Studying Genetic Variation I: Laboratory Techniques

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Genetic variation in other lectures

- **Population genetics, patterns of human genetic variation, linkage disequilibrium, HapMap, genome-wide association studies - Lynn Jorde**
- **Linkage analysis, genome-wide linkage studies, haplotype analysis, susceptibility to cancer- Elaine Ostrander**
- **Origins of genetic variants, types of variants, discovery methods, use of databases, HapMap, linkage disequilibrium - Jim Mullikan**

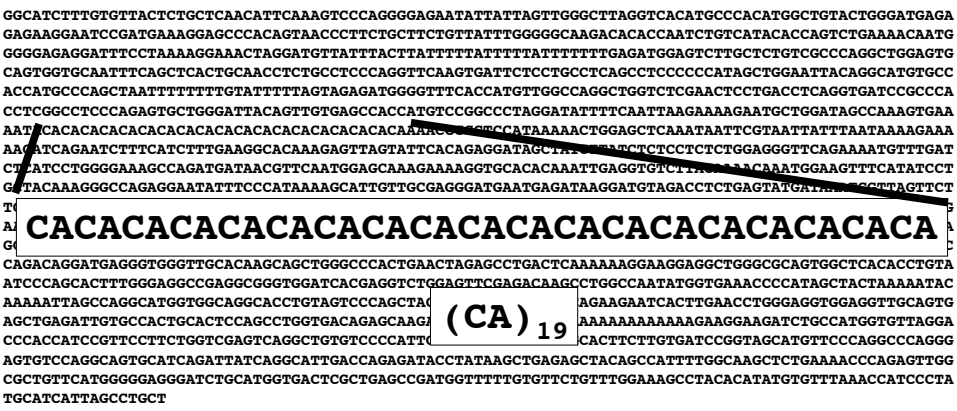
Human Genetic Variation

- **Types of variants**
- **Methods for scoring variants**
- **Genome-wide scoring of SNPs**
- **Structural variants**

Human Genetic Variation

- **Sequence repeats**
- **Single nucleotide polymorphisms**
- **Insertions and deletions**
- **Other structural variation**

Microsatellite

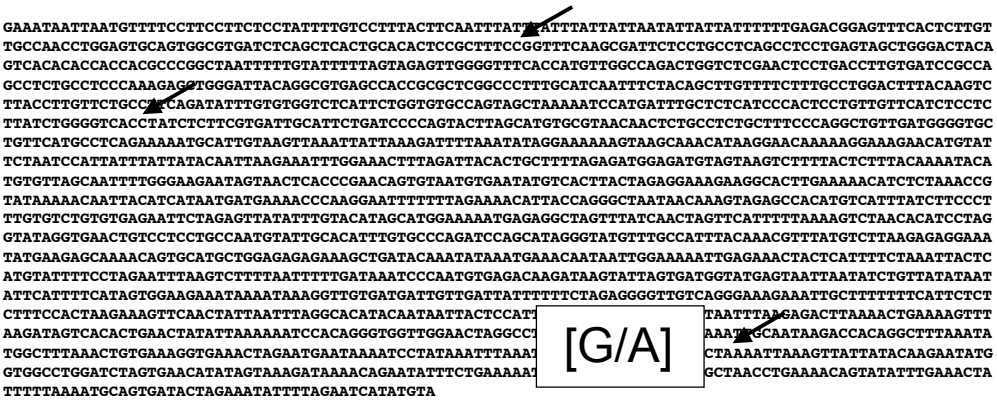


Example dinucleotide marker named
AFM059XA9 and D3S1262

Microsatellites

- **Many alleles, highly informative**
- **>50,000 in human genome**
- **Relatively high mutation rate**
- **Used to build first framework map**

Single nucleotide polymorphisms (SNPs)



Three SNPs are located at positions 49,752,348, 49,752,721 and 49,754,018 (hg17).

SNPs

- **Less polymorphic/informative**
- **More stable inheritance**
- **~1 SNP with frequency greater than 1% per 300 nucleotides (10 million in genome)**
- **Mutation at CpG 10-fold higher rate**
- **Exist in coding regions**

Deletion/insertion polymorphisms (indels)

- **One to many nucleotides present or not**
- **Example:**

AGTATCTTCACAGAAATGACCATA

AGTATCTTCACAAGAAATGACCATA

AGTATCTTCACA[-/A]GAAATGACCATA

Indel polymorphisms

Another example:

CAGACTCAATAAGCATGTTTTTACAGACTCAATAAGCATGTTT
TTTTTTTTTTTTTTTTTTTGAGACGGAGTCTCGCTCTGTCGCCCA
GGCTGGAGTGCAGTGGCGCGATCTCGGCTCACTGCAAGCTC
CGCCTCCCGGGTTCACGCCATTCTCCTGCCTCAGCCTCCCGA
GTAGCTGGGACTACAGGCTCCCGCCACCACGCCCGGCTAAT
TTTTTGTATTTTTAGTAGAGACGGGGTTAGCATGTTTTT

CAGACTCAATA[LARGEINSERTION/-]AGCATGTTTTT

Structural variation

- **Includes deletions, insertions, duplications, inversions, translocations**
- **~1 million > 1 bp, at least 1500 > 1kb**
- **Many small indels are in linkage disequilibrium with nearby SNPs**
- **Some deletions and rearrangements recur between repeated sequences**

Human Genetic Variation

- **Types of variants**
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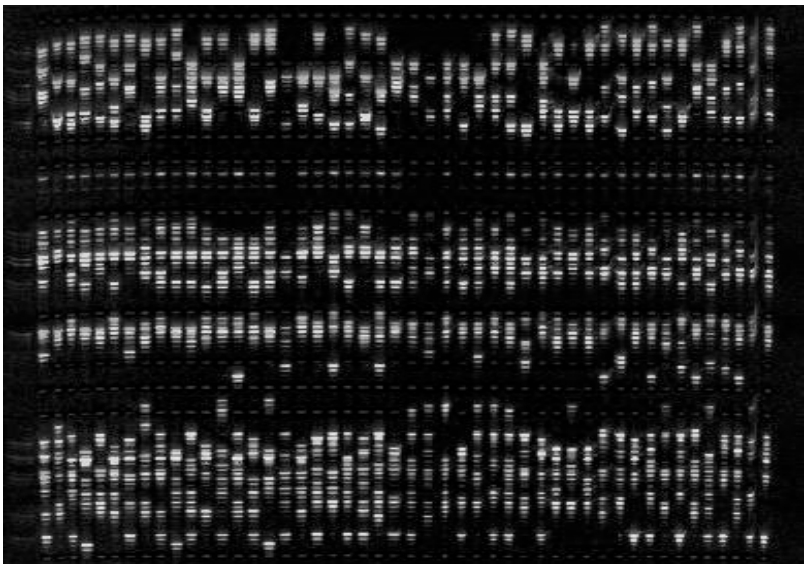
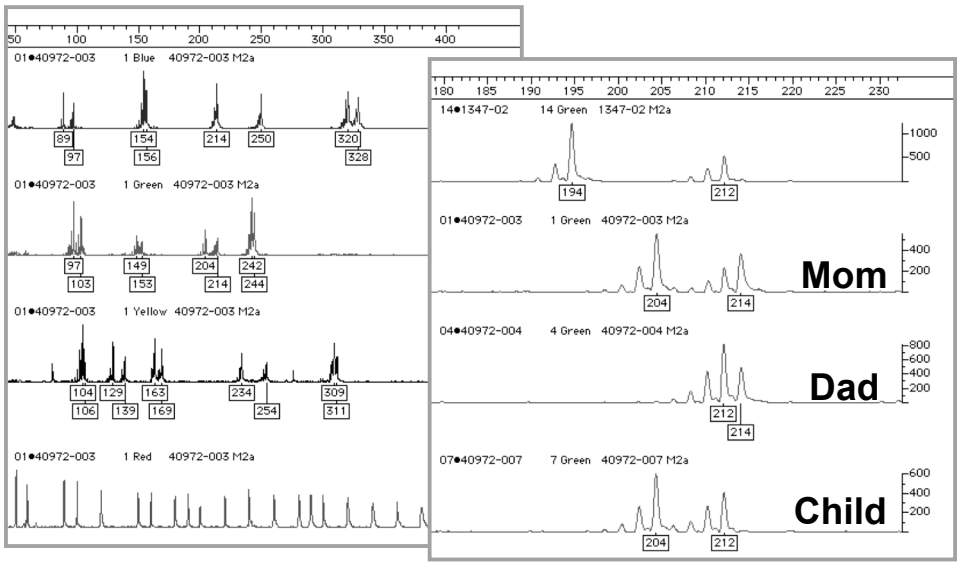
Scoring variants

- **Scoring = genotyping = typing**
- **Laboratory technique depends on**
  - **Type of variant**
  - **Fixed or custom set of variants**
  - **Number of variants**
  - **Number of samples**

Scoring Microsatellites



Scoring Microsatellites



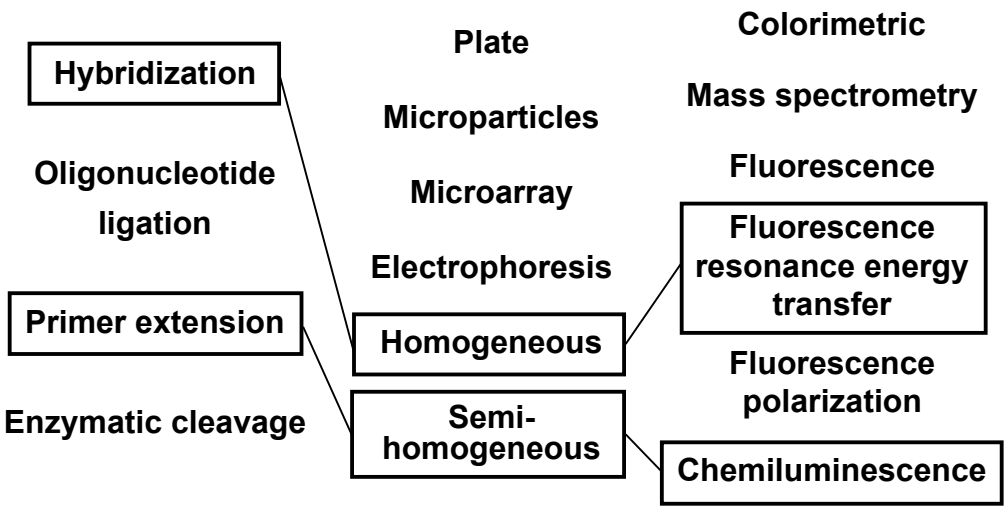
Scoring SNPs

- **Genotype accuracy**
- **Cost of assays and specialized instrument(s)**
- **Assay development time and ease**
- **Ability to automate**

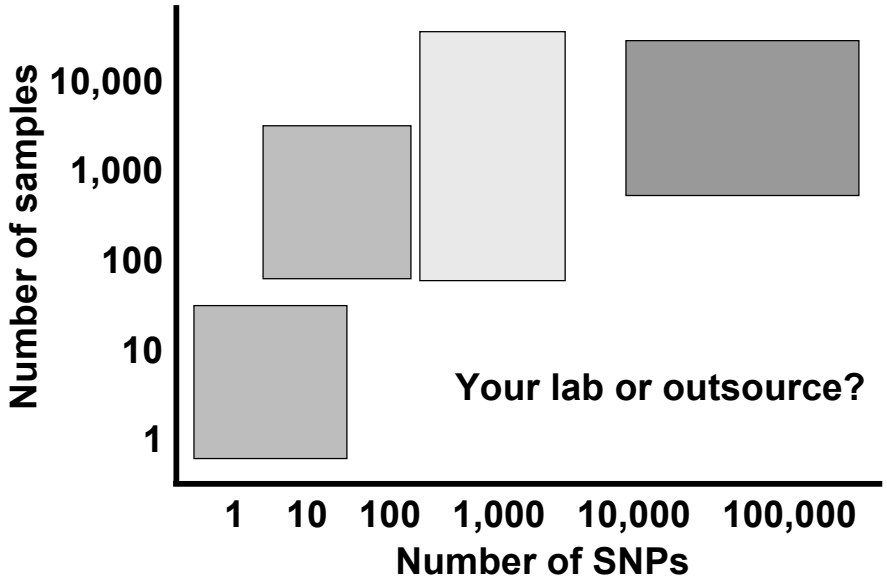
Scoring SNPs (2)

- **Time to perform assays**
- **Ability to multiplex**
- **Data accumulation and analysis**
- **Allele frequency quantification**

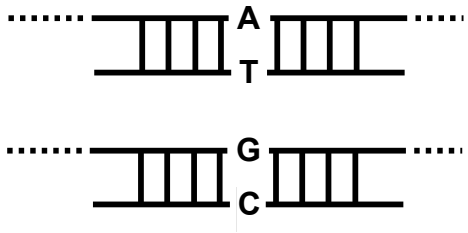
Overview of SNP typing methods



Which SNP genotyping method?

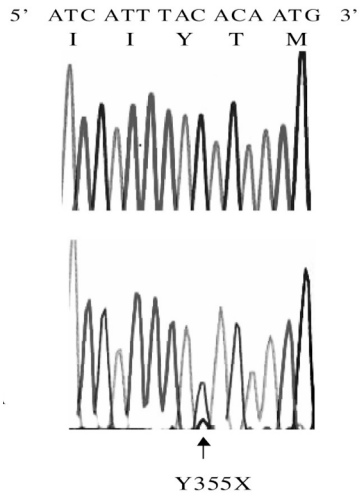


Example SNP



Sequencing

PPARG Y355X

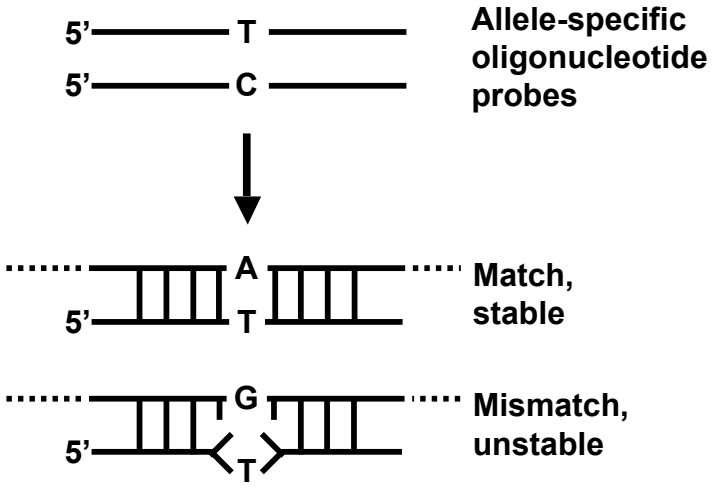


Francis et al. 2006 BMC Med Gen 7:3

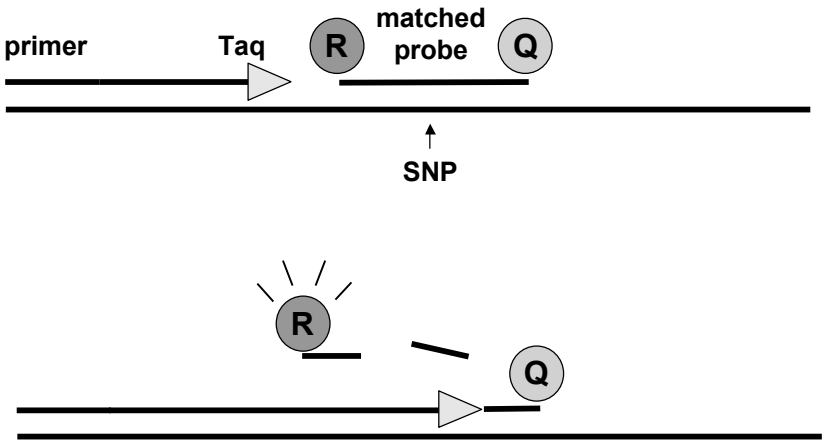
Sequencing

- **Advantages:**
  - **Instrumentation widely available**
  - **Easy and fast for small studies**
- **Disadvantages**
  - **Expensive for many SNPs or samples**
  - **Local sequence affects success**

Hybridization



Fluorescence resonance energy transfer (FRET)



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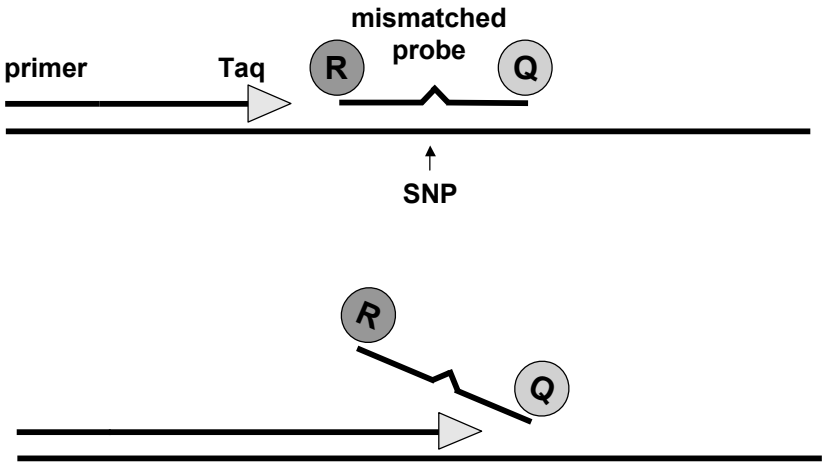
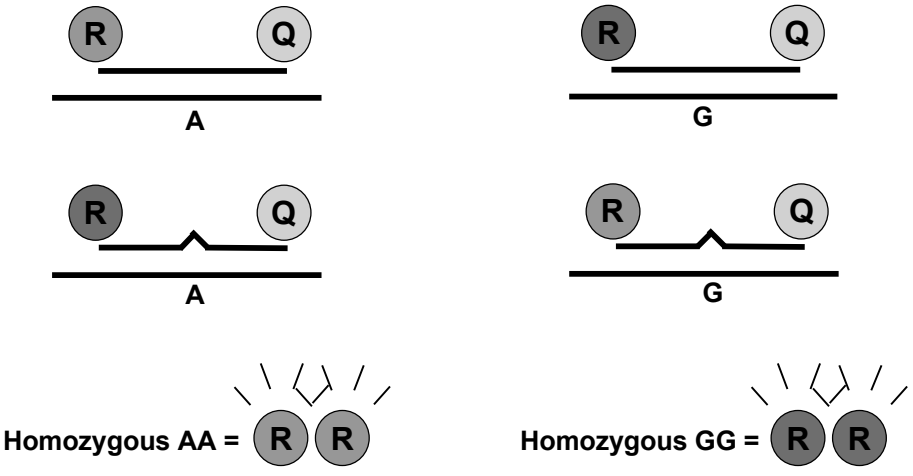
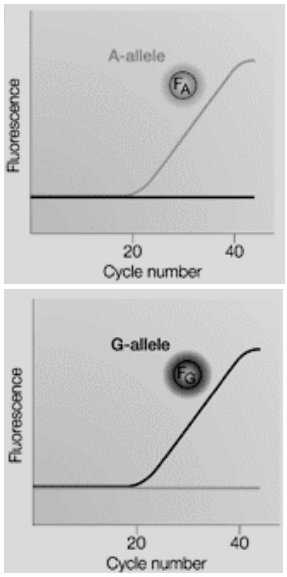
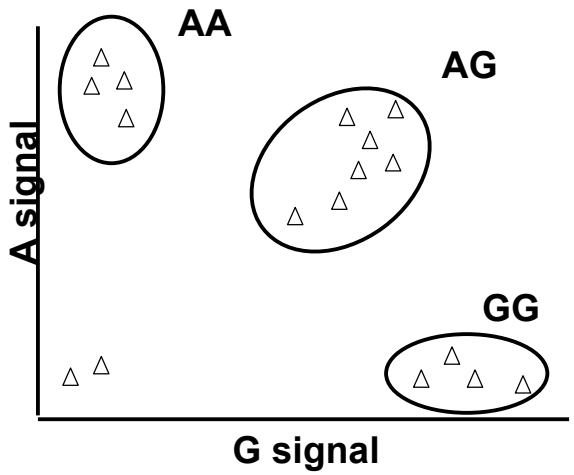


TaqMan competing probes



TaqMan genotype scoring





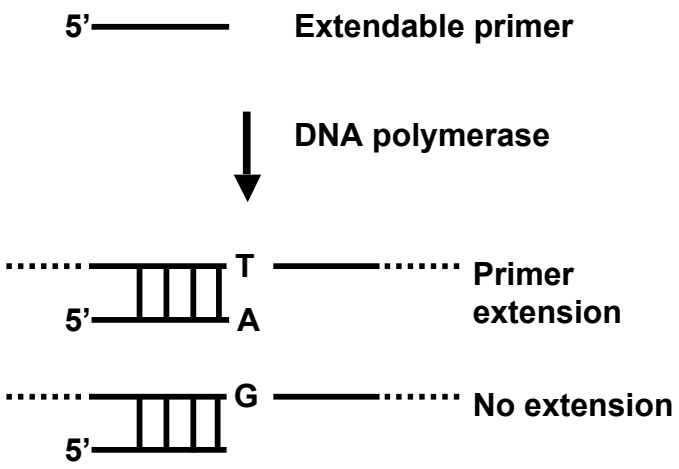
TaqMan

- **Advantages:**
  - **Simple to perform**
  - **Closed-tube system**
  - **Accurate quantification**

- **Disadvantages**
  - **Expensive probes**
  - **No multiplexing**
  - **Assays require optimization**

appliedbiosystems.com

Primer extension = Minisequencing



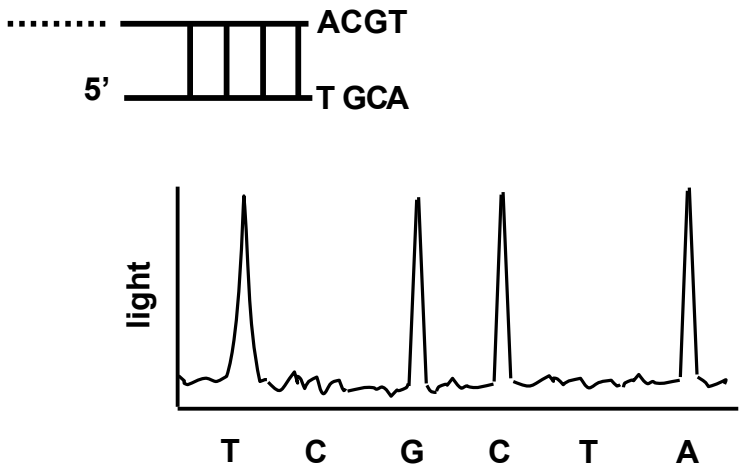
Pyrosequencing

- **Four enzymes**
  - **DNA polymerase**
  - **ATP sulfurylase--converts pyrophosphate to ATP**
  - **Luciferase--converts ATP to light**
  - **Apyrase--degrades excess nucleotides**
- **Nucleotides added sequentially**

pyrosequencing.com

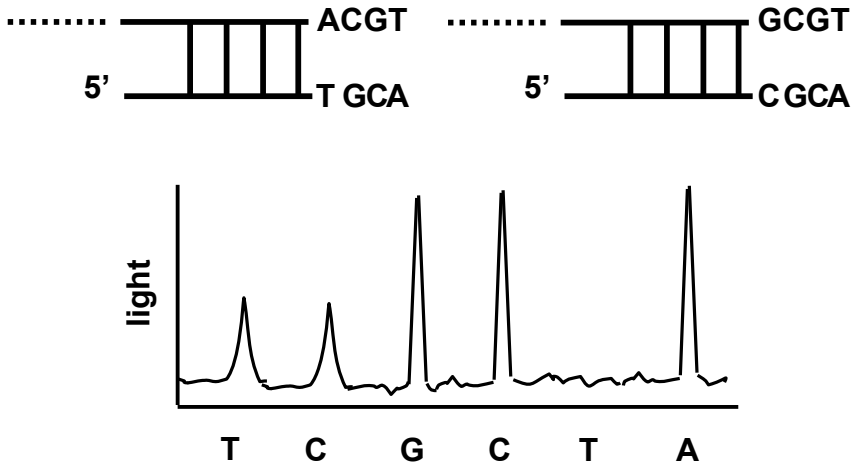
Pyrosequencing

…[A/G]CGT…



Pyrosequencing

…[A/G]CGT…



Pyrosequencing

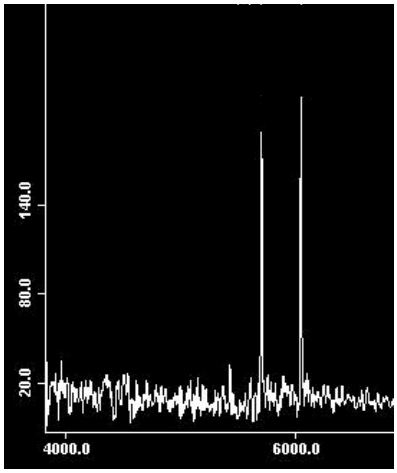
- **Advantages:**
  - **Accurate**
  - **Accurate allele frequency estimation**
  - **Robust for closely spaced SNPs**
- **Disadvantages**
  - **Expensive reagents**
  - **Requires post-PCR processing**

pyrosequencing.com

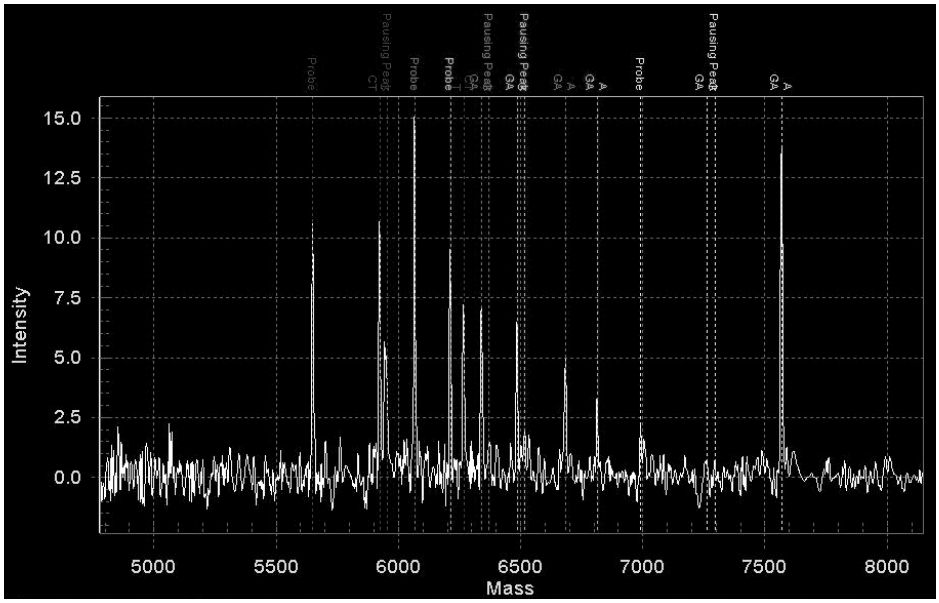
Primer extension mass spectrometry

Primer extension reactions designed to generate different sized products

	Mass in Daltons
GGACCTGGAGCCCCCACC	**5430.5**
GGACCTGGAGCCCCCACCC	**5703.7**
GGACCTGGAGCCCCCACCTG	**6047.9**



Mass spectrometry multiplexing



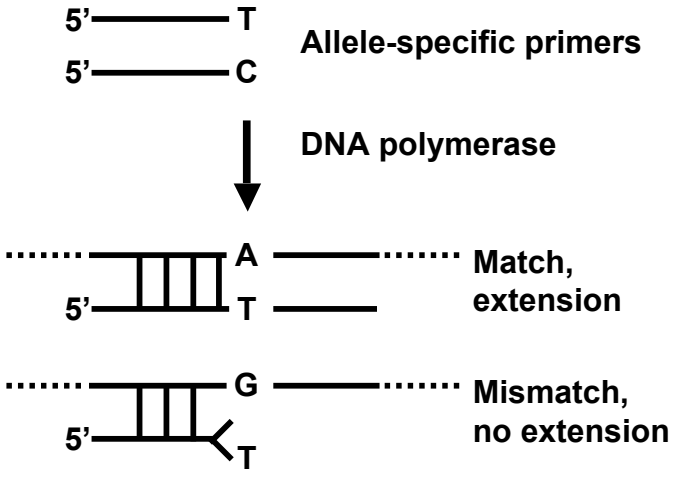
Primer extension mass spectrometry

- **Advantages:**
  - **Accurate**
  - **Automated assay design**
  - **Fast automated data collection**
  - **Multiplexing capacity**

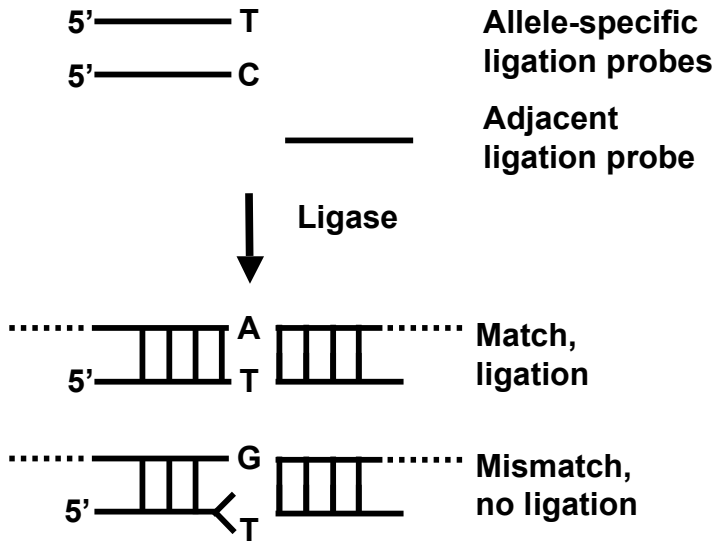
- **Disadvantages**
  - **Expensive instruments, consumables**
  - **Extensive post-PCR processing**

sequenom.com

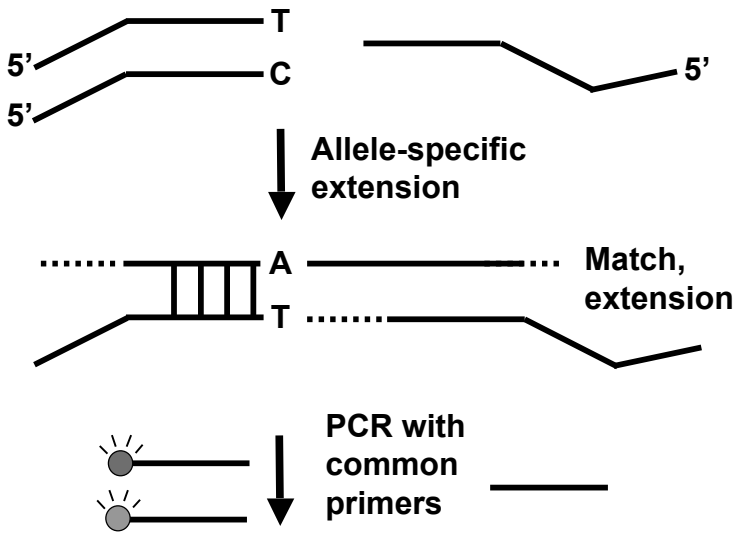
Allele-specific PCR



Oligonucleotide Ligation Assay (OLA)

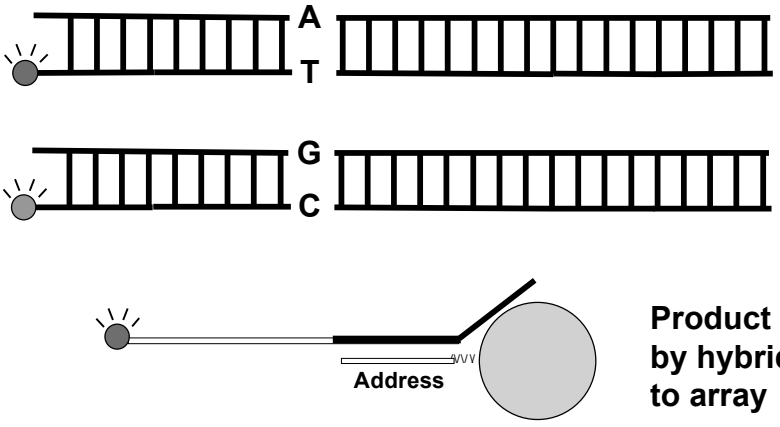


GoldenGate: Allele-specific extension

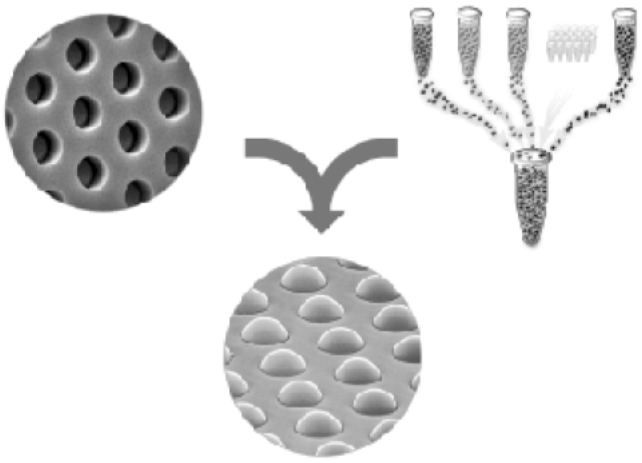


illumina.com

GoldenGate: Allele-specific extension



GoldenGate genotyping technology



Illumina GoldenGate

- **Advantages:**
  - **Very highly multiplexed**
  - **Accurate**
  - **Low cost per genotype**
- **Disadvantages**
  - **Not cost-effective for small studies**
  - **Limits to SNPs that can be designed**

illumina.com

Quality control of genotype data

- **High genotype success**
- **Accurate duplicate genotypes**
- **No genotypes in no DNA controls**
- **Allele frequencies similar to databases**
- **Accurate on a second platform**

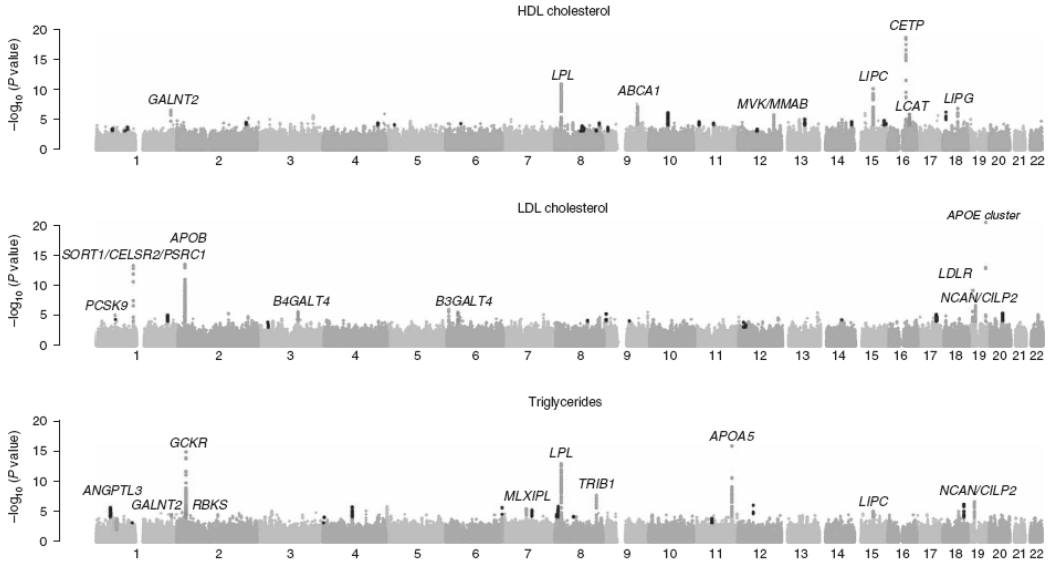
Quality control of genotype data

- **Test whether data are consistent with Hardy-Weinberg Equilibrium (HWE): $p^2 + 2pq + q^2 = 1$**
- **Calculate observed frequencies p and q**
- **Use p and q to calculate expected genotype frequencies**
- **Compare observed and expected genotype frequencies by X^2 test with 1 degree of freedom**

Human Genetic Variation

- **Types of variants**
- **Methods for scoring variants**
- **Genome-wide scoring of SNPs**
- **Structural variants**

Genome-wide association

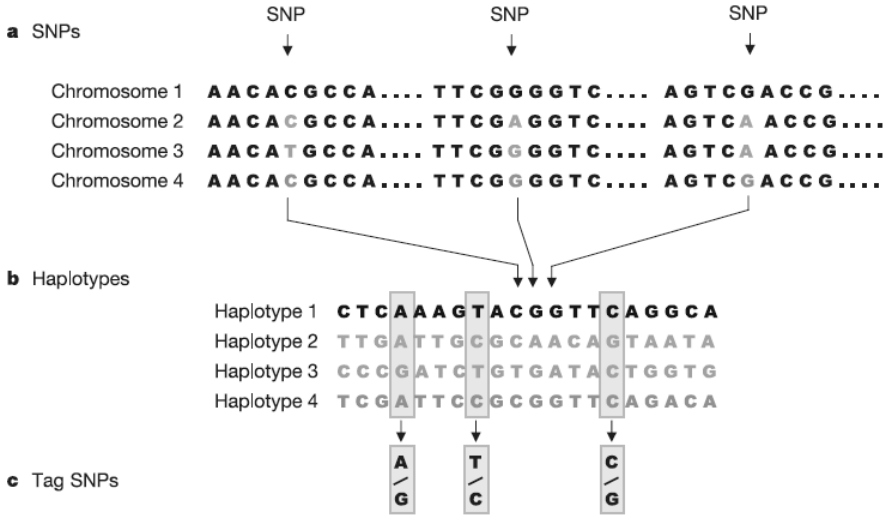


Willer, Sanna et al., (2008) Nat Gen 40:161

Genome-wide SNP panels

- **10,000 - 1 million SNPs per experiment**
- **Affymetrix, Illumina, Perlegen**
  - **Random SNPs**
  - **Selected haplotype tag SNPs**
  - **Coding or nonsynonymous SNPs**

Selecting 'haplotype tag' SNPs



International HapMap Consortium (2003) Nature 426:789

Haplotype map project

Genotype data on ~4 million SNPs

269 samples from four populations

- **Utah Caucasians with ancestry in N & W Europe (CEU)**
- **Han Chinese from Beijing (CHB)**
- **Japanese from Tokyo (JPT)**
- **Yoruban from Ibadan, Nigeria (YRI)**

www.hapmap.org

Affymetrix GeneChip Array

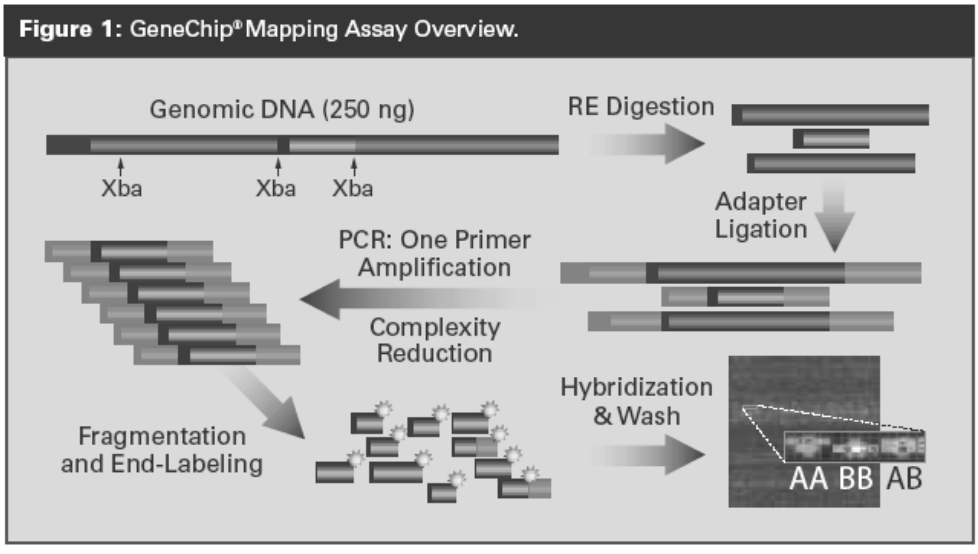
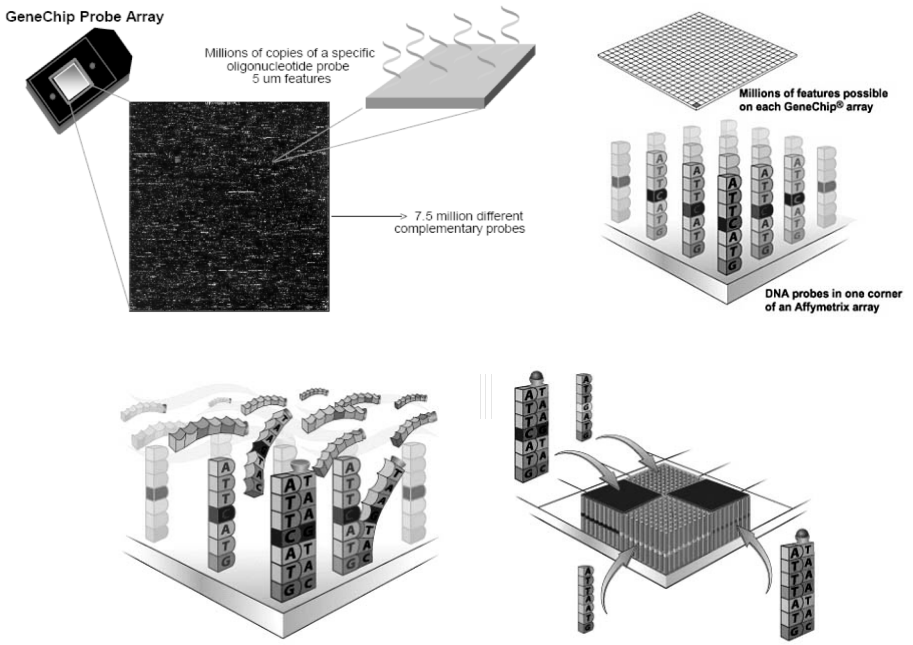
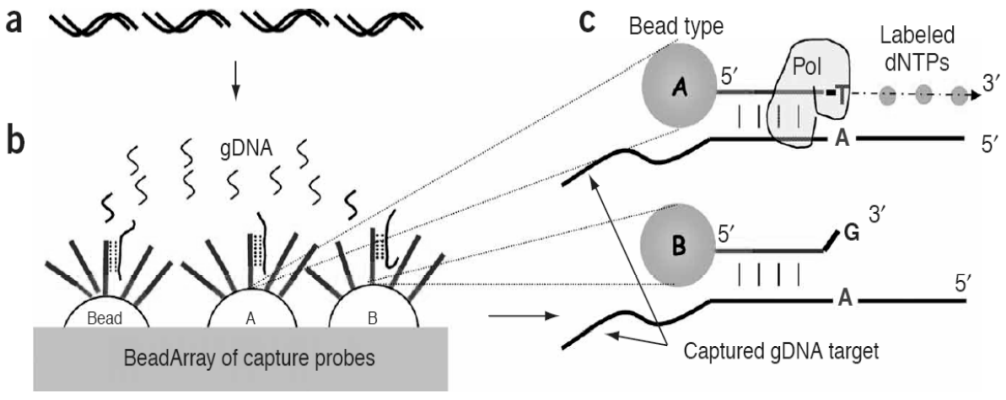


image from affymetrix.com

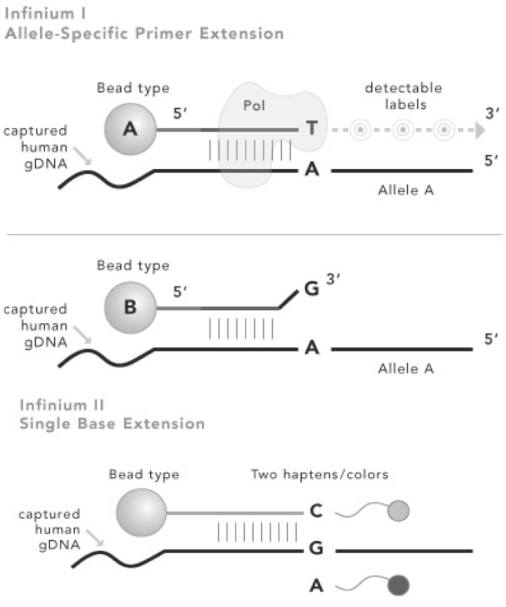


Illumina Infinium Assay



Gunderson et al. (2005) NatGen 37:549

Illumina Infinium Assays



Illumina.com

Global genomic coverage

Table 1 Global coverage (%) by SNP chips

SNP chip	*CEU*	*CHB+JPT*	*YRI*
SNP Array 5.0	64	66	41
SNP Array 6.0	83	84	62
HumanHap300	77	66	29
HumanHap550	87	83	50
HumanHap650Y	87	84	60
Human1M	93	92	68

Li et al. (2008) EJHG advance online publication

Local genomic coverage

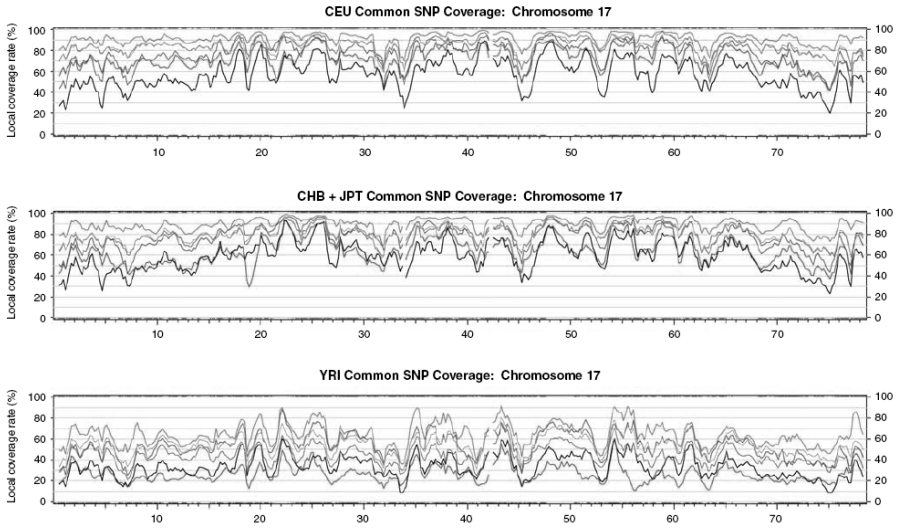
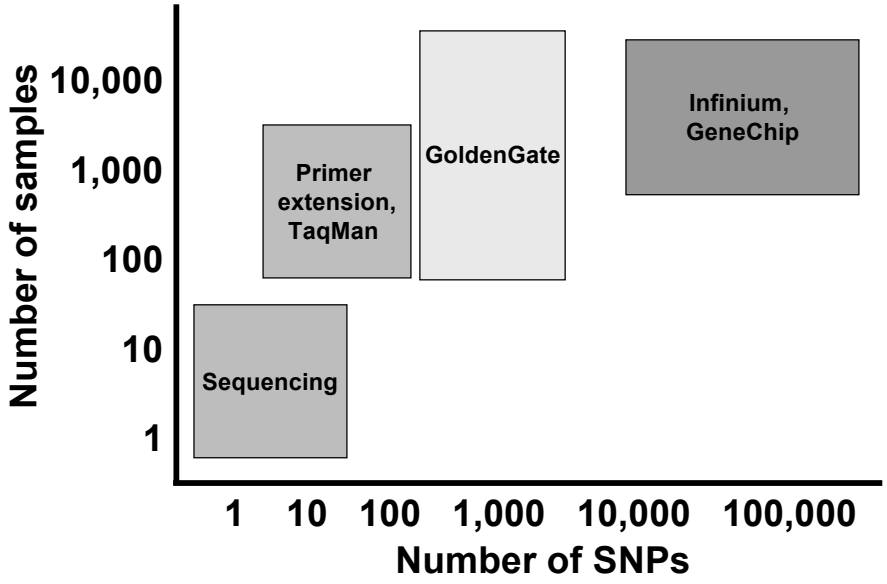


Figure 1 Local coverage map for each HapMap population for chromosome 17. The six SNP chips that were evaluated are SNP Array 5.0 (black), SNP Array 6.0 (blue), HumanHap300 (red), HumanHap550 (green), HumanHap650Y (cyan), and Human1M (purple). The red bars at the top and bottom indicate the transcription regions of known protein coding genes.

Li et al. (2008) EJHG advance online publication

Which SNP genotyping method?



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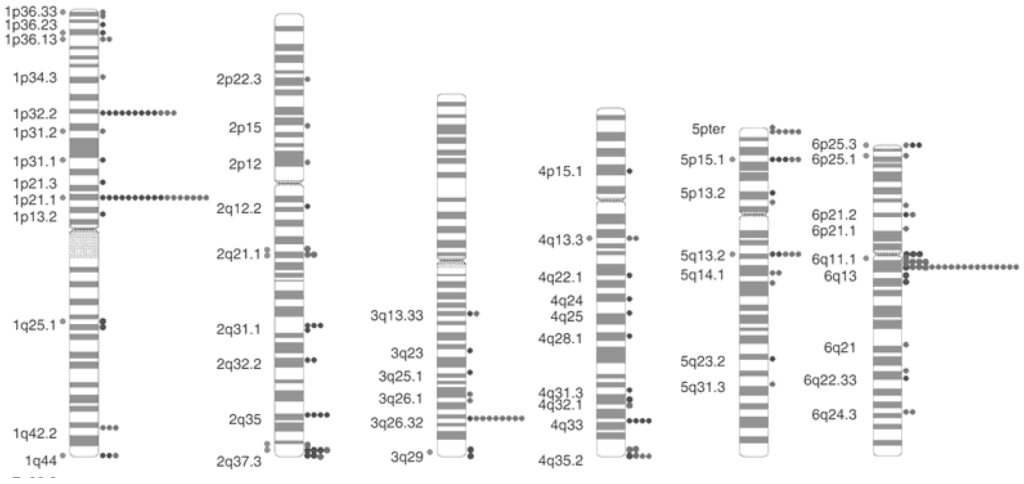
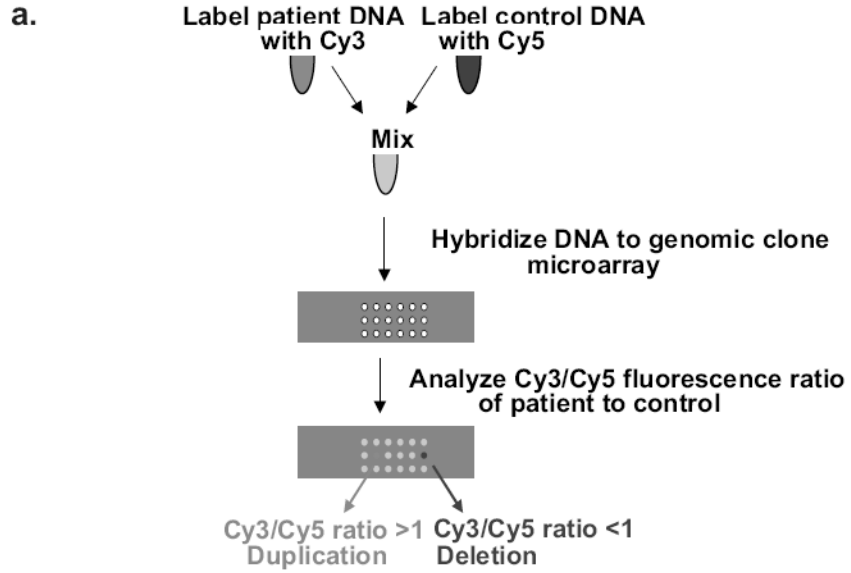
Structural variants span the genome



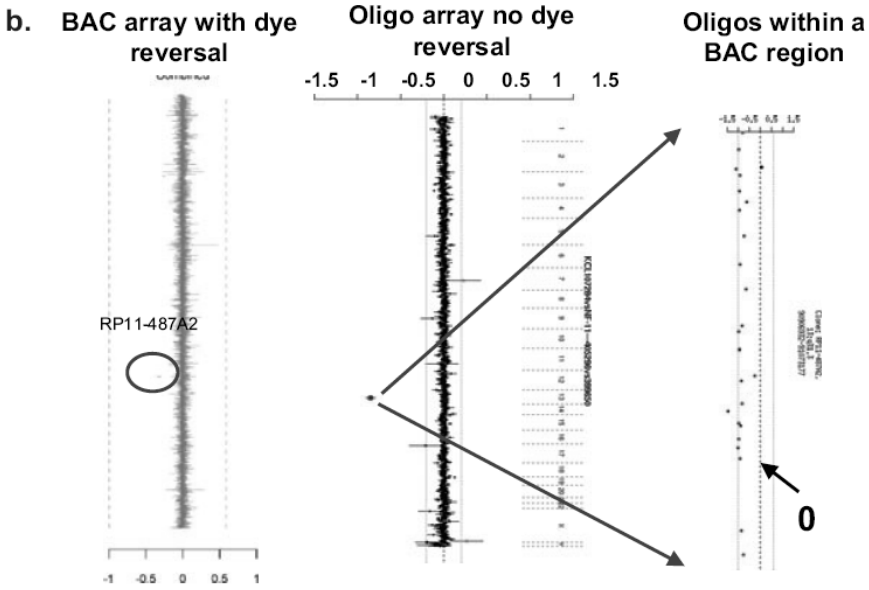
Iafrate et al. (2004) NatGen 36:949

Comparative genomic hybridization



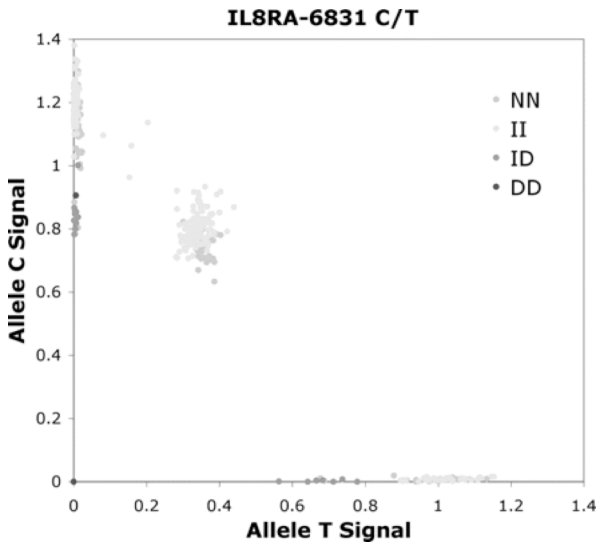
Beaudet & Belmont (2008) Annu Rev Med 59:113-129

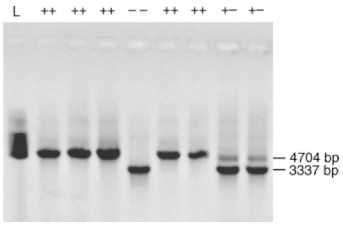
Comparative genomic hybridization



Beaudet & Belmont (2008) Annu Rev Med 59:113-129

Allele intensity in SNP genotyping





Carlson et al. (2006) HMG 15:1931

Future

- **Faster, cheaper, easier genotyping**
- **Next generation sequencing as genotyping**
- **Genome maps of structural variants**
- **Discovery of new susceptibility genes for complex traits**