



MACQUARIE
University

BIOL3120 –Human Genetics and Evolutionary Medicine

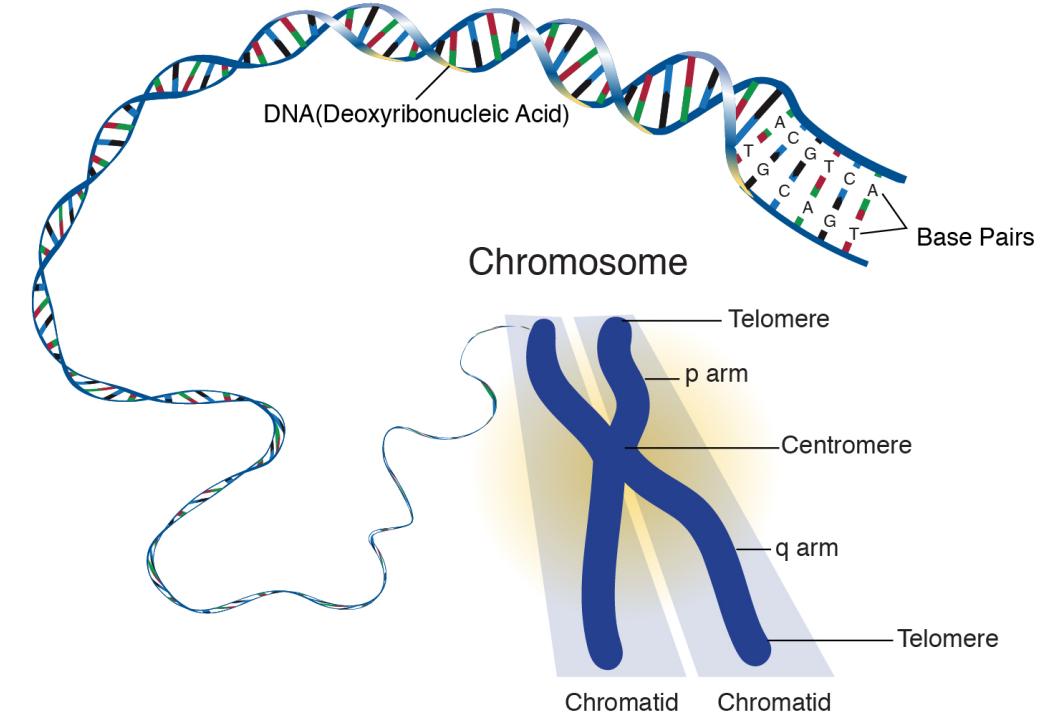
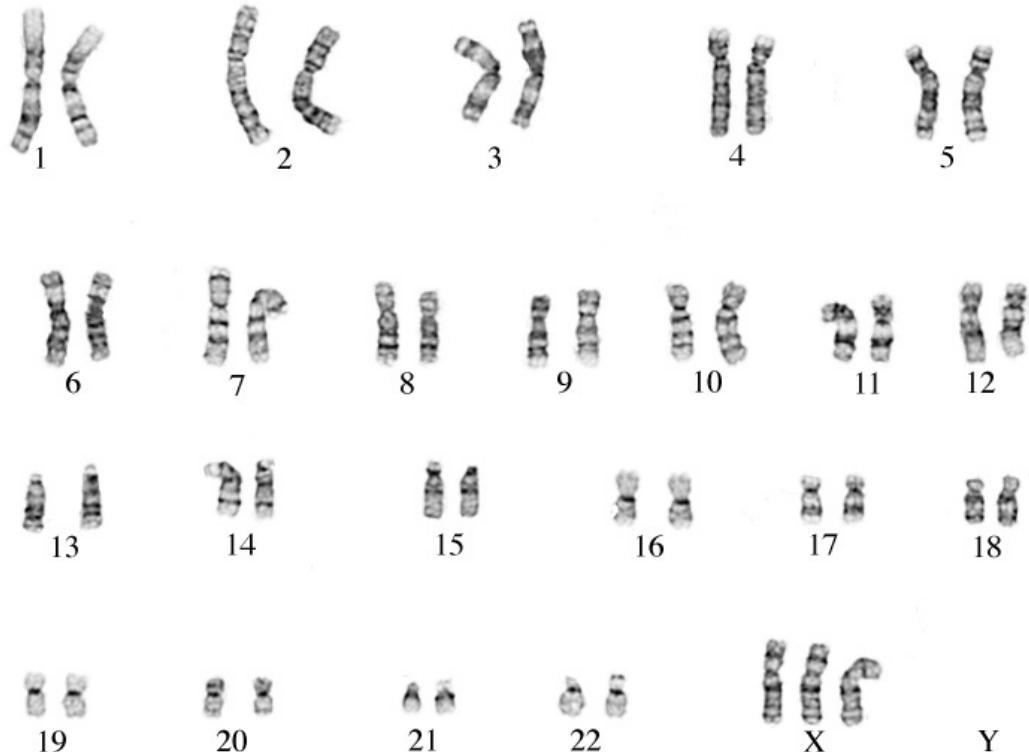
Genetic Testing Techniques





6	Genetic Testing Techniques GWAS	Problem Set 4	Problem Set 4 (5%)	Explain the importance of new techniques in human genetics for understanding human disease Solve problems in human genetics using appropriate analytical methods and a variety of up to date resources
Recess				
Recess		Pracs for External Students only		
7	Treatment for Genetic Conditions Epigenetics and Imprinting	Problem Set 5	Problem Set 4 (5%) & Problem Set 5 (5%)	Explain the importance of new techniques in human genetics for understanding human disease Solve problems in human genetics using appropriate analytical methods and a variety of up to date resources

Detection of chromosomal changes vs Detection of nucleotide changes in individuals



BIOL3120 –Genetic Testing Techniques

LEARNING OBJECTIVES



On successful completion of this lecture, you will be able to:

- Identify and understand the technologies that detect chromosomal mutations
- Identify and understand the technologies that detect nucleotide mutations

Overview

- Detection of chromosomal changes
 - Karotype
 - Non-Invasive Prenatal Testing (NIPT)
 - Microarrays
- Detection of nucleotide changes
 - Mutation panels
 - Genetic linking & mapping
 - Sequencing
 - Sanger sequencing
 - Next generation sequencing
 - Triplet repeat primed PCR
 - Multiplex Ligation-dependent Probe Amplification (MLPA)



Chromosomal Changes

Karyotype

- A karyotype is an individual's collection of chromosomes.
- The term also refers to a laboratory technique that produces an image of an individual's chromosomes.
- A picture of an individual's chromosomes
- The karyotype is used to look for abnormal numbers or structures of chromosomes.

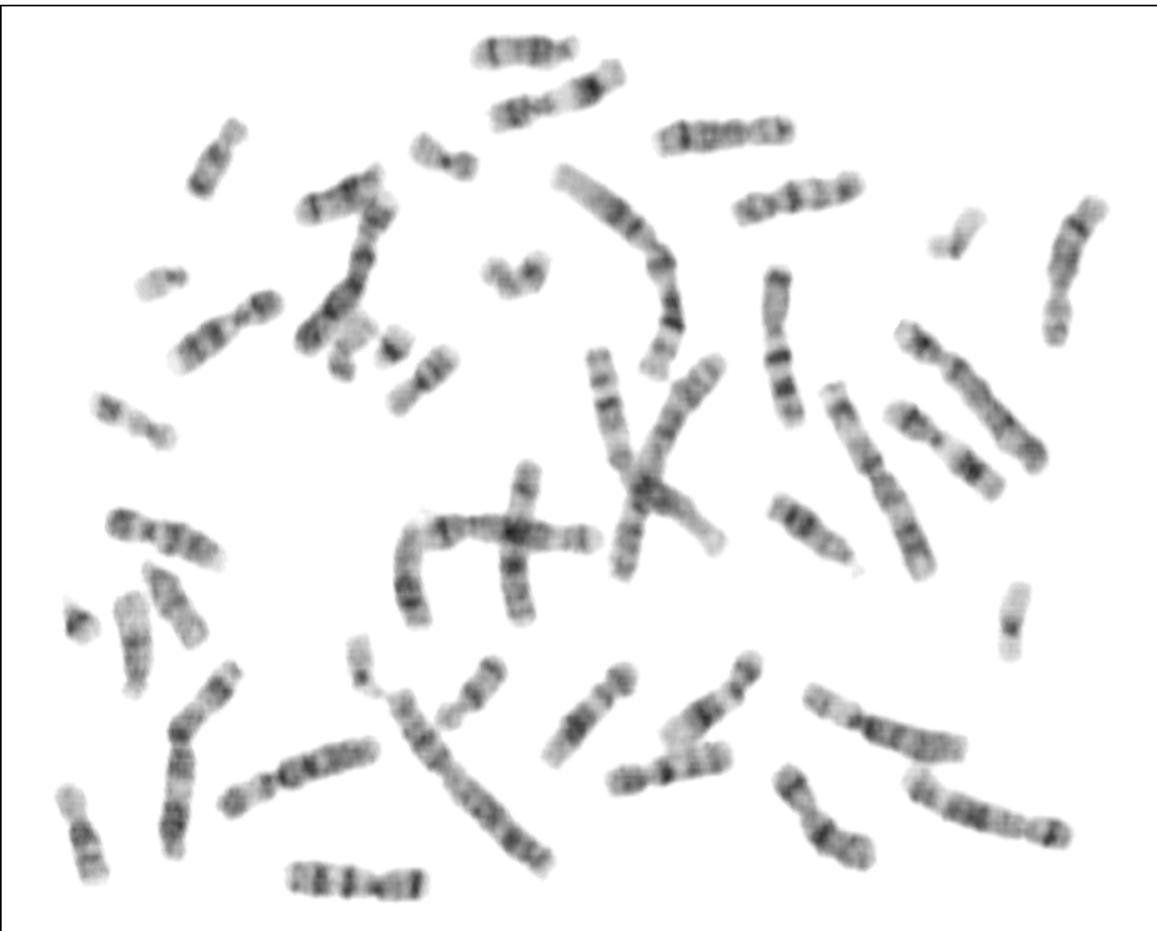
Basic Technique

- Using cells in tissue culture
- Pretreating cells in a hypotonic solution, which swells them and spreads the chromosomes
- Arresting mitosis in metaphase by a solution of colchicine
- Squashing the preparation on the slide forcing the chromosomes into a single plane
- Cutting up a photomicrograph and arranging the result into an indisputable karyogram.

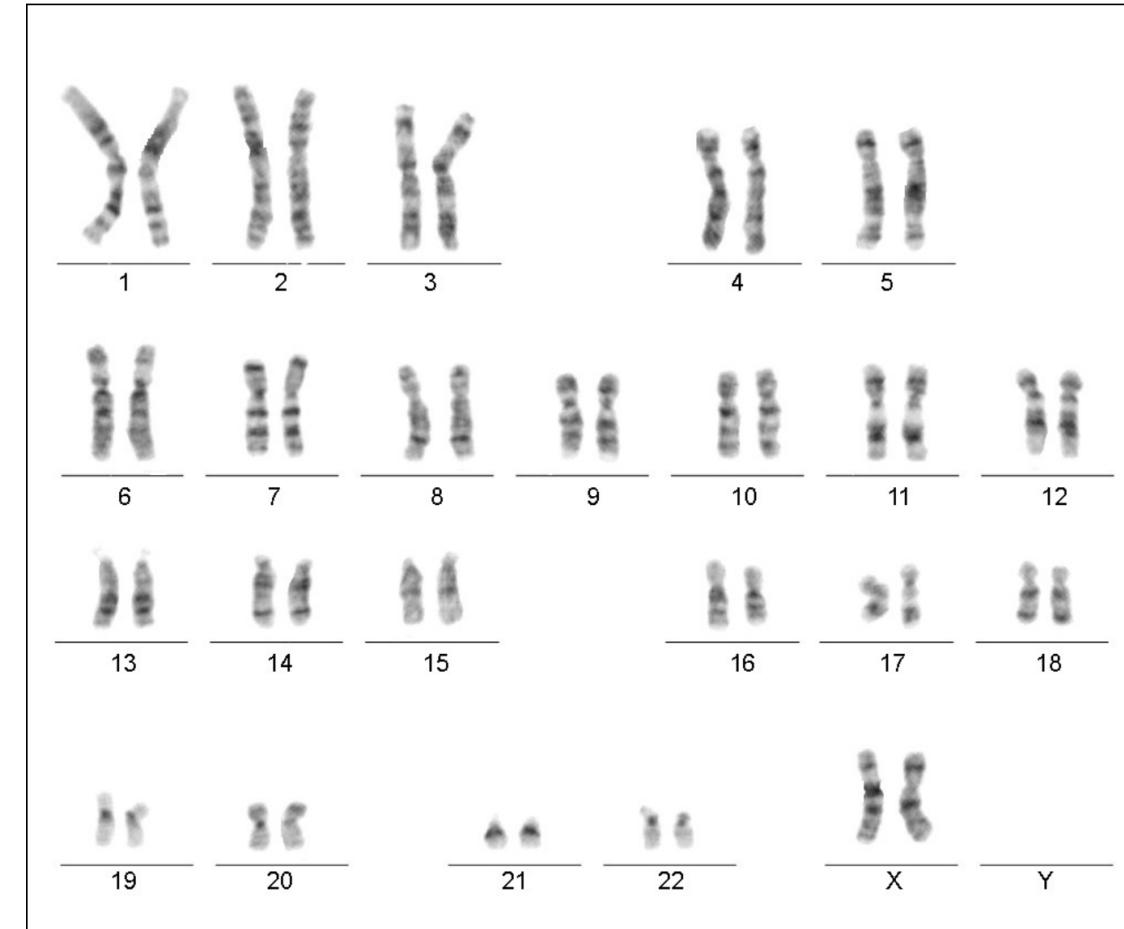
JOE HIN TJIO & ALBERT LEVAN, 1956

Karyotype

Metaphase Image

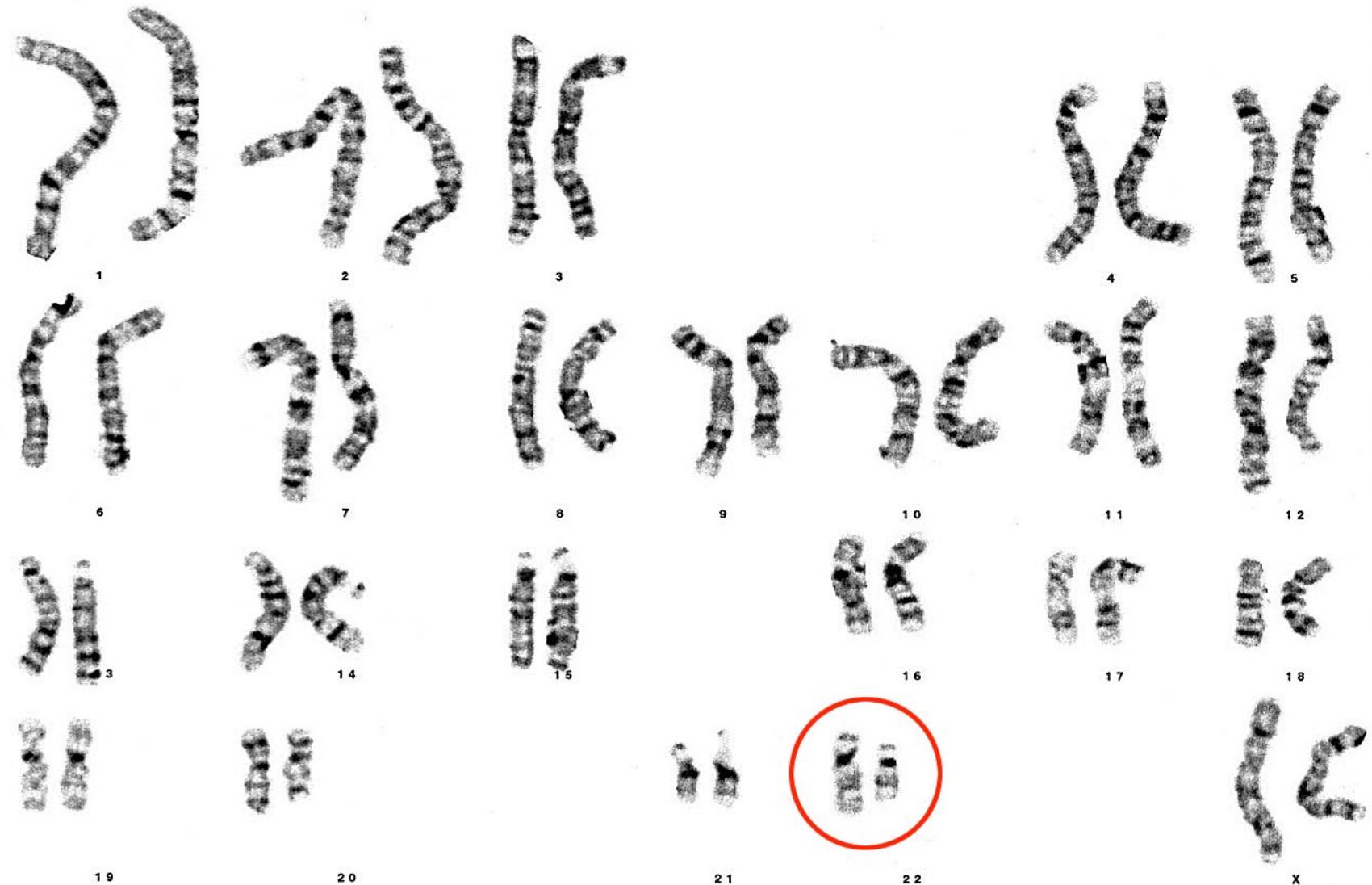


Karyotype Image

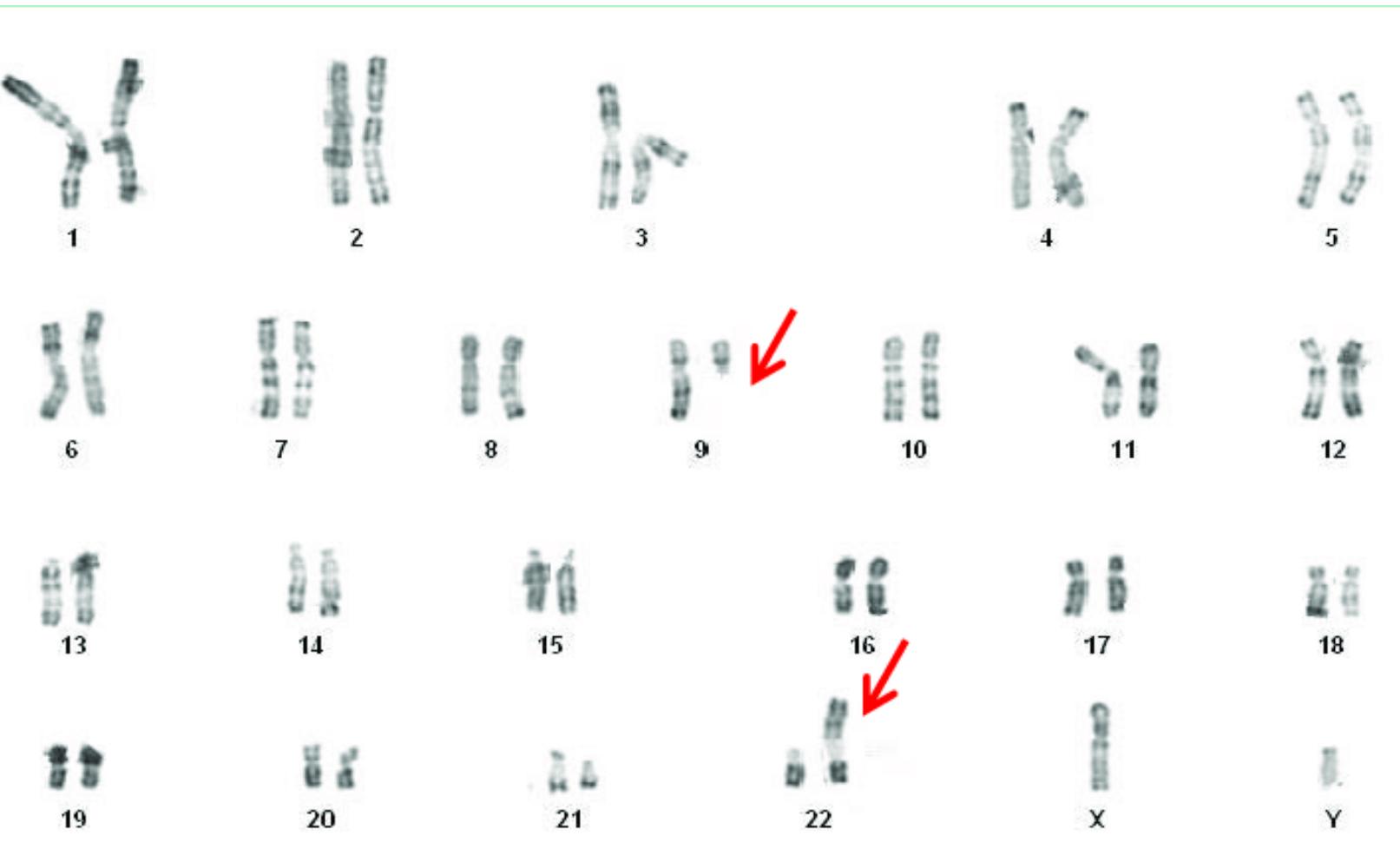


Karyotype

- ~5Mb and larger deletions/duplications detectable
- Can detect Translocations
- Mosaicism for detectable changes (count cells)

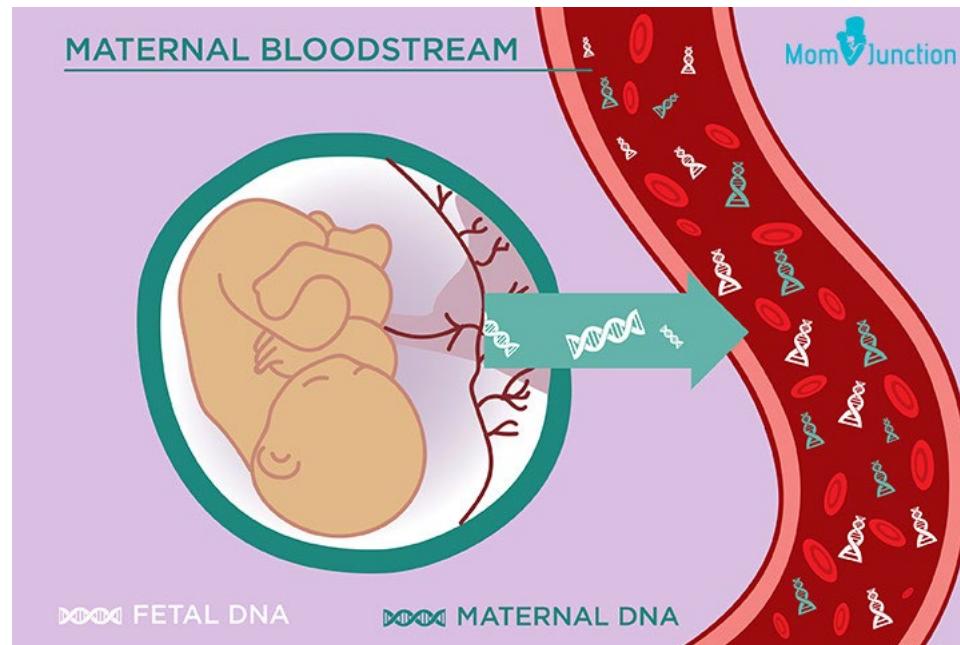


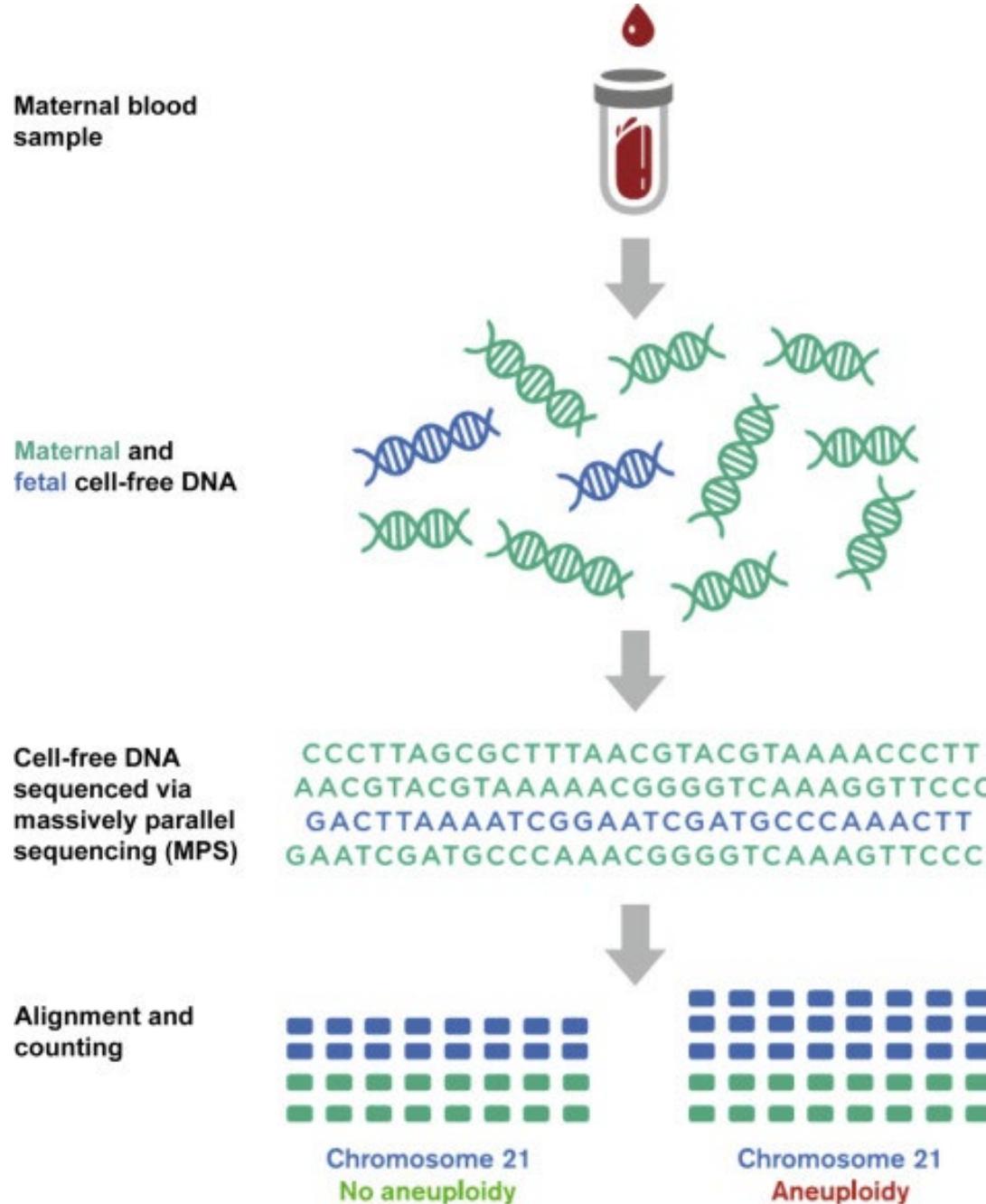
Karyotype



Non-Invasive Prenatal Testing (NIPT)

- Also called Non-invasive prenatal screening (NIPS)
- Fetal DNA present in mother's bloodstream cfDNA = cell-free DNA
- From 10 weeks





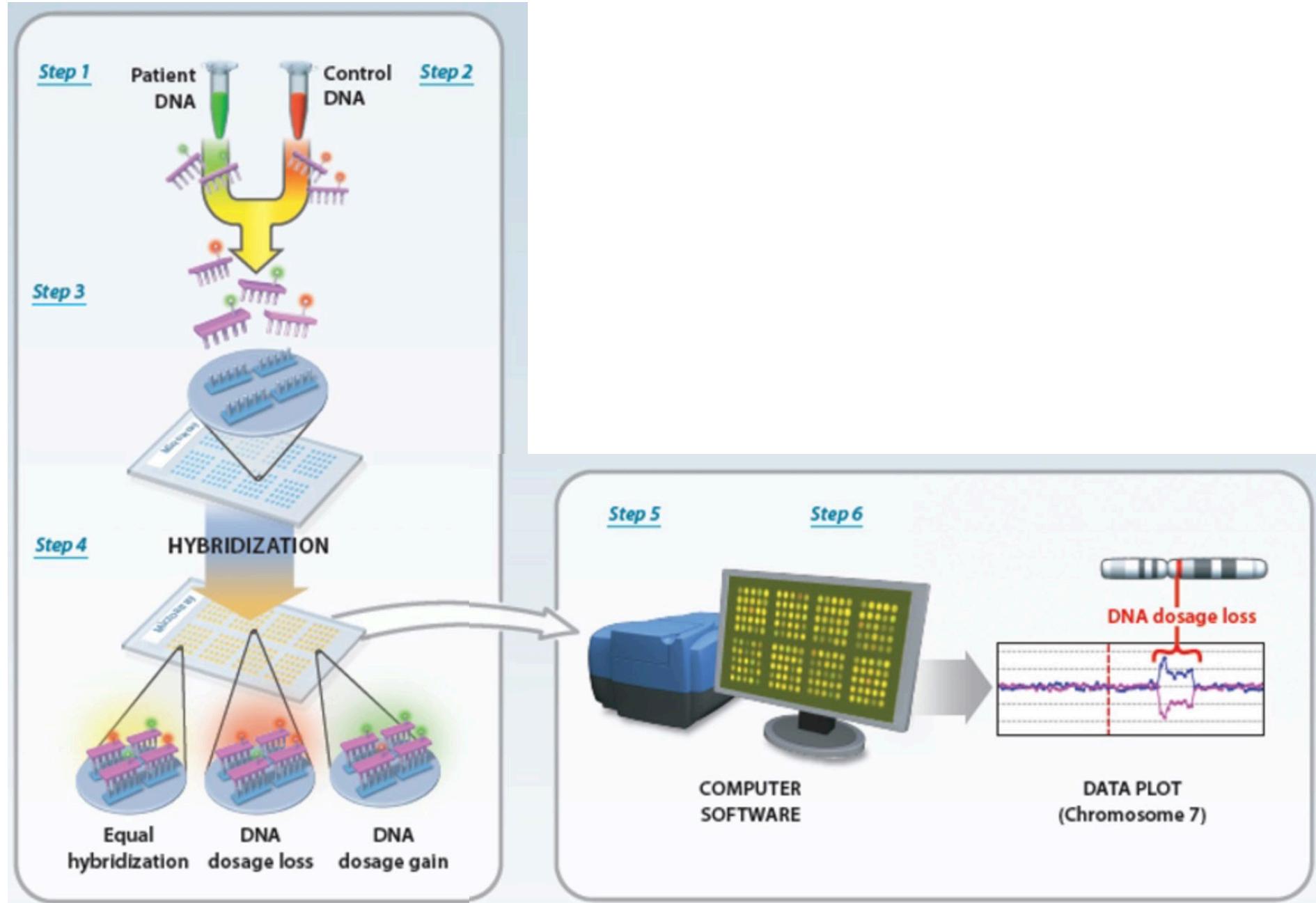
- Massively Parallel Sequencing = Next Generation Sequencing = High Throughput Sequencing
- ≠ Sangar sequencing
- To determine chromosomal aneuploidy, the most common method is to count all cfDNA fragments (both fetal and maternal).
 - If the percentage of cfDNA fragments from each chromosome is as expected, then the fetus has a decreased risk of having a chromosomal condition (negative test result).
 - If the percentage of cfDNA fragments from a particular chromosome is more than expected, then the fetus has an increased likelihood of having a trisomy condition (positive test result).
 - A positive screening result indicates that further testing (called diagnostic testing, because it is used to diagnose a disease) should be performed to confirm the result.

NIPT developing

- Some services now offering deletion/duplication
- Complete genome from cfDNA?
- Cancer detection

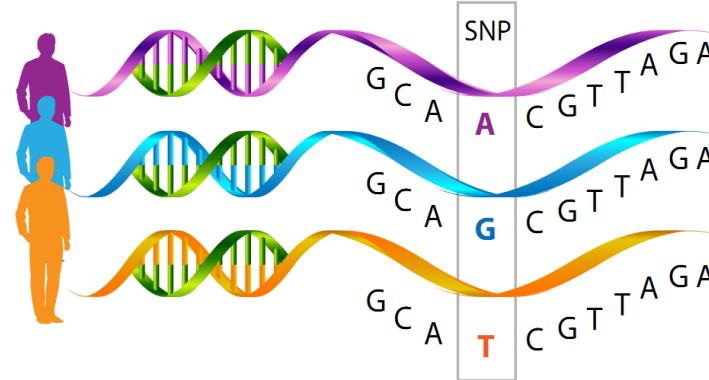
Microarray

- Detect the expression of thousands of genes at the same time
- DNA microarrays are microscope slides that are printed with thousands of tiny spots in defined positions, with each spot containing a known DNA sequence or gene
 - Referred to as gene chips or DNA chips
 - Each spot containing a known DNA sequence is referred to as a DNA probe
- cDNA is taken from an experimental sample and a reference sample and labelled with a fluorescent probe 
- The cDNA molecules bind the DNA probes on the chip
- Chip is then scanned to detect the fluorescence



Microarray

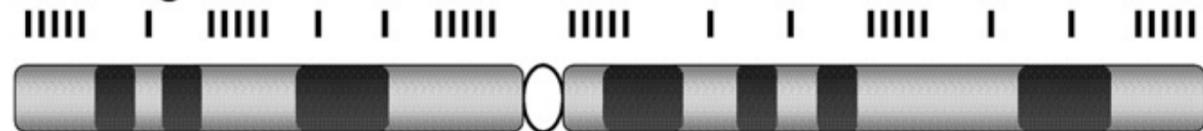
- Comparative genomic hybridisation
 - Detects
 - Copy Number Variants over ~50kb
 - Homozygosity / uniparental disomy
 - Can't detect
 - Balanced translocations
 - Inversions etc
 - Don't know where duplications are



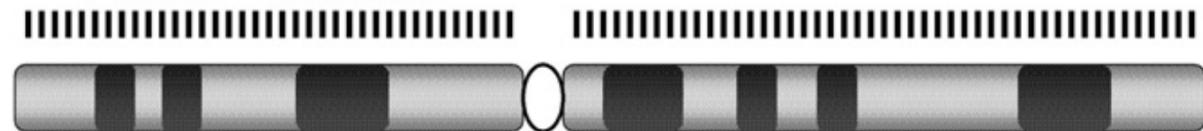
A Targeted



B Targeted with Backbone

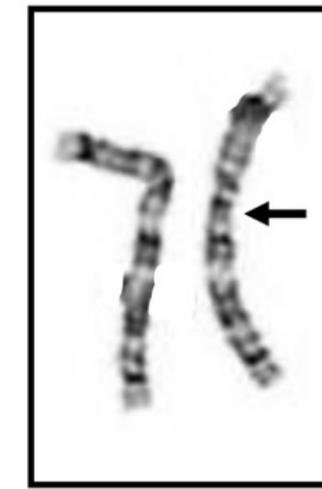
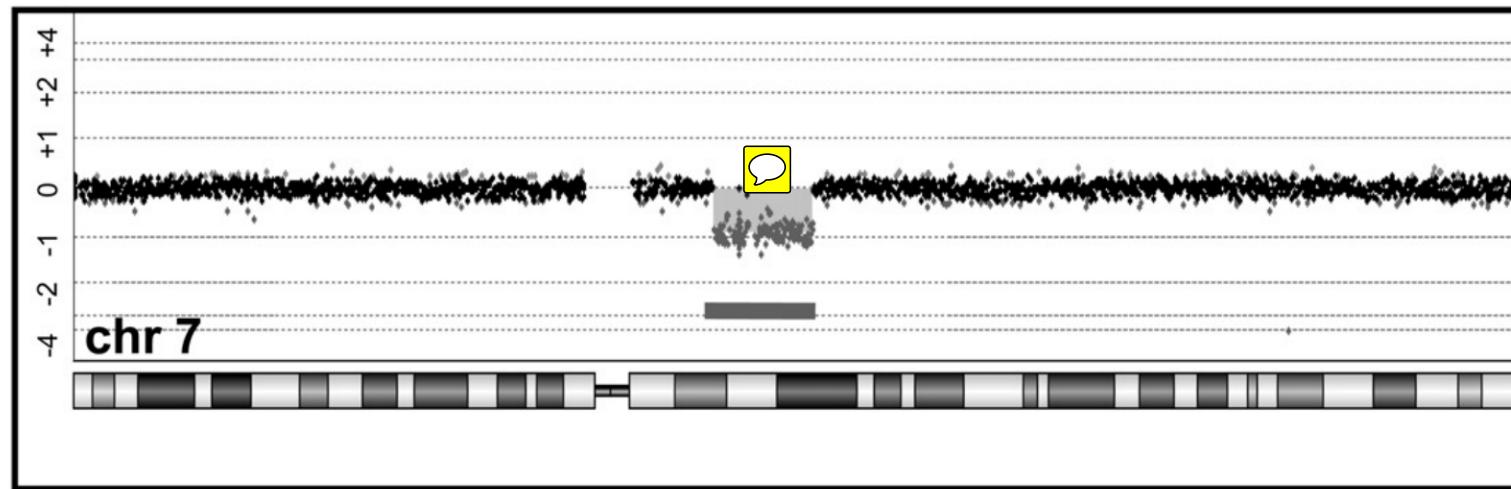


c Whole Genome

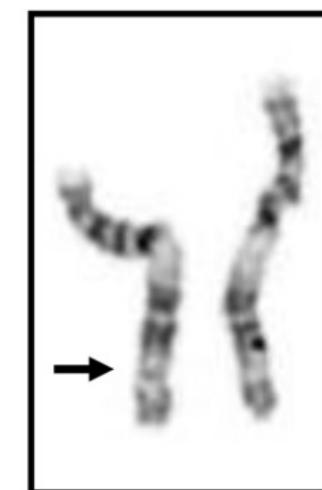
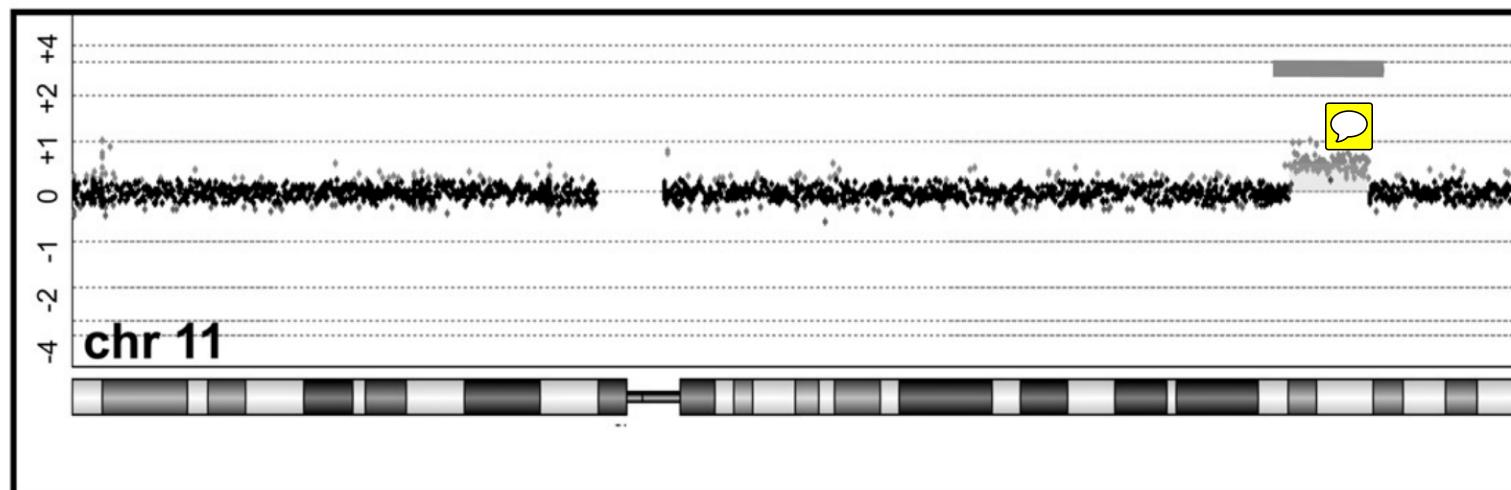


Microarray results

A



B



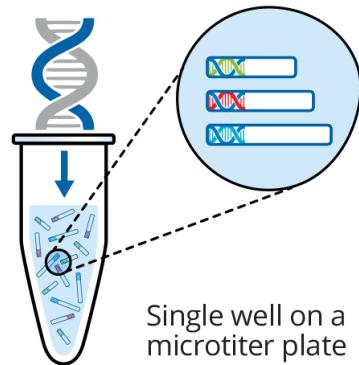


Nucleotide Mutations

Old School Methods

Targeted mutation panels

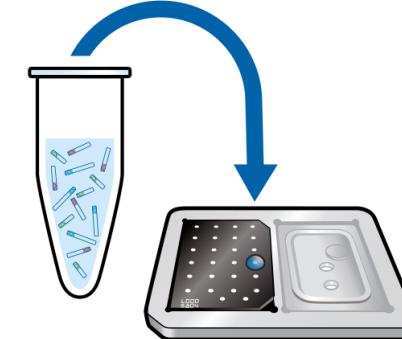
- Pre-set specific mutations to search for 
- Does not generate sequence – just tests for presence of mutations
- Quick and cheap



Endpoint PCR

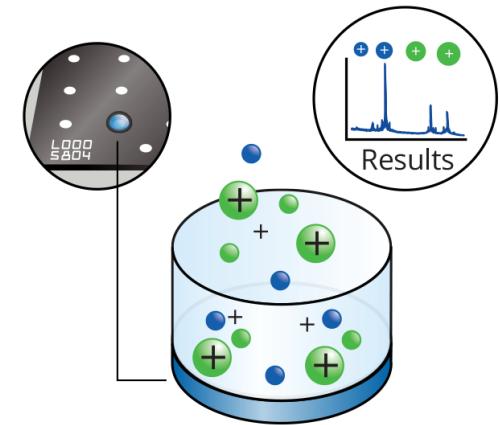
Amplify and extend up to 40 target-specific DNA fragments in a single reaction.

Sample Process Journey



Transfer Analyte

Transfer a small amount of sample to a single pad on the SpectroCHIP® Array.



Detection and Analysis

Multiple tests can be run on a single SpectroCHIP Array. Hundreds of mutations can be tested per sample.

* Use multiple reactions for >40 targets if required.

Targeted mutation panels

Cystic Fibrosis Test

Overview

Cystic fibrosis (CF) is an inherited condition affecting breathing and digestion. CF causes the build-up of thick mucus which traps bacteria, resulting in recurrent infections that damage the lungs. Thick mucus in the gut also makes digestion of food difficult. People with CF require daily physiotherapy to clear mucus from their lungs, frequent courses of antibiotics and need to take medicine to help with digestion. There is no cure for CF but better treatments are under research and development.

We screen for 175 cystic fibrosis transmembrane conductance regulator (CFTR) variants and 178 in diagnostic tests. [Download the full list of cystic fibrosis variants.](#)

Targeted mutation panels



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W vcgs.org.au

VCGS variant list for cystic fibrosis screening & diagnostic testing

Exon/intron number	Nucleotide change (NM_000492.3)	Protein change (NP_000483.3)	Diagnostic/screening
4	c.350G>A	p.Arg117His	Diagnostic assay only
Intron 9	c.1210-34TG[11_13]	polyTG tract	Diagnostic assay only
Intron 9	c.1210-12T[5_9]	polyT tract	Diagnostic assay only
Intron 2	c.165-3C>T	No protein name	Diagnostic and prepair® screening
Intron 2	c.165-1G>A	No protein name	Diagnostic and prepair® screening
3	c.166G>A	p.Glu56Lys	Diagnostic and prepair® screening
3	c.169T>G	p.Trp57Gly	Diagnostic and prepair® screening
3	c.170G>A	p.Trp57*	Diagnostic and prepair® screening
3	c.171G>A	p.Trp57*	Diagnostic and prepair® screening
3	c.175dupA	p.Arg59Lysfs*10	Diagnostic and prepair® screening
3	c.174_177delTAGA	p.Asp58Glufs*32	Diagnostic and prepair® screening
3	c.178G>T	p.Glu60*	Diagnostic and prepair® screening
3	c.200C>T	p.Pro67Leu	Diagnostic and prepair® screening
3	c.223C>T	p.Arg75*	Diagnostic and prepair® screening
3	c.233dupT	p.Trp79Leufs*32	Diagnostic and prepair® screening
2	c.254G>A	p.Glu85Glu	Diagnostic and prepair® screening

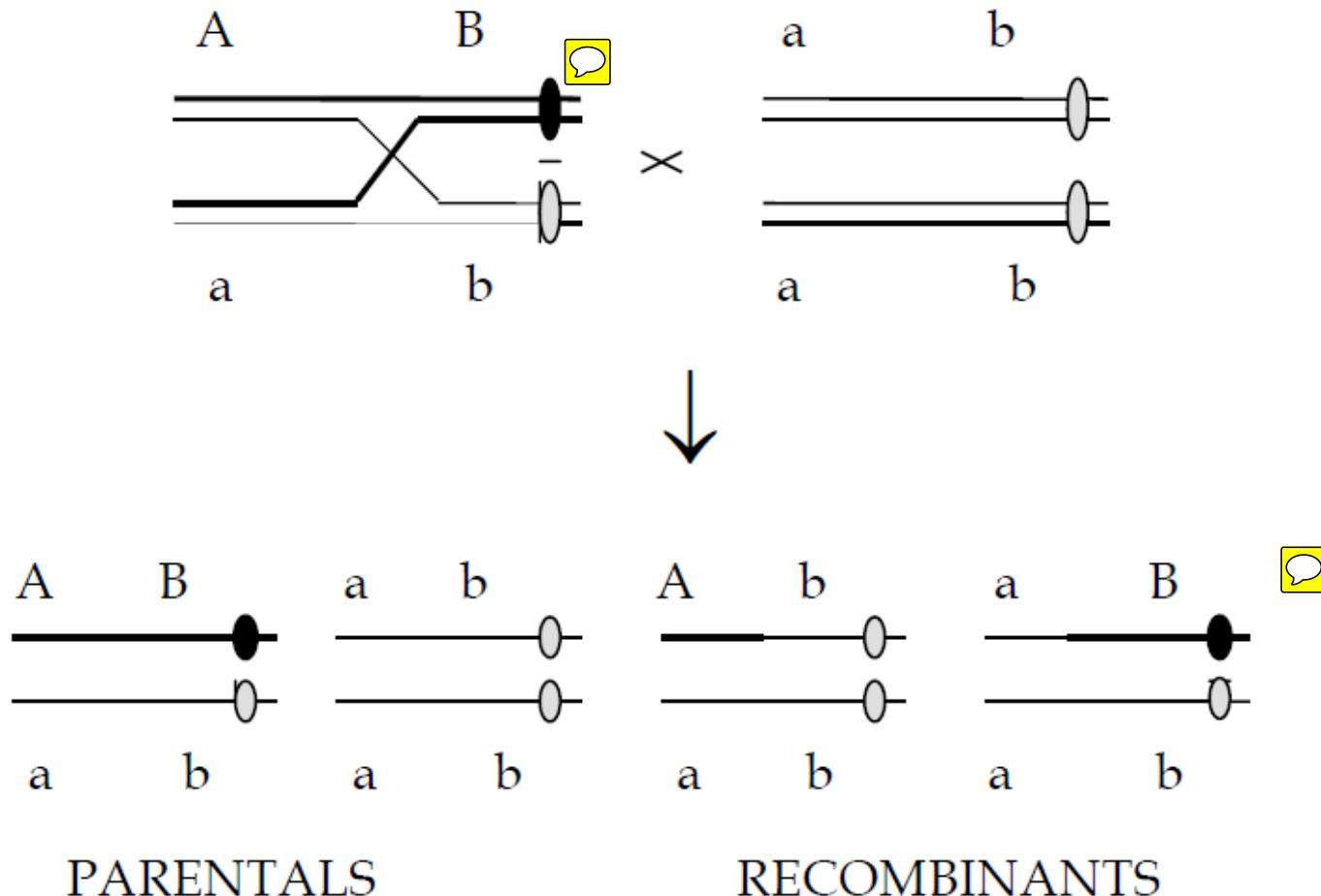
Genetic Linkage and Mapping



- Technique used to determine the locus of a disease linked mutation
- Basic principles of genetic linkage:
 - Linkage results from the location of genes on chromosomes
 - Gene loci on the same chromosome are inherited together
 - (sometimes)
 - Linkage is deduced from the progeny by observing the arrangement of alleles (haplotype)
 - Linkage phase: arrangement of alleles on each chromosome in a double heterozygote
- Individuals who have inherited a disease linked mutation are unlikely to be recombinant in that location

Genetic Linkage and Mapping

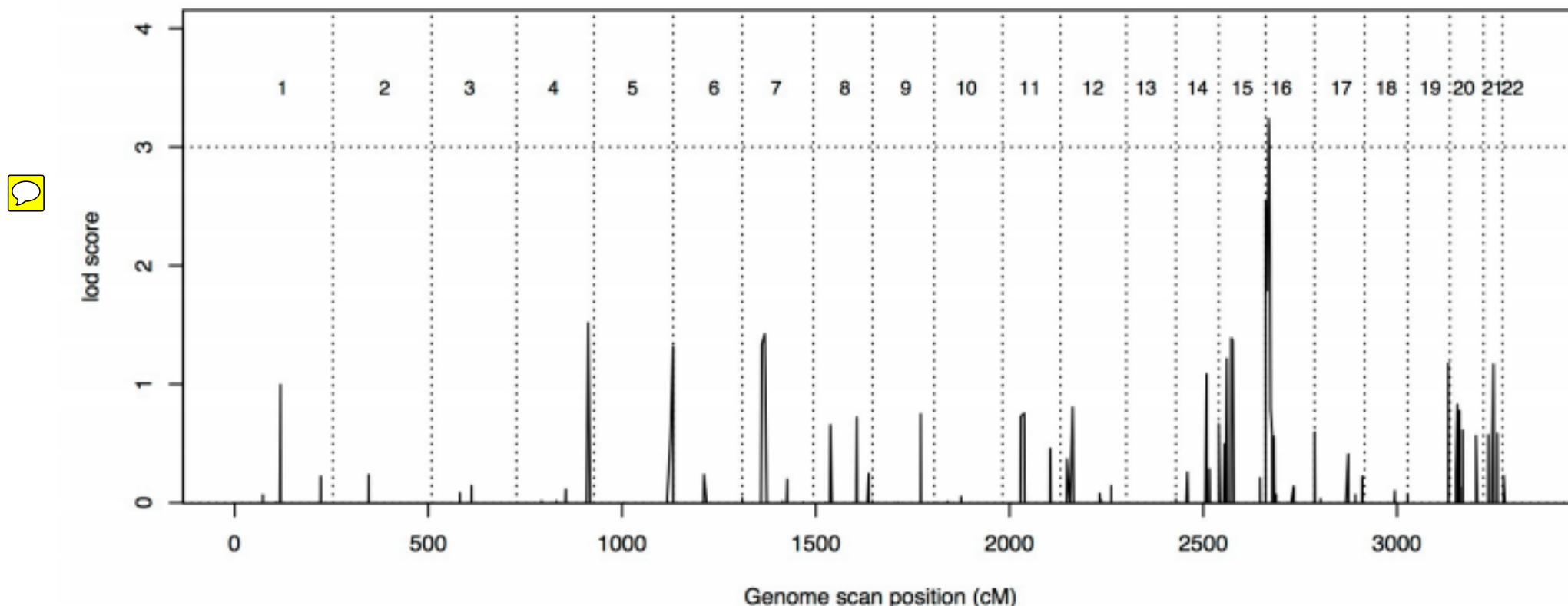
Basic testcross for linkage:



Genetic Linkage and Mapping

a

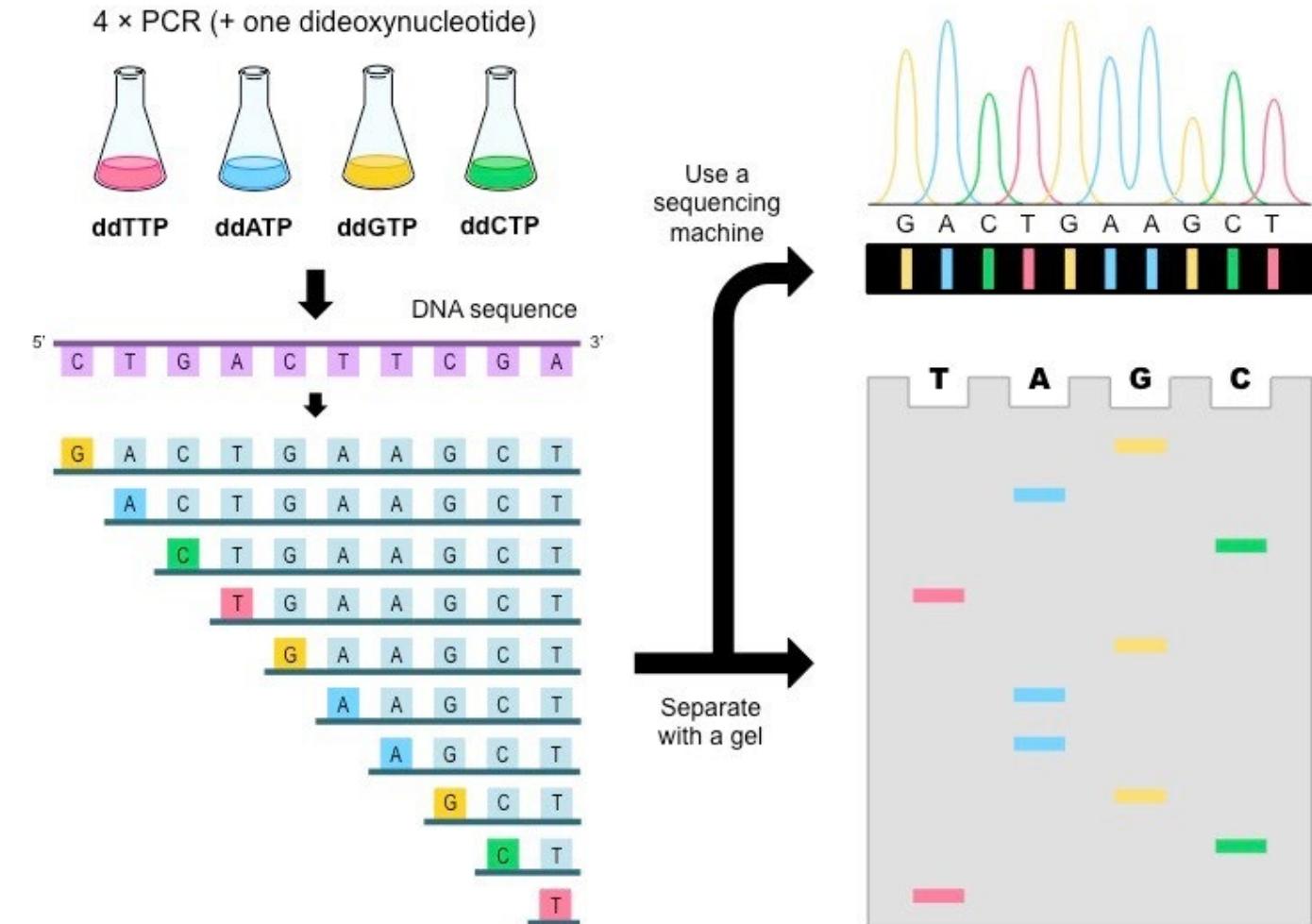
Genome-wide microsatellite 2-point linkage analysis



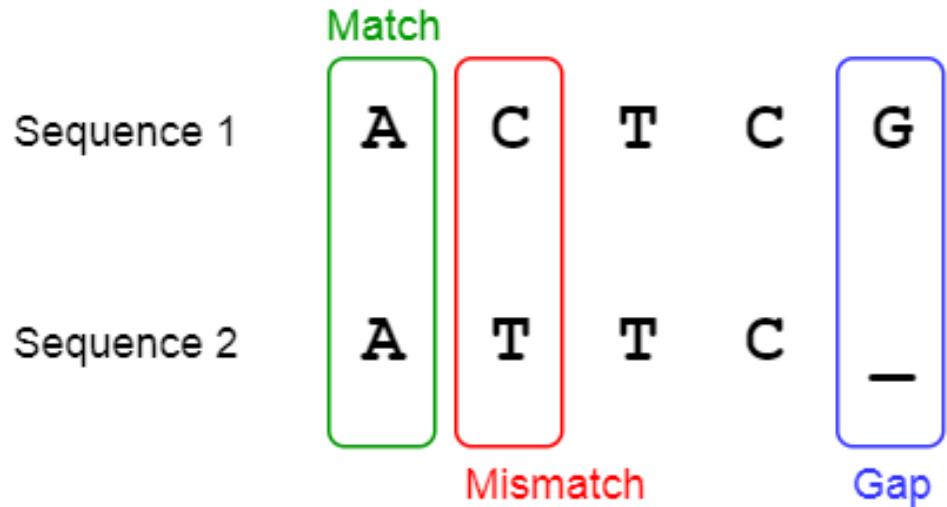
Sanger sequencing



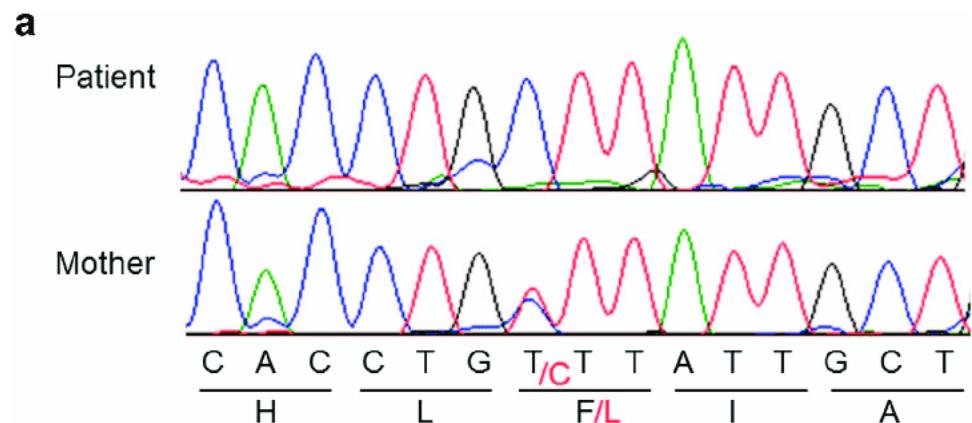
- Requires target region amplified by PCR – can be picky
- ~1kb limit per sequence – can build up overlapping reads to cover larger area



Sanger sequencing: compare to expected



- Heterozygous:



Sanger sequencing

- Large genes require a lot of work
- Titin gene (TTN)
 - 365,719bp total gene size (including introns)
 - Exons: 80,781bp across 365 exons
- DMD gene (Duchenne muscular dystrophy)
 - 2,220,390 bp including introns
 - ~14kb mRNA



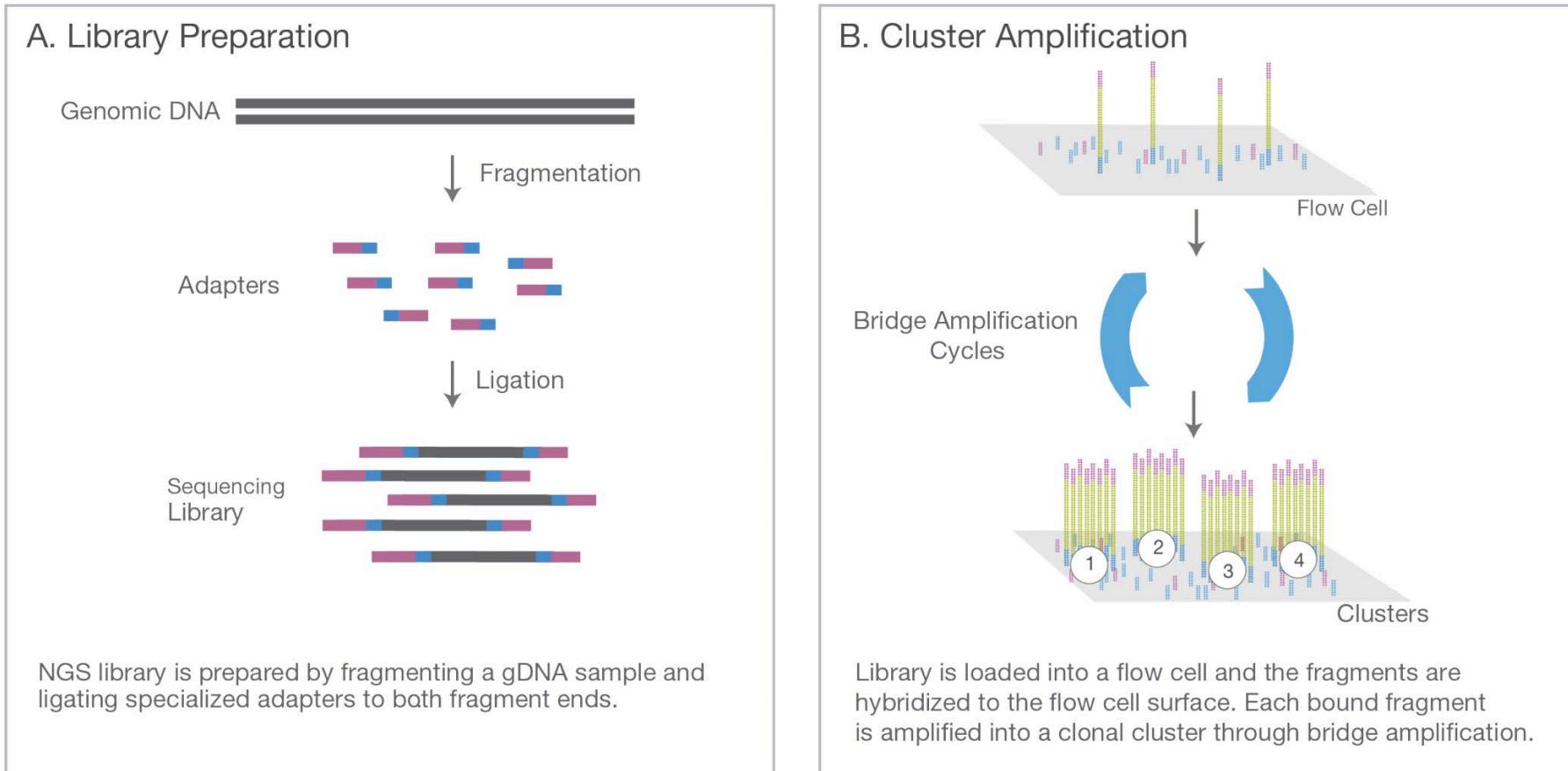
Nucleotide Mutations

Newer Technologies

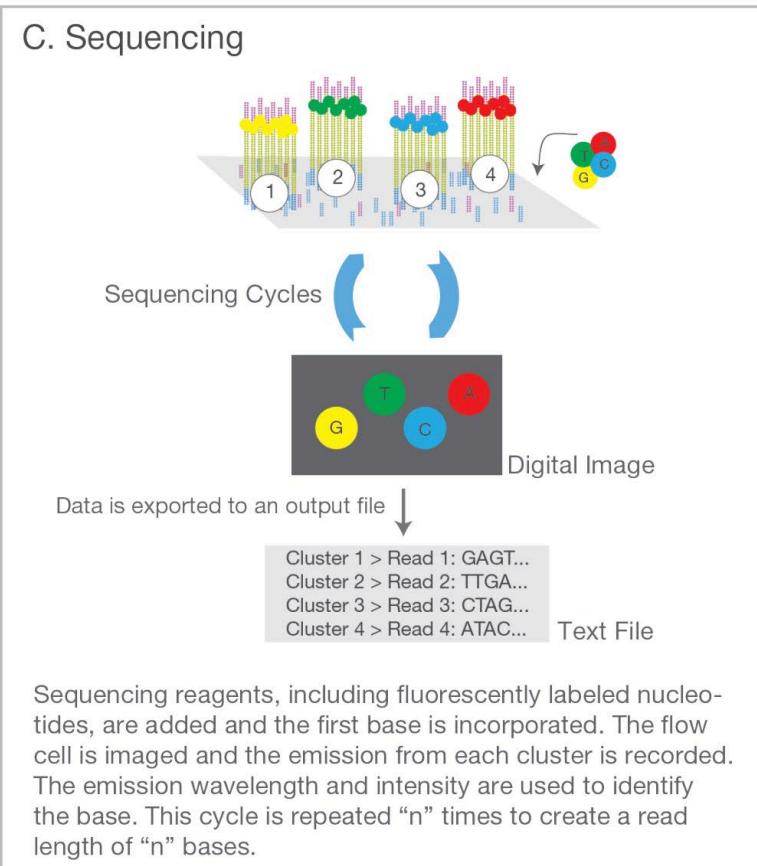
Next-generation sequencing (NGS)

- Sequence large regions of or entire genome
- Often cheaper than sequencing individual genes
 - Means we are more likely to test multiple genes at once (up to hundreds) 
 - Means we are more likely to find variants 

Next-generation sequencing: generation of reads



Next-generation sequencing: generation of reads

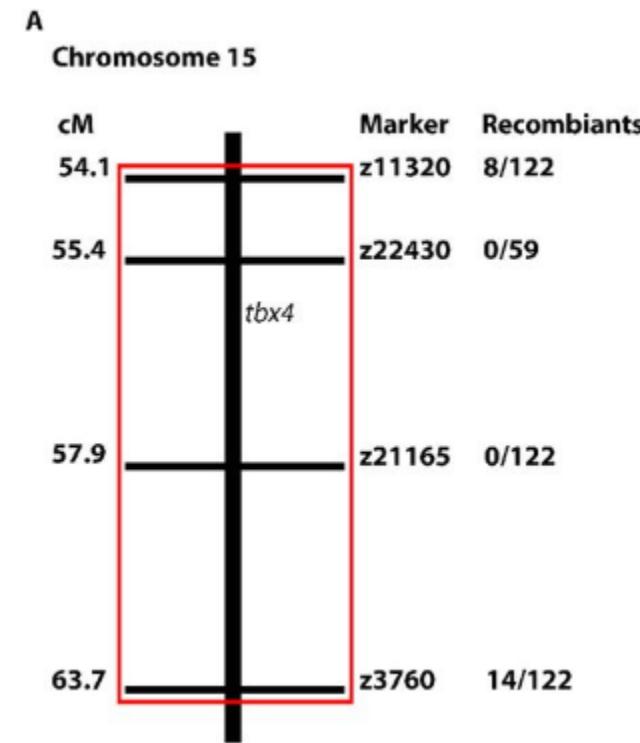


Next-generation sequencing: alignment of reads

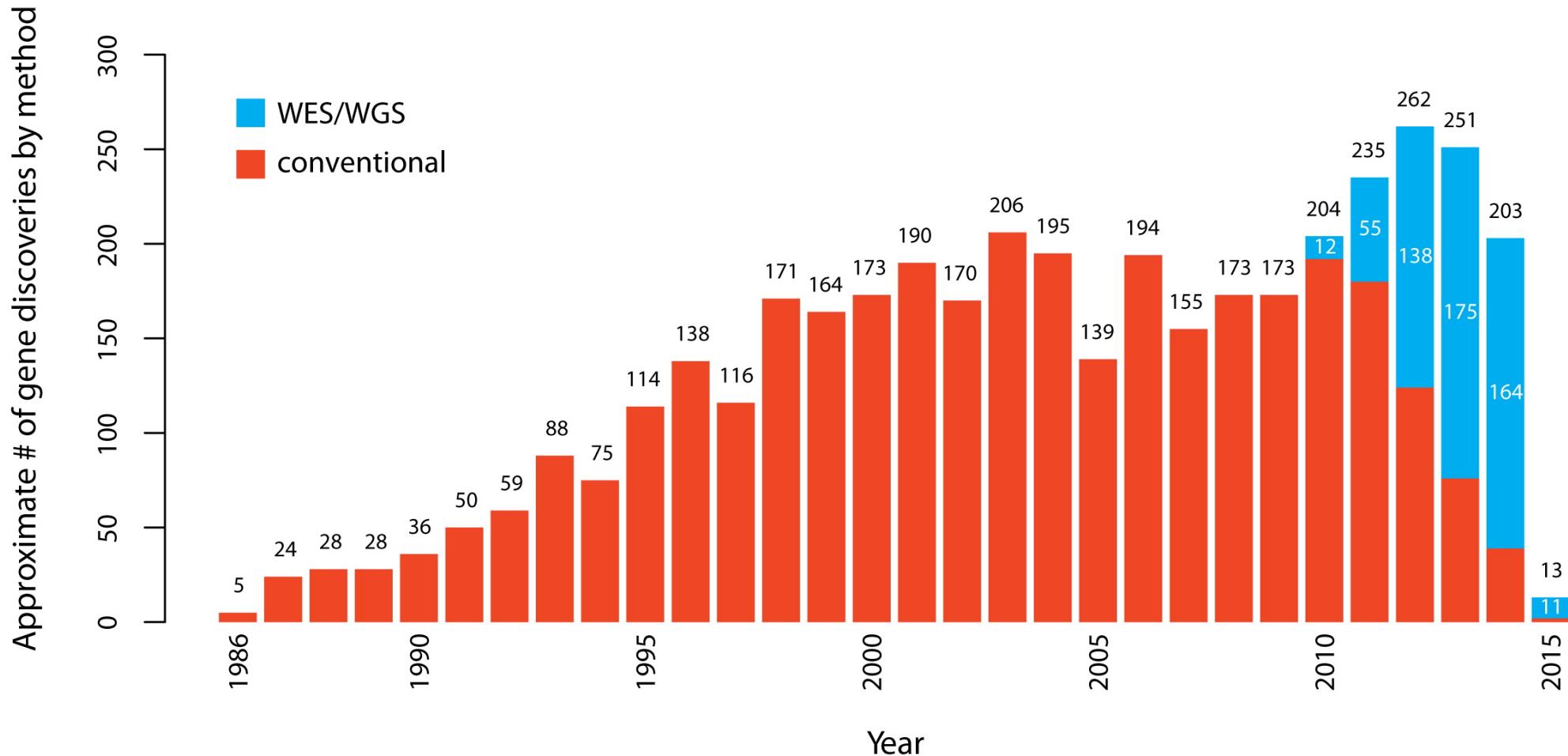


Next-generation sequencing

- Whole genome sequencing (WGS) = entire genome
- Whole exome sequencing (WES) = every known exon, usually with 10 base pairs either side
- Panel sequencing = selection of target genes (tens to hundreds)
- Targeted-enrichment next generation sequencing 



Since the introduction of WES and WGS in 2010, the pace of discovery of genes implicated in Mendelian phenotypes per year has increased substantially, and the proportion of discoveries made by WES or WGS (blue) versus conventional approaches (red) has steadily increased:

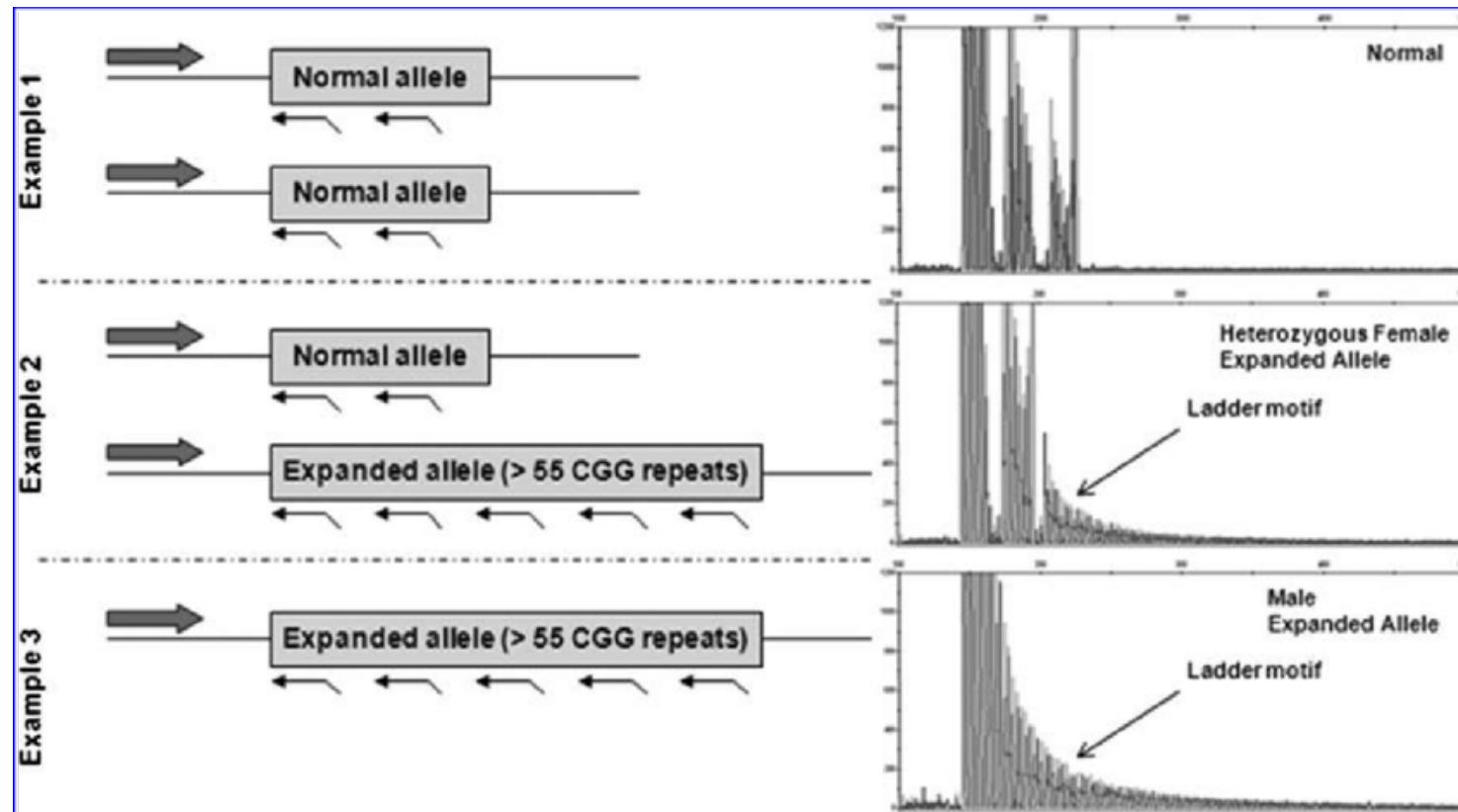


Next-generation sequencing

- Coverage/cost 
- Problems with deletions/duplications, and repeat sequences 

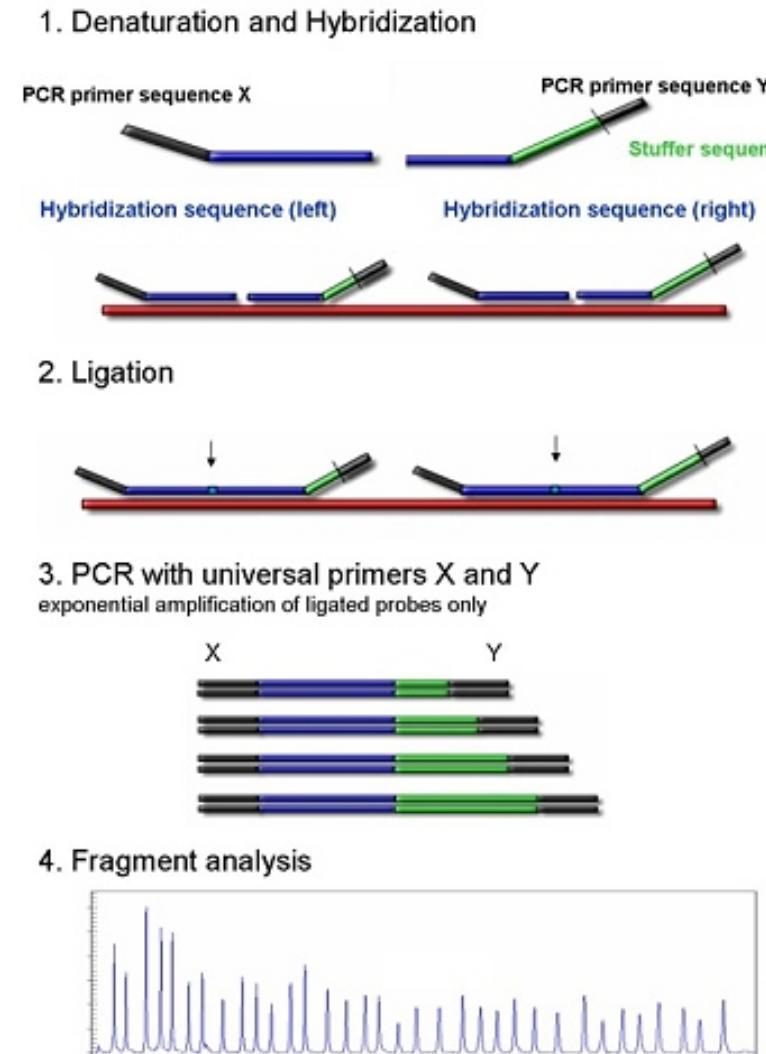
Triplet repeat primed PCR: how it works

- Detects expansion repeats
- Chimeric or triplet repeat primed PCR is defined as a PCR method that generates different sized amplicons due to multiple annealing sites on the template.



Multiplex ligation-dependent probe amplification (MLPA)

- Detects deletions/duplications in a target gene (often complements NGS) 
- Between next gen sequencing and microarray in deletion/duplication, sizewise
- Small probes throughout gene, amplification will only work if exact sequence is present
- Extra or missing regions show up as higher/lower amplification for that particular probe



BIOL3120 –Genetic Testing Techniques

LEARNING OBJECTIVES



On successful completion of this lecture, you will be able to:

- Identify and understand the technologies that detect chromosomal mutations
- Identify and understand the technologies that detect nucleotide mutations