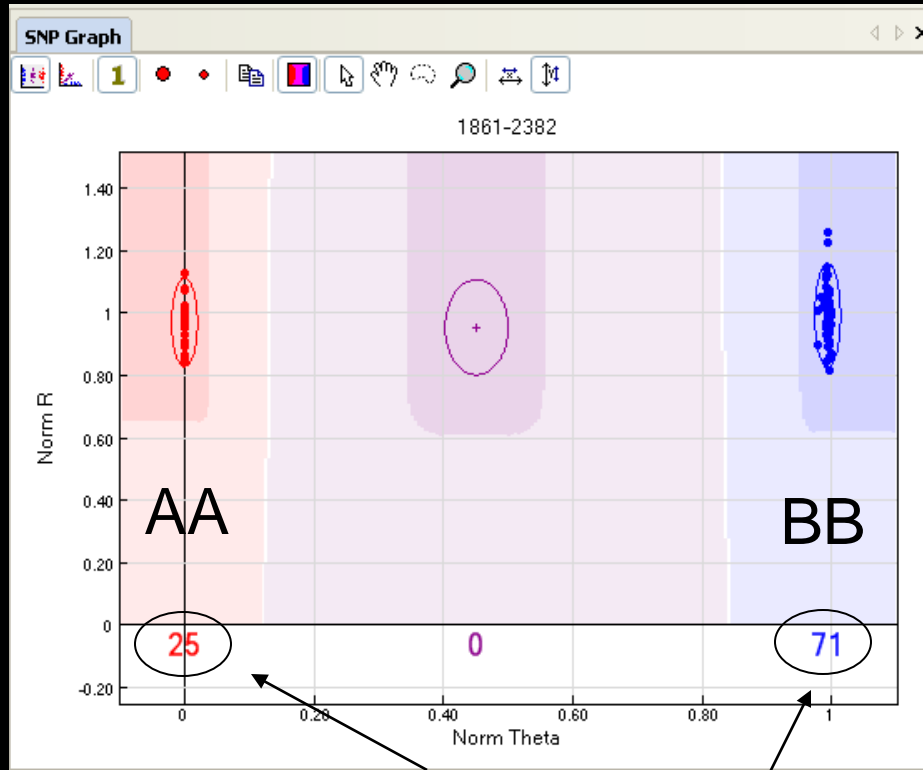
$$R = A + B$$

Clustering



- GenCall score reflects the distance of a data point to the centroid of a cluster
- GenCall score is a representation of the confidence with which a genotype call is made (GenTrain score and data to model fit)
- Dark shaded regions are call regions (GenCall score > 0.25 for GoldenGate and 0.15 for Infinium)

An Example of Barley SNP Genotype Calls



Number of individuals with that genotype

