Chromosomal Recombination and Rearrangement

MCT
Fort Wayne Center
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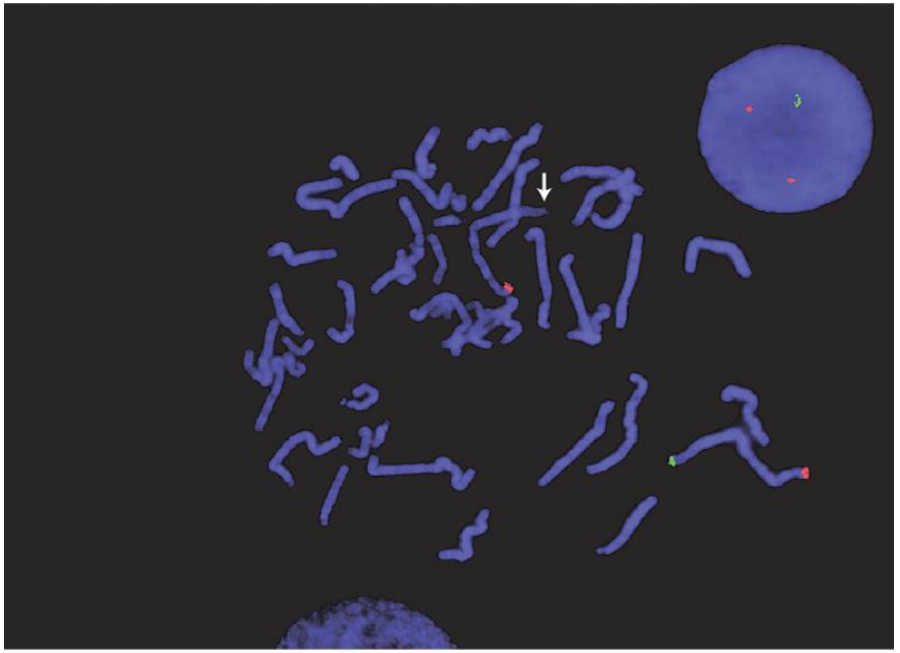
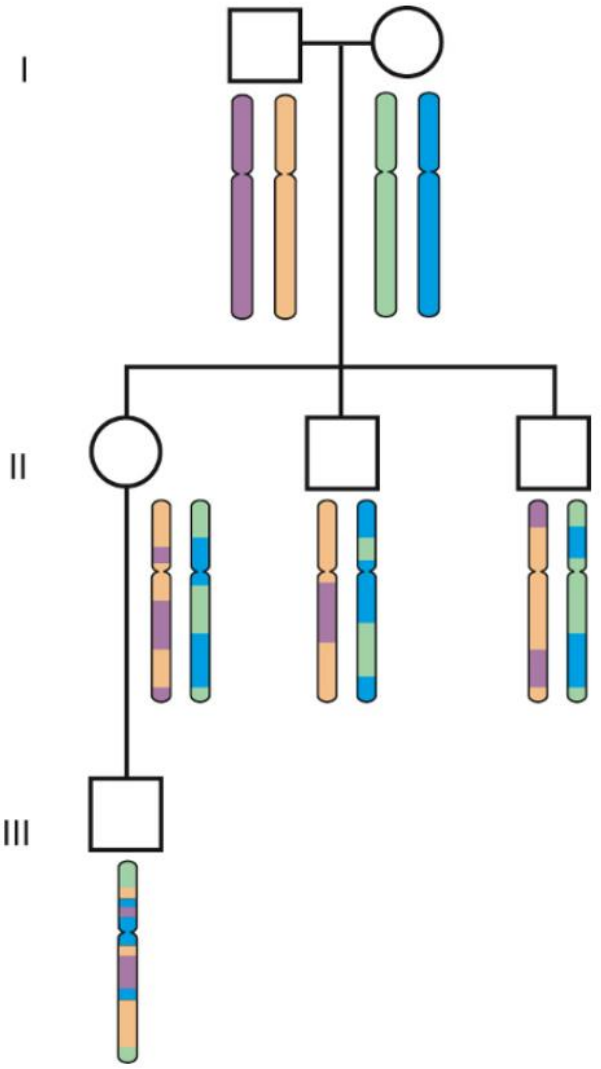


Learning Objectives

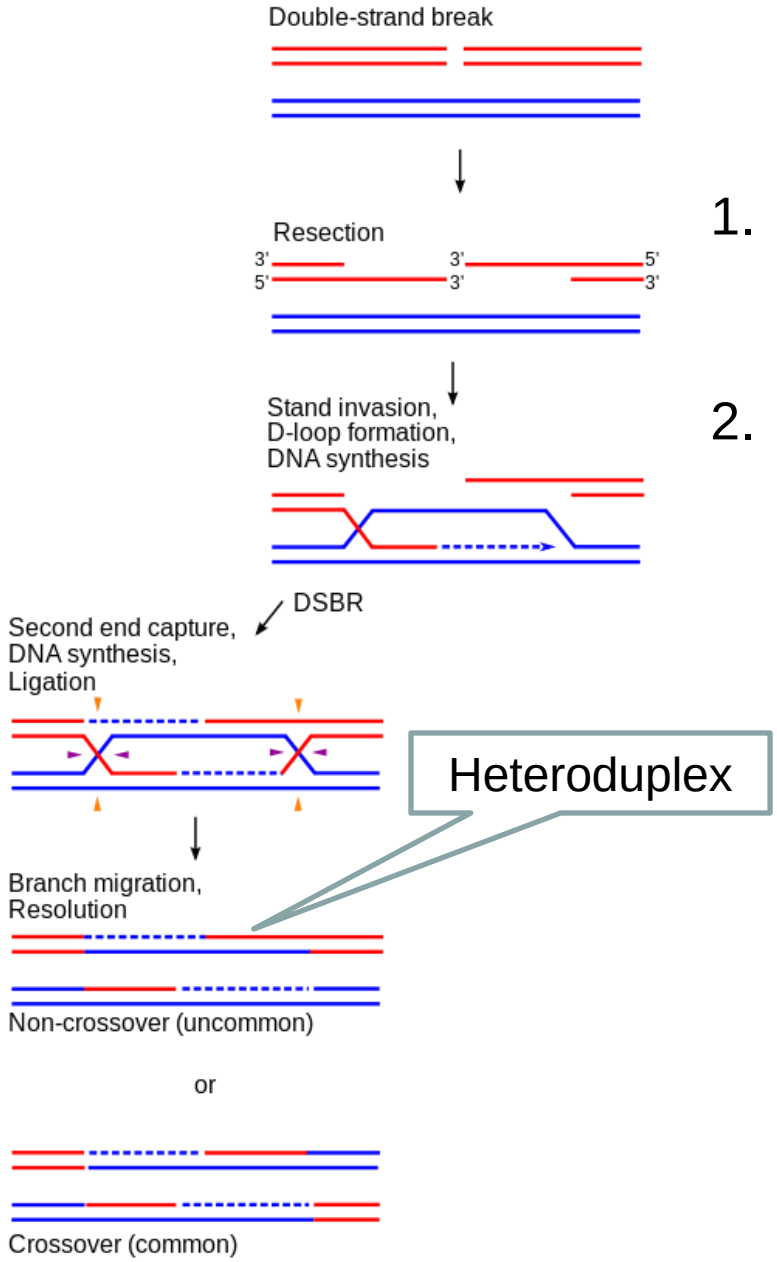
- Describe the process of homologous recombination and how it is regulated.
- Discuss how homologous recombination during meiosis produces genetic diversity and can contribute to chromosomal rearrangement and genetic disease.
- Explain chromosomal non-disjunction's role in cancer and genetic disease. Recognizing the clinical signs of trisomic (Down, Edward, Patau, Klinefelter) and monosomic genetic disorders (Turner)*.
  - Lecture based on Thompson and Thompson chapter 5.
  - * For the last SLO please review Cell Division lecture and read appropriate sections of Thompson & Thompson chapter 6

Homologous Recombination

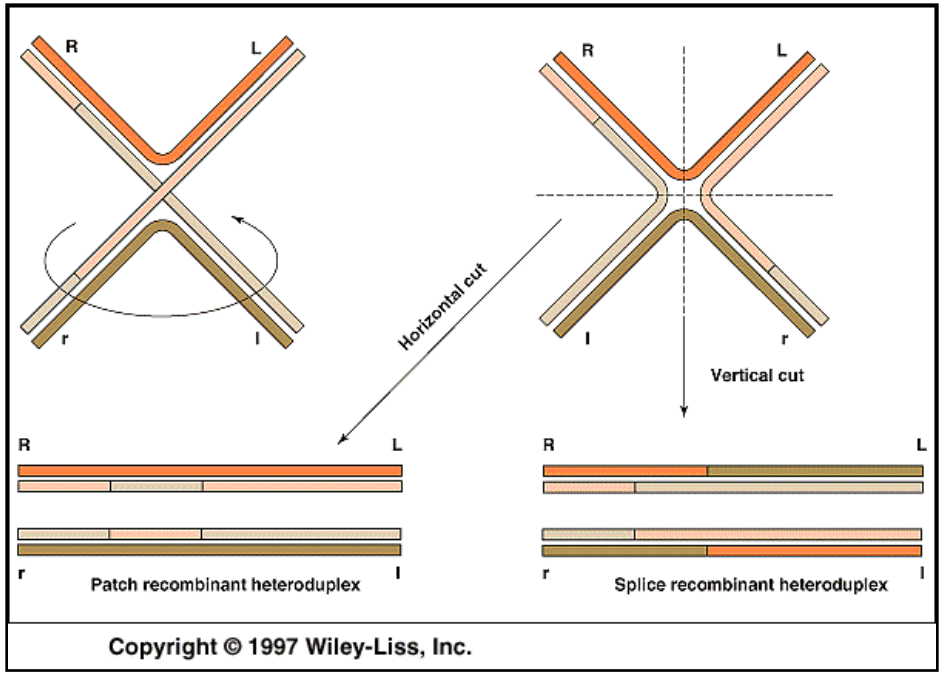
- Provides mixing of alleles inherited from parents during meiosis.
- Genes that are significantly separated on a particular chromosome are inherited as independently as genes that are on separate chromosomes.
- Recombination also has a very important cellular role in the repair of DNA damage.
- Can generate inappropriate rearrangements



Homologous Recombination



1. The region of **heteroduplex** can be expanded by holiday junction migration.
2. Either non-crossover (patch) or crossover (splice) recombination can result from a recombination event.



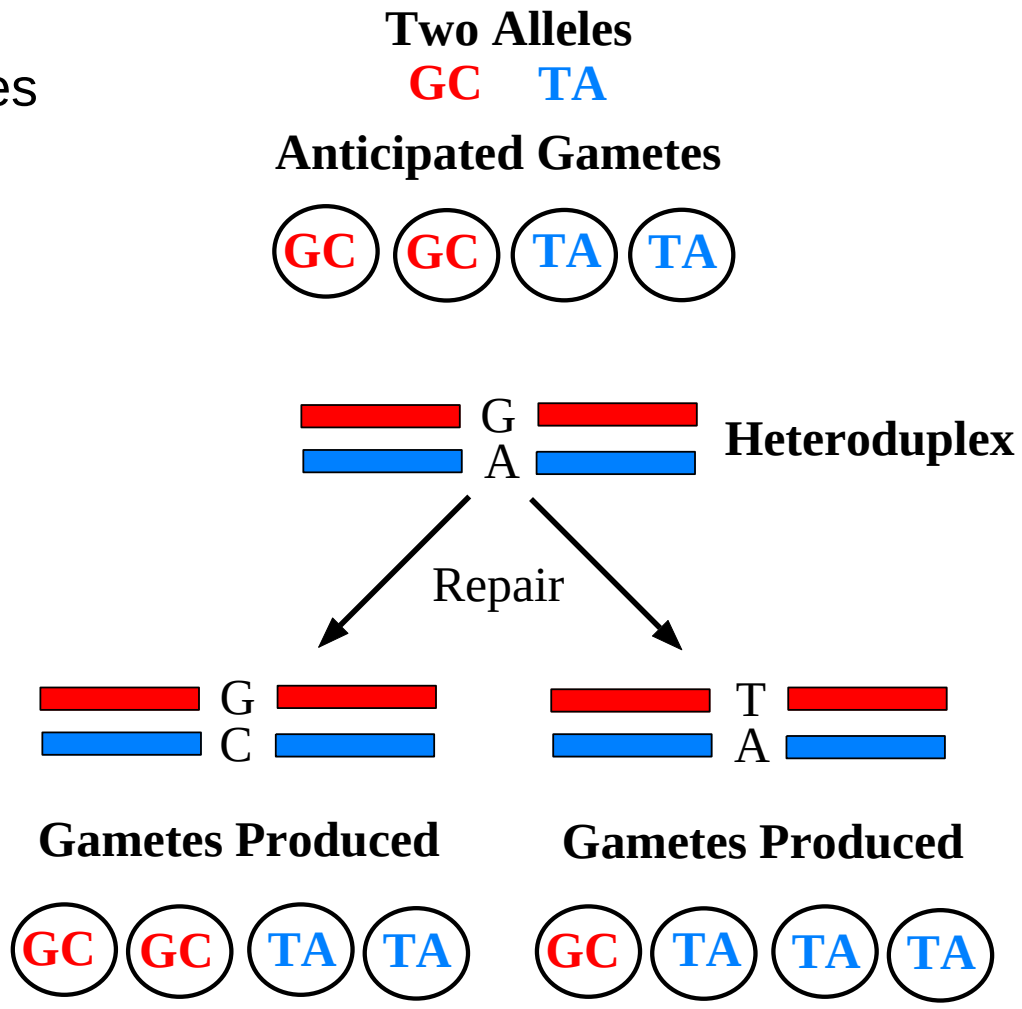
Recombination Can Cause Gene Conversion

Homologous chromosomes have minor sequence differences.

Recombination produces regions of **heteroduplex** where there may be base pair mismatches.

Repair of these mismatches can cause **Gene conversion.**

Conversion can be beneficial, harmful or just reestablish the heterozygous state.

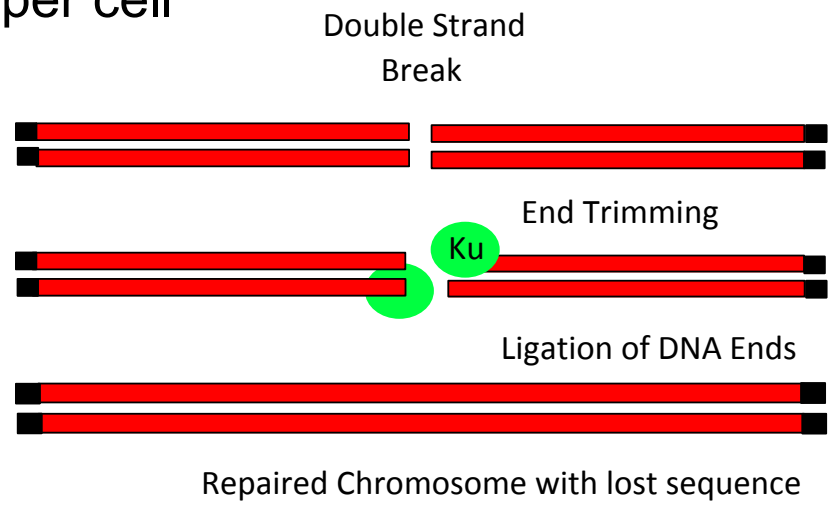


Repair of DNA Double Strand Breaks

- A serious form of DNA damage is DNA **double strand** breakage.
- Broken ends are recognized by the lack of a teleomere.
- DNA damage results in a cell cycle delay to allow repair prior to replication or mitosis.

Strand breaks are repaired by two mechanisms:

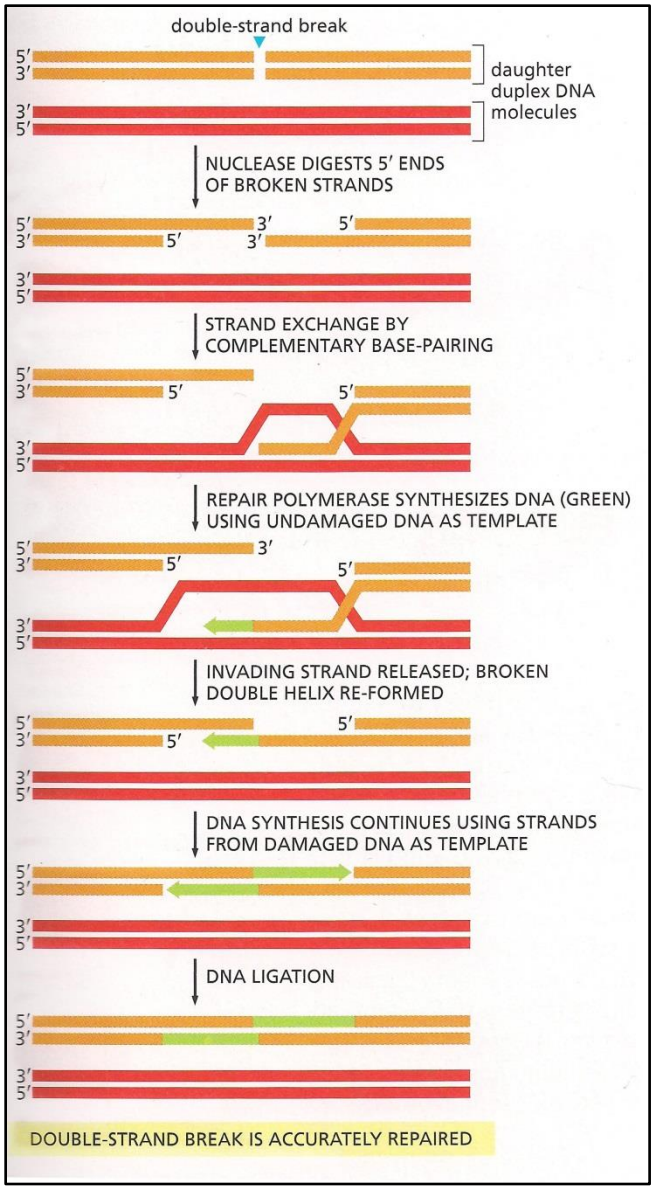
- Nonhomologous end joining
  - Acceptable since most DNA is non-coding?
  - By age 70, 2000 repair "scars" per cell
  - Most common in interphase cells before DNA replication
  - Can join ends that do not go together forming altered chromosomes!
  - Dominate form of break repair in humans.



Repair of DNA Double Strand Breaks

Break Repair by Homologous Recombination

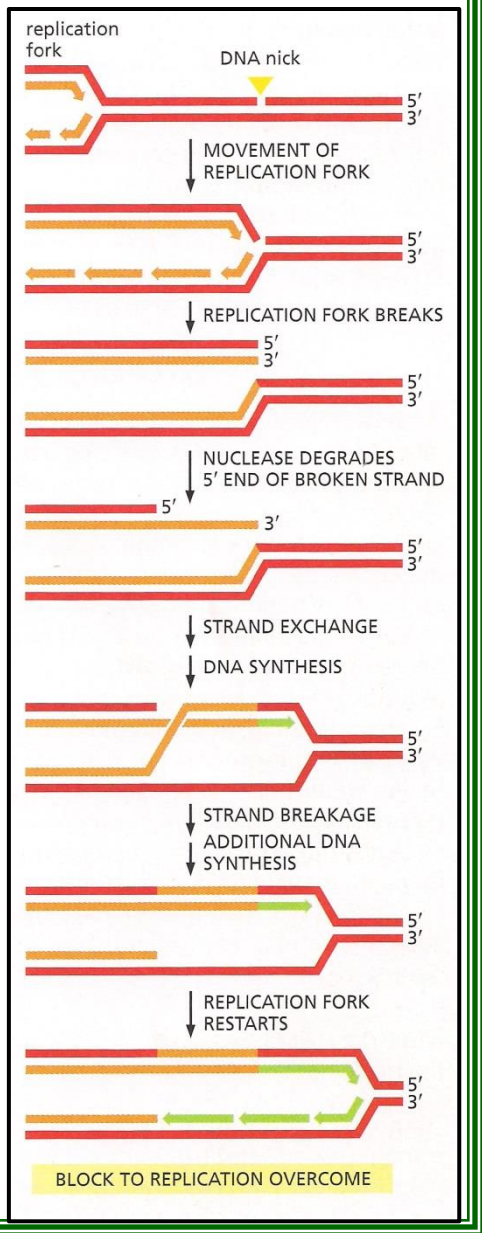
- Used during and after DNA replication when duplicate homologous chromosomes are present. (S phase and G2)
- Homologous chromosome serves as template for 3’ terminal extension of broken ends.
- Strand invasion occurs but invading strand is then displaced without patch or strand exchange.
- Overlapping region in damaged chromosome allows accurate repair without sequence loss.



DNA Strand Breaks in Replication

Break Repair by Homologous Recombination

- A strand nick will cause a chromosome break when a replication fork reaches it.
- End processing forms single strand 3’ end.
- Strand exchange with DNA synthesis
- Strand breakage and repair synthesis
- Replication can then continue normally on the reformed replication fork.

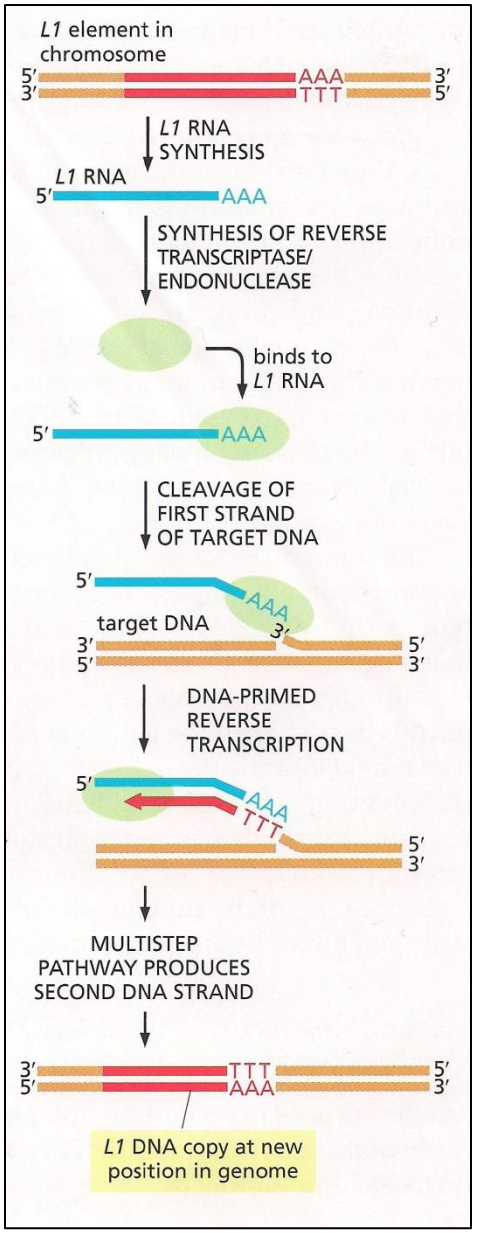


Mobile Genetic Elements

- **Transposable Elements** are potentially mobile sequences found in the genomes of humans an other organisms.
- There are three types of transposable elements:
  - DNA-only transposons
    - Frequent in bacteria where they can move antibiotic resistance genes
    - Drosophila P element
    - Transposase recognizes short repeated terminal repeats and will cut sequence out of one site and insert into another site forming a short repeat at the site of insertion due to staggered cut of target site.
    - Present in human genome, but not thought to be mobile.
  - Retroviral-like Retrotransposons
    - Have long terminal repeats
    - Reproduce like retrovirus, by means of an RNA intermediate.
    - However, they do not form viral particles
    - RNA encodes reverse transcriptase that generates DNA copy of RNA.
    - DNA can insert into a new chromosomal site
    - Similar to retrovirus life cycle, but can not invade a new cell, just a new site.

Mobile Genetic Elements

- Three types of transposable elements;
  - Non-retroviral retrotransposons
    - These include the abundant LINE and SINE sequences found in the human genome like Alu sequences.
    - Most sequences in the human genome are immobile, but some can still move.
    - An Alu sequence is thought to move once per every 100-200 human births.
    - Movement of this type of element causes gene mutations (Estimated frequency of 2 out of every 1000 mutations in humans)
    - More common in mice accounting for about 10% of mutations
  - Misaligned normal recombination between close repeat sequences can cause duplications or deletions.



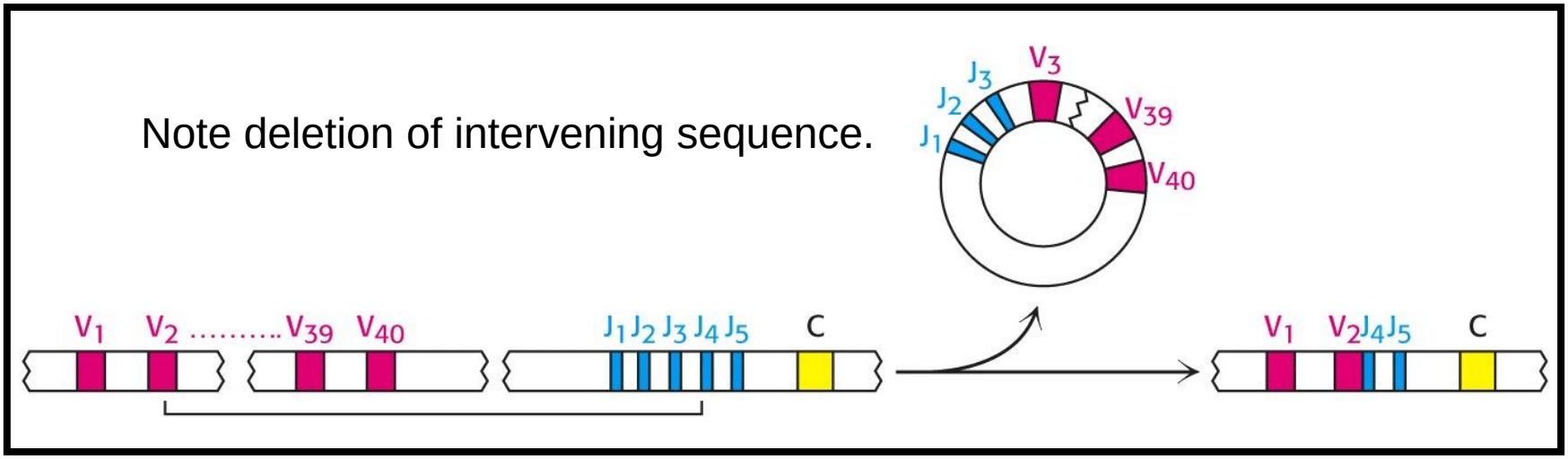
Conservative Site Specific Recombination

- This type of recombination can reversibly move DNA
- The mechanism is used by DNA viruses
- It requires short conserved sequences at the site of excision and at the site of insertion.
- The enzymes function like topoisomerases in that they can break and then ligate the DNA without the need for ATP or DNA ligase.

- Conservative site-specific rearrangements can cause:
  - Sequence excision
  - Sequence Insertion
  - Sequence Inversions

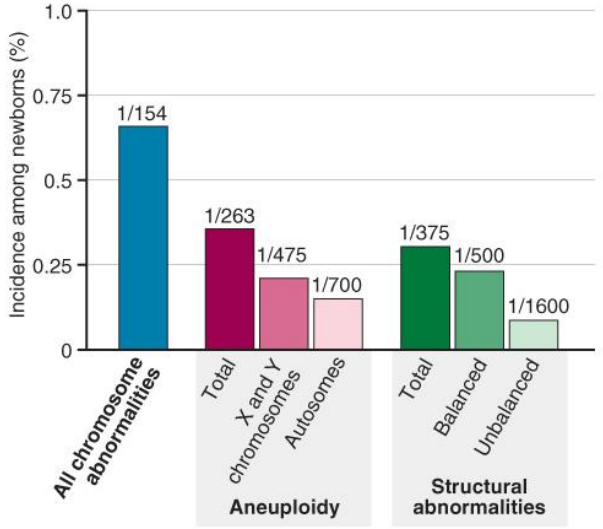
Site-Specific Recombination and the Immune System

- Four site specific recombinations are needed to generate a mature antibody molecule in humans.
- The genes for the responsible enzymes lack introns and the proteins have sequence homology with the transposases that move DNA only transposons.
- Mutations in the human lymphocyte specific V(D)J recombinase genes **RAG1** or **RAG2** can cause **SCID** (Severe Combined Immunodeficiency).



Genetic Disorders

The three major classes of genetic disorders:



- Chromosomal Disorders
  - Wrong number of chromosomes
  - Structurally altered chromosomes
- Single-gene Disorders
  - Patterns of inheritance vary.
- Multifactorial Disorders
  - Most of the common human diseases.

Karyotype Analysis of Cancer

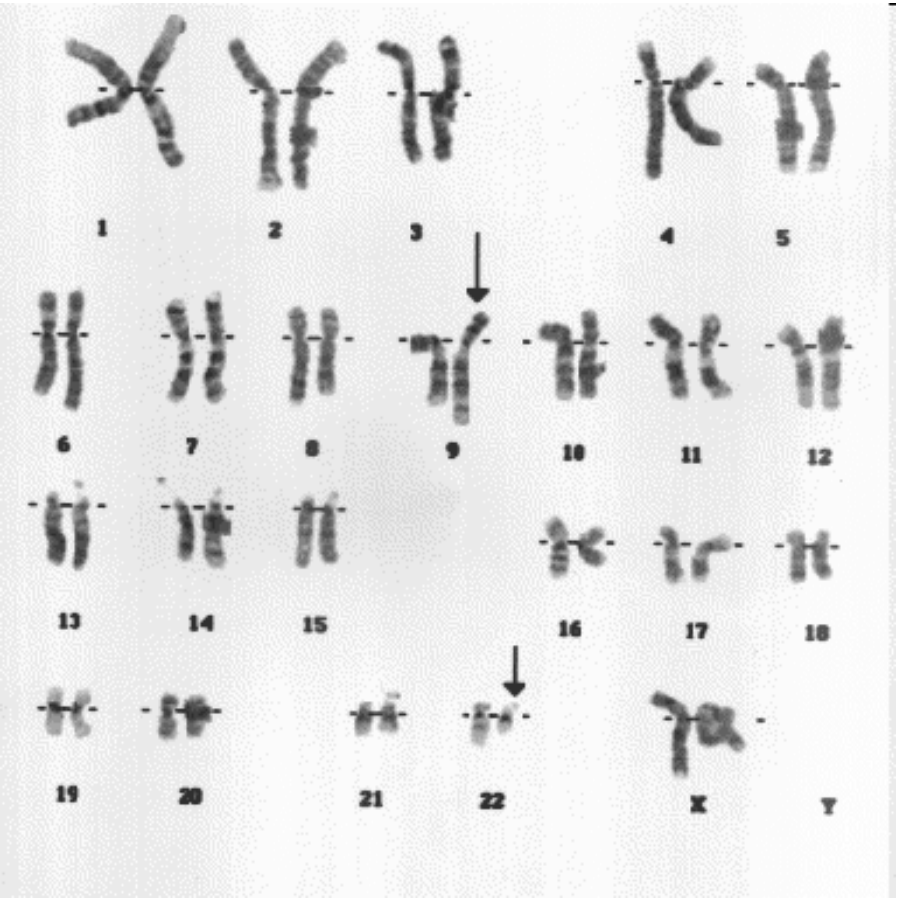
Example:

95% of cases of chronic myleogenous leukemia (CML) involve this translocation between chrmosomes 9 and 22.

t(9;22)(q34;q11)

Results in the production of the BCR-ABL Fusion protein.

Philadelphia Chromosome



Chromosomal Abnormalities

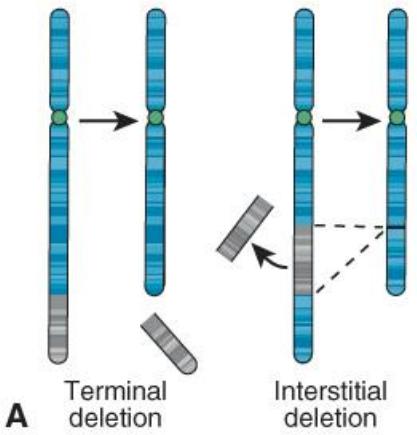
Structural Abnormalities

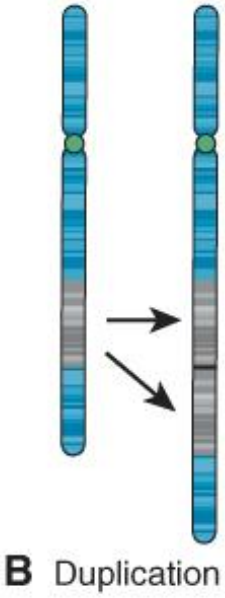
- Result from inappropriate recombination or chromosome breakage followed by reconstitution in an abnormal arrangement.
- Occur spontaneously, but are induced by certain chemicals, viruses and ionizing radiation.
- Can result in a chromosomal rearrangement, deletion or insertion.
- There are two forms of rearrangement:
  - Unbalanced, genetic material has been deleted, added or duplicated.
    - Often problematic, monosomic for deleted region or trisomic for duplicated region.
    - Clinical consequences reflect the result of haploinsufficiency or overexpression.
  - Balanced, all genetic material present, just rearranged.
    - Usually not problematic for carrier, but potentially a problem for offspring.

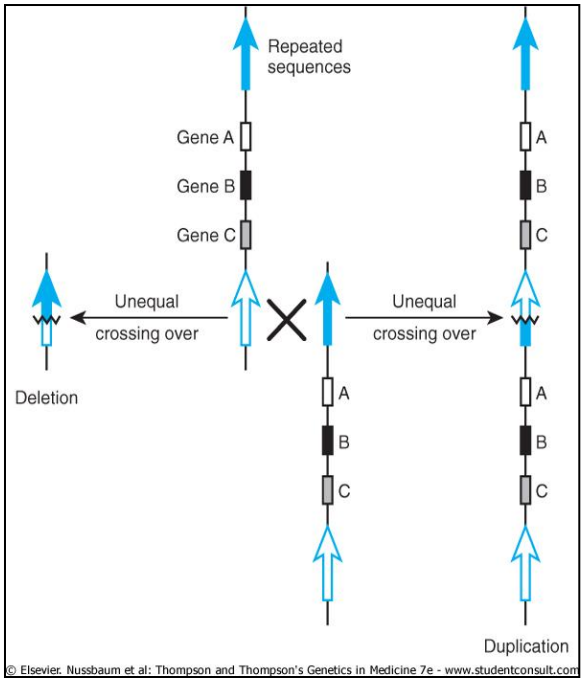
Chromosomal Abnormalities

Unbalanced Rearrangements

- Deletions (del)
  - Terminal
  - Interstitial
- Deletions and duplications
  - Can result from unequal crossover events
- **See Thompson & Thompson table 5.1 for list of abbreviations.**

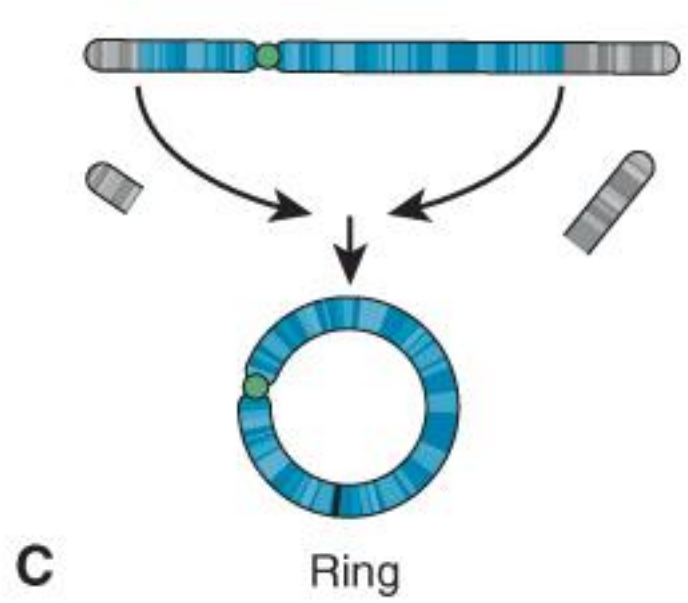






Chromosomal Abnormalities

- Ring Chromosomes (r)
  - Often present in addition to normal chromosomal complement.
  - Supernumerary chromosomes or **marker chromosomes** (mar).
  - Hard to identify by banding due to small size.
  - **FISH** analysis is often helpful.
  - Frequency of 1/2500
  - Odds of serious risk to fetus can be very low to nearly 100%, depending on source of extra chromosome.
  - A relatively high fraction derive from the sex chromosomes and chromosome 15.
  - Some are mitotically stable despite lacking the normal centromere. (Have neocentromere).
  - Often lack teleomeres.
  - Large ring chromosomes are rare.
    - Problem at mitosis if sister chromatids are intertwined.
    - Breakage and fusion may form smaller rings.



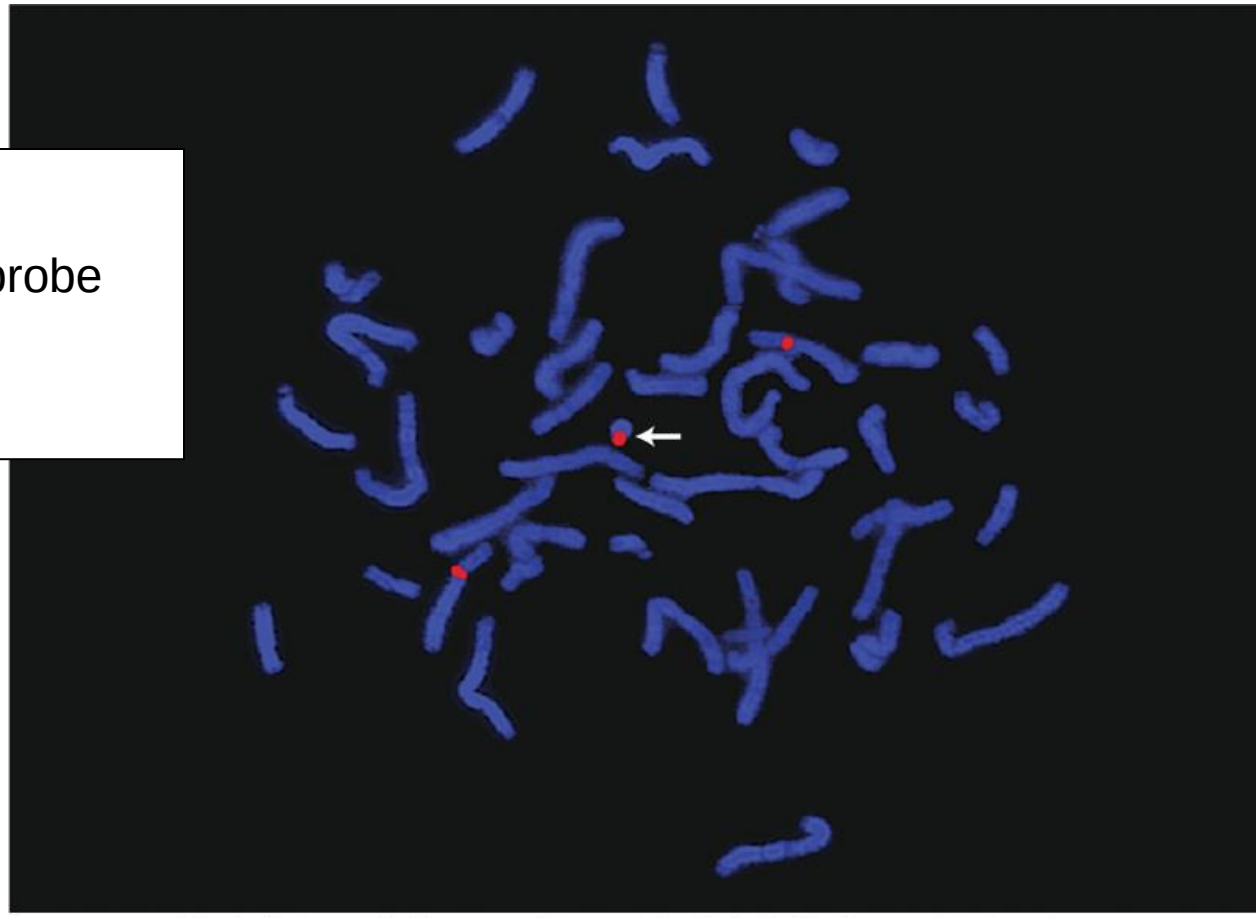
FISH Analysis

- FISH can help identifiy the source of odd chromosomes.

Unidentified, mar

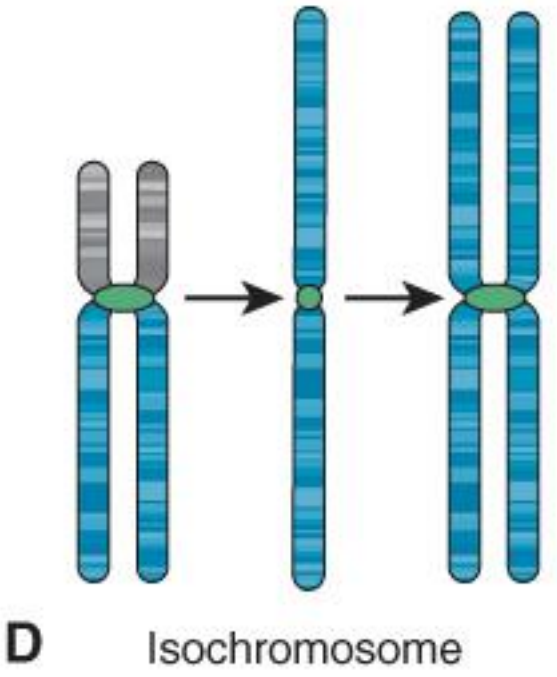
Centromeric α-satellite probe for chromosome 8.

Diagnosis? 46 + r8



Chromosomal Abnormalities

- Isochromosomes (i)
  - One arm deleted and remaining arm is duplicated.
  - Common in tumors
  - If chromosomal content is normal otherwise, monosomy for missing arm, trisomy for duplicated arm.
  - **iXq** most common form.
  - **Note Xi denotes inactive X**

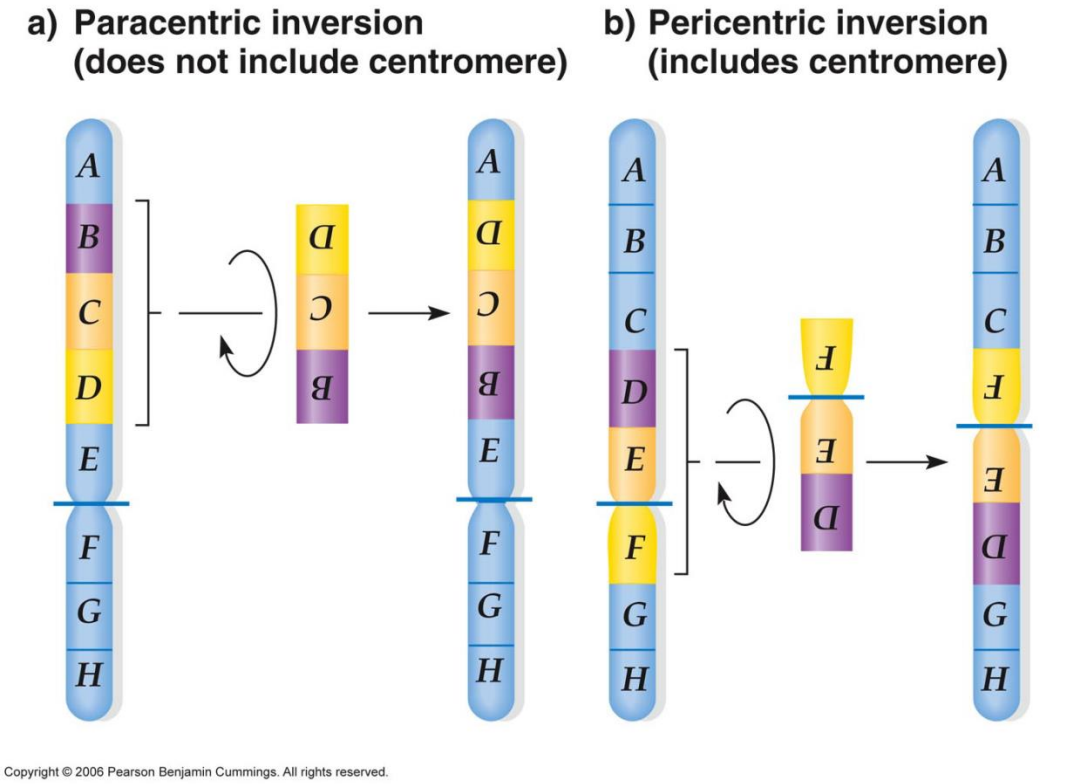


D Isochromosome

- Dicentric Chromosomes (dic)
  - Contain two centromeres
  - **Mitotically unstable** unless one centromere is inactivated (pseudodicentric)

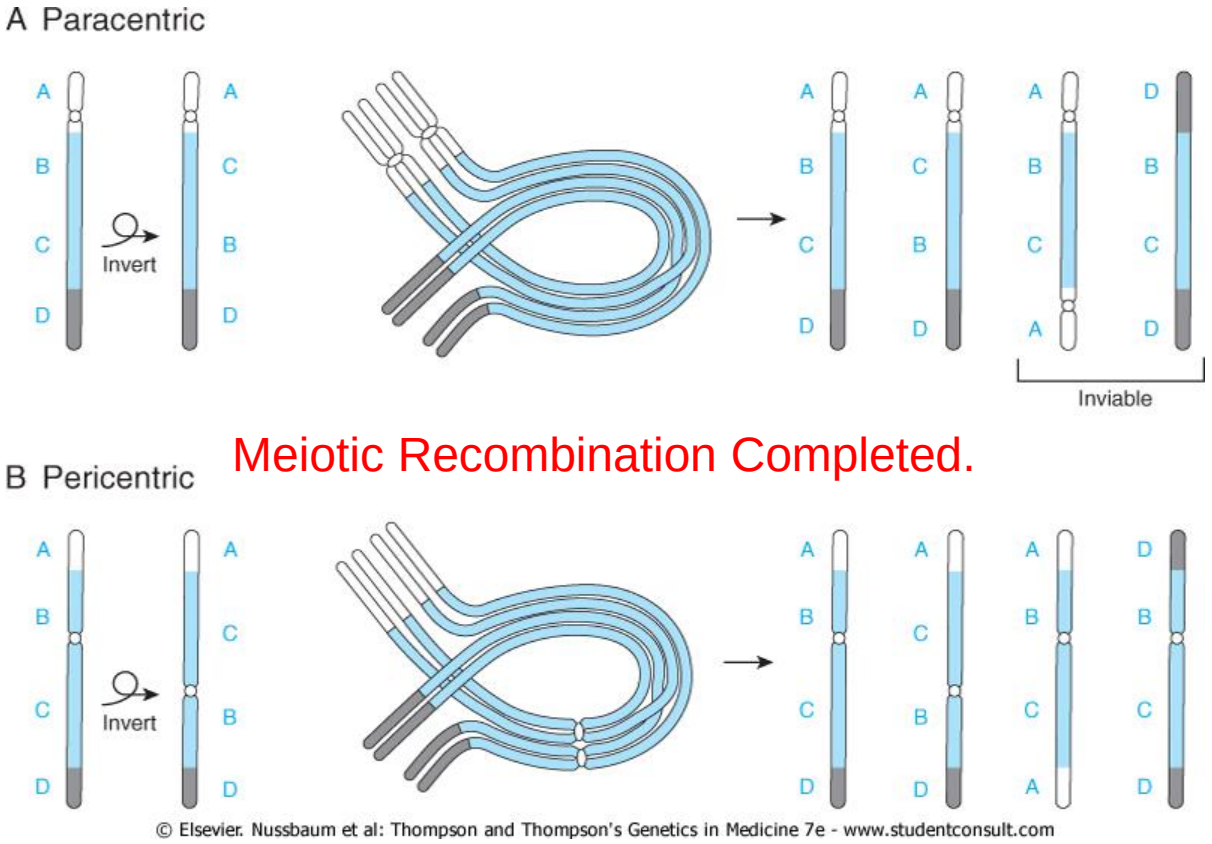
Chromosomal Abnormalities
Balanced Rearrangements

- Inversions
- Usually no phenotype unless a gene is damaged at one or both breakpoints.
- Medical significance comes from the risk of having offspring with unbalanced karyotypes.



Para (beside)

Peri (around, surrounding)

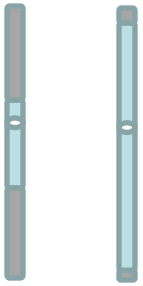


- Paracentric (centromere not inverted, breaks both in same arm)
  - Recombination and meiosis will produce gametes with the normal chromosome, the balanced inversion along with gametes containing a dicentric and acentric chromosomes (latter are mostly inviable).
- Pericentric (inverted region includes centromere, break in each arm).
  - Recombination and meiosis will produce gametes with the normal chromosome, the balanced inversion along with gametes containing deletions and duplications of the regions outside the inversion.
  - Risk of passing on unbalanced karyotype is in general 5-10% (specific for each inversion).

Pericentric Inversions:

- If inversion is physically short, the duplicated and deleted segments are physically long
  - Individuals with **small** pericentric inversions have a higher frequency of sterility, miscarriage, stillbirths, with a lower frequency of abnormal, viable offspring

- If inversion is physically long, (compared to the total length of the chromosome), the duplicated and deleted segments are physically short
  - Individuals with **long** pericentric inversions have a lower frequency of sterility, miscarriage, stillbirths, but have a higher frequency of abnormal viable offspring



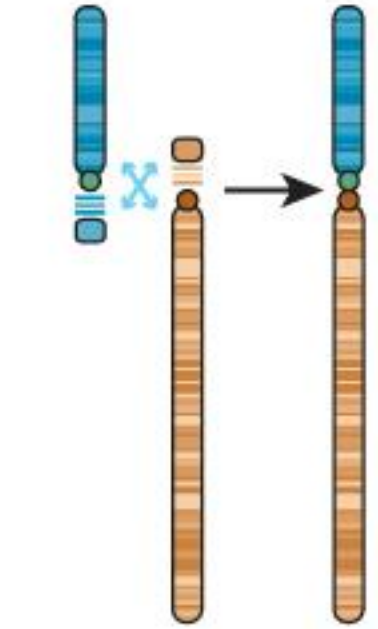
Chromosomal Abnormalities

Insertions (ins)

- Nonreciprocal translocation.
- Rare since three or more breaks are required.
- High risk of abnormal child.
- Prenatal diagnosis is indicated.

Robertsonian Translocations (rob)

- Involve **acrocentric** chromosomes
- Reduce chromosome number to 45 for balanced karyotype.
- Loss of short arm is covered by remaining acrocentric chromosomes.
- rob(13q14q) and rob(14q21q) are the two most common.
- Risk of unbalanced karyotype in offspring.



E Robertsonian translocation

#13, 14, 15, 21, 22

Robertsonian Chromosomes

Acrocentric Chromosomes

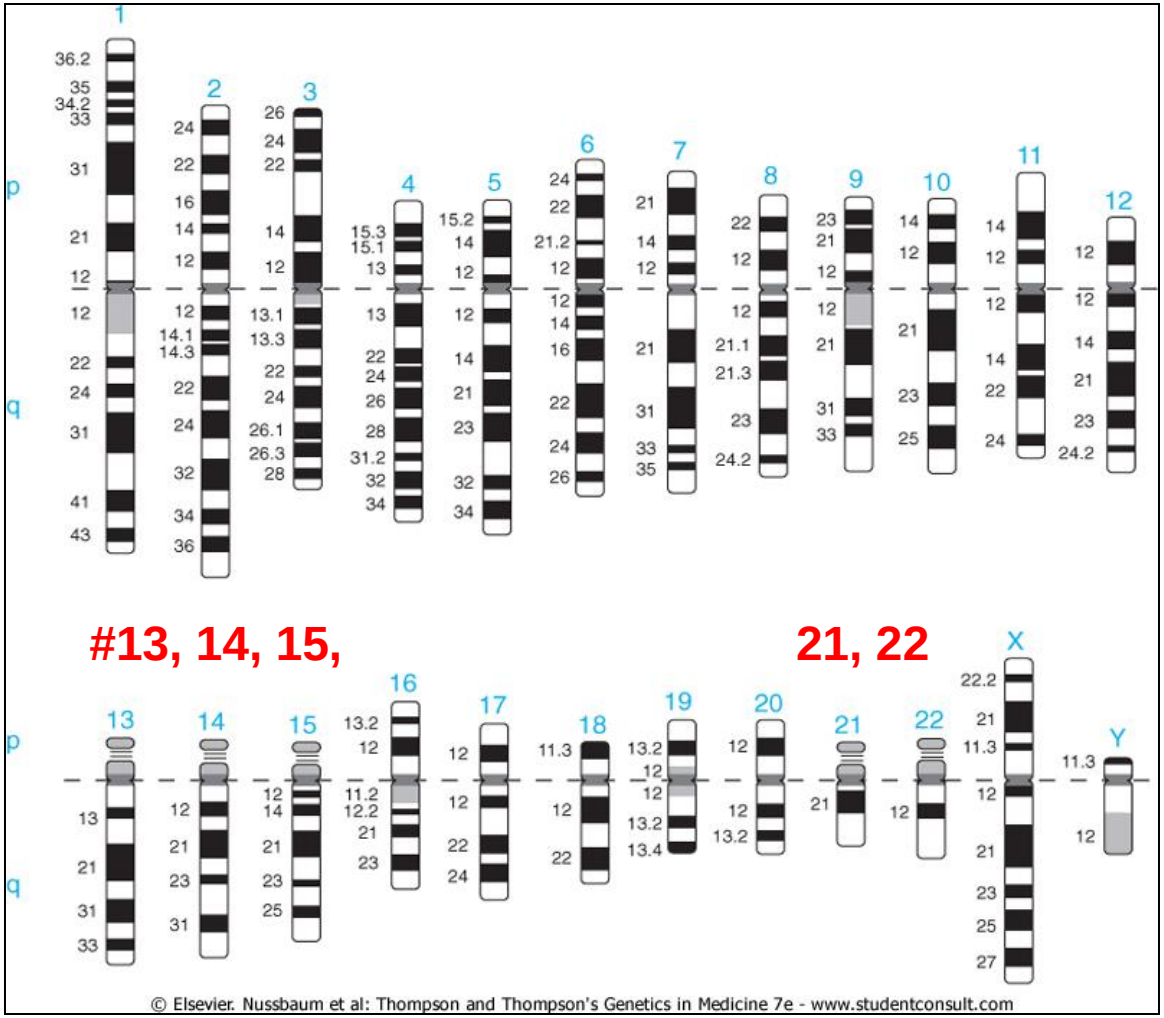
Have centromere located near one end of chromosome.

All have small distinctive masses of chromatin on short arm.

The short p arm region contains hundreds of copies of the ribosomal RNA genes. (Satellite DNA).

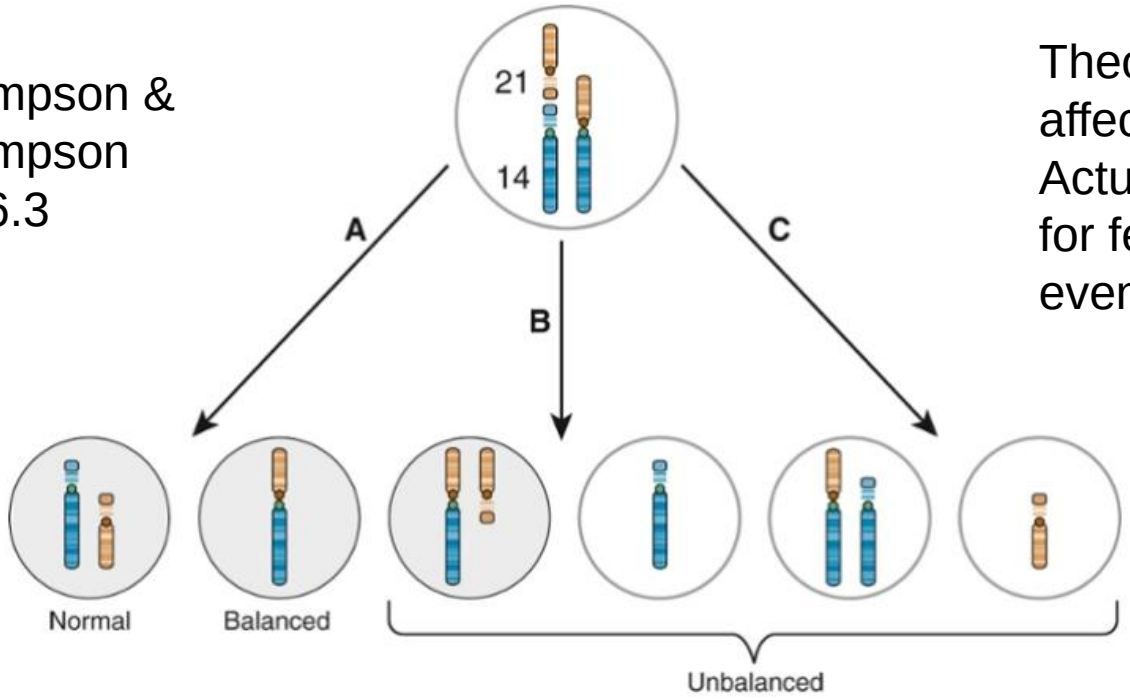
A Robertsonian translocation (rob14q21q for example) will still leave three chromosomes with ribosomal RNA genes. So in this case the loss of DNA is tolerated.

Adults with a Robertsonian translocation involving chromosome 21 are at higher risk of having a child with Down Syndrome.



Robertsonian Chromosomes

Thompson & Thompson Fig 6.3



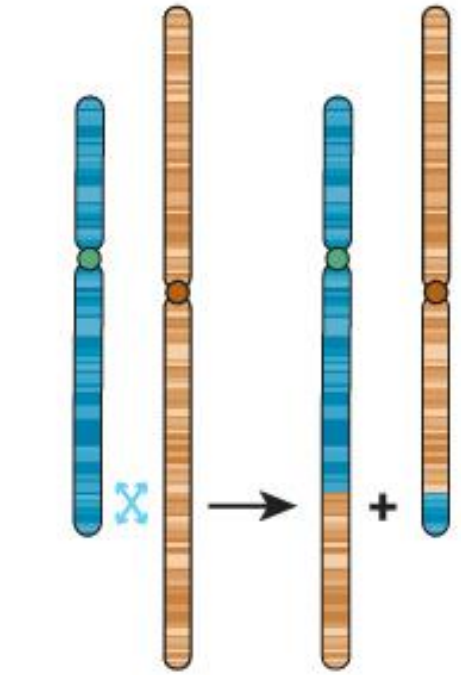
Theoretical risk of affected child is 1 in 3. Actual risk is 10 to 15% for female carriers and even less for males.

- Six types of gametes can be formed:
  - 1 Normal
  - 1 Balanced
  - 4 Unbalanced (Only the first of which is likely to be viable, but would produce a child with Down Syndrome)

Balanced Chromosomal Abnormalities

Reciprocal Translocations (t)

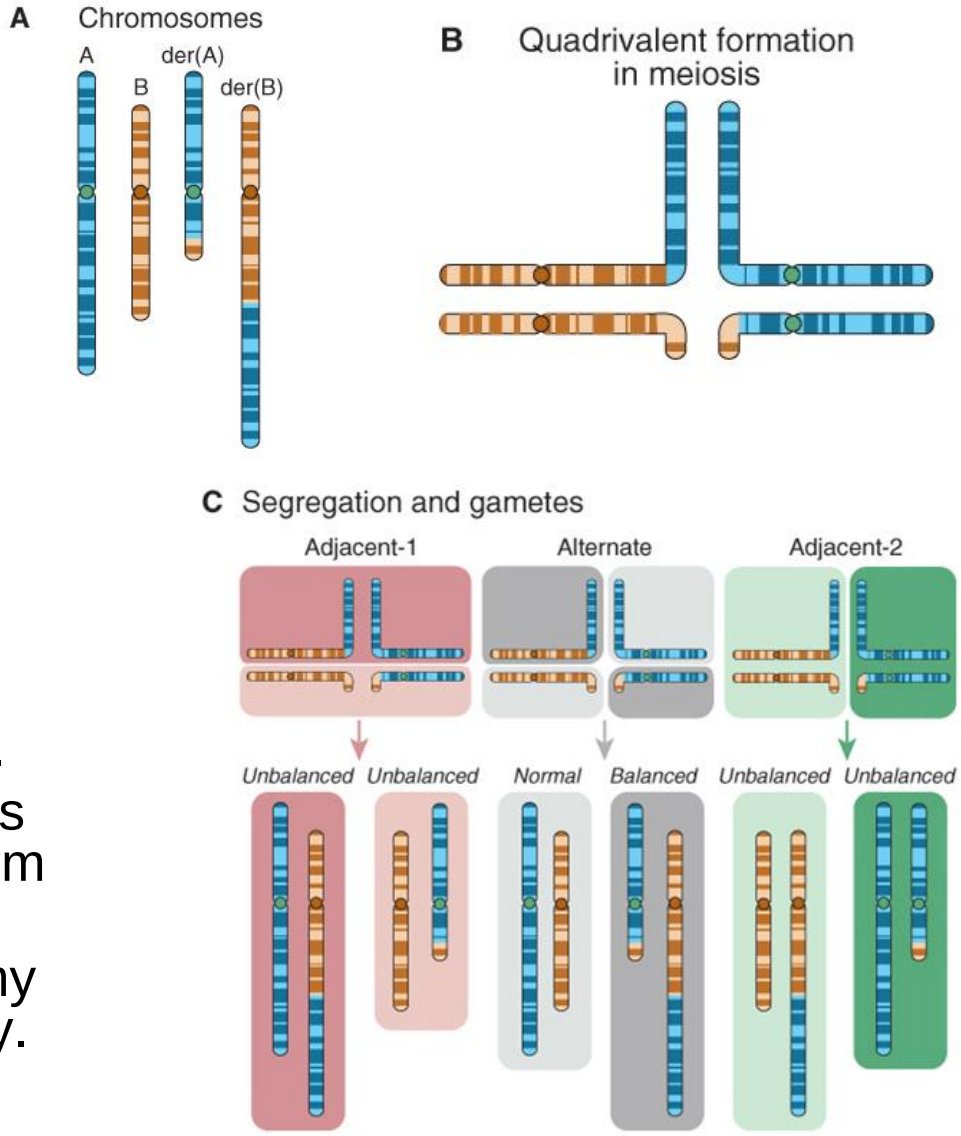
- Reciprocal exchange between nonhomologous chromosomes.
- Number of chromosomes unchanged.
- Derivative chromosomes produced (der)
- 1/600 newborns
- Associated with **high risk** of unbalanced gametes and abnormal offspring.



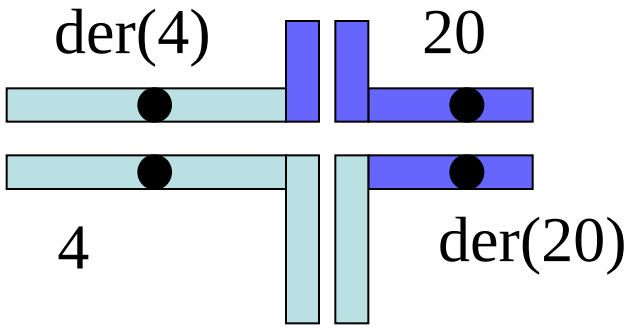
Chromosomal Abnormalities

Reciprocal Translocations

- Chromosomal pairing at **meiosis I** produces a quadrivalent.
- At anaphase the chromosomes can be segregated (2:2) in three ways, only one of which results in gametes with balanced genomic content.
- 3:1 sorting of chromosomes also occurs (5-20% of sperm of a translocation carrier).
- More often results in trisomy of a region than monosomy.



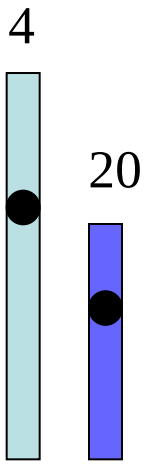
Segregation of Reciprocal Translocations



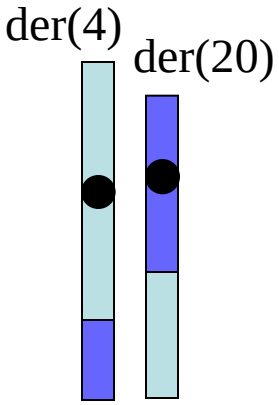
Alternate

Adjacent-1

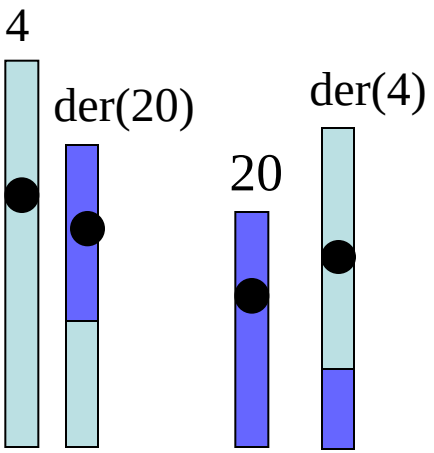
Adjacent-2



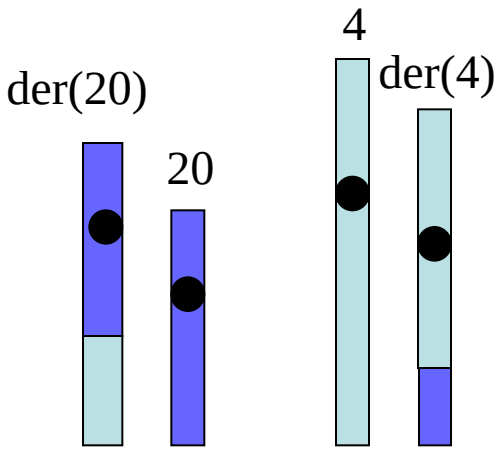
Normal



Balanced



Unbalanced



Unbalanced

Other Analysis Methods

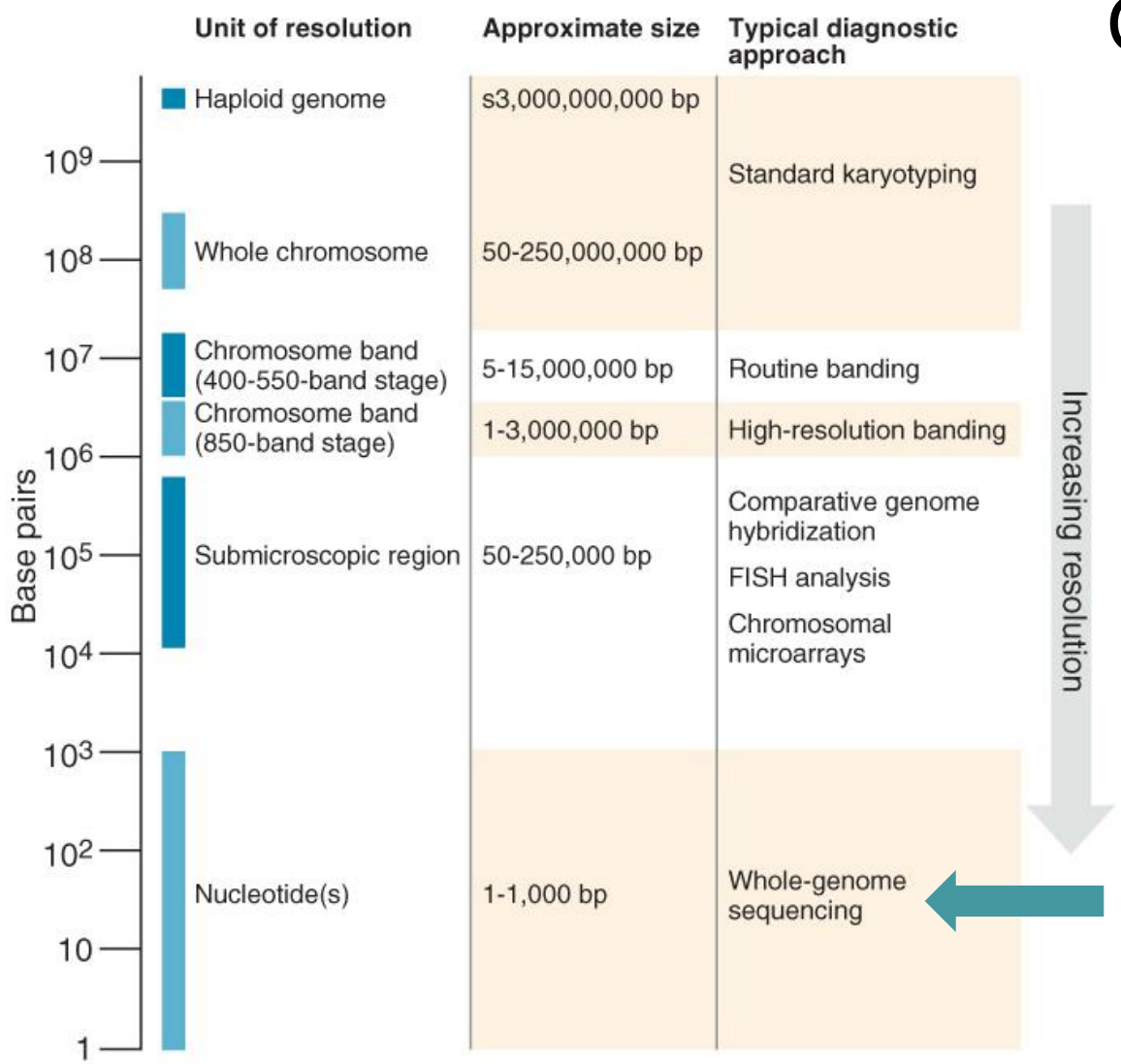


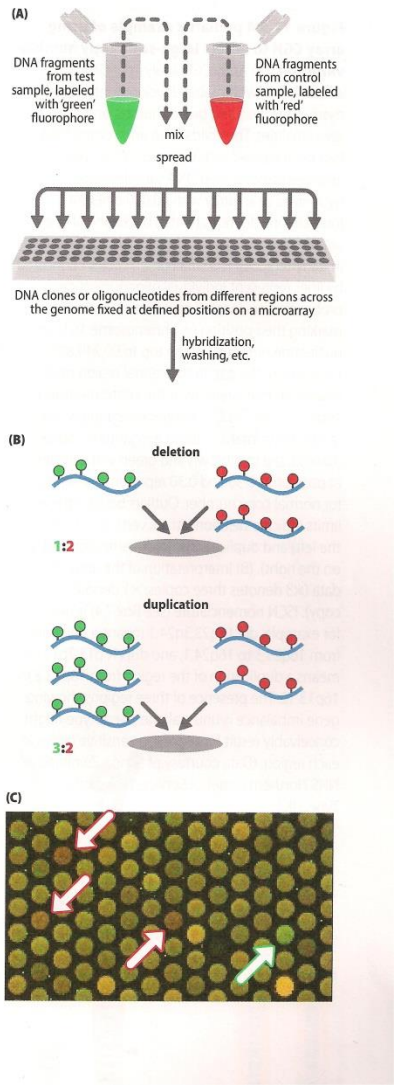
Figure 5-1 Spectrum of resolution in chromosome and genome analysis. The typical resolution and range of effectiveness are given for various diagnostic approaches used routinely in chromosome and genome analysis. FISH, Fluorescence in situ hybridization.

The resolution of standard karyotyping is limited and so various molecular methods are used to improve the resolution of chromosome analysis.

Molecular Karyotyping by CGH
Comparative Genome Hybridization

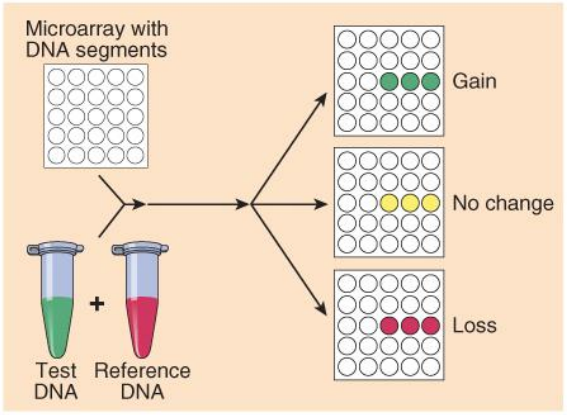
- **Microarray based method.**
  - Samples can be genomic DNA or cDNA

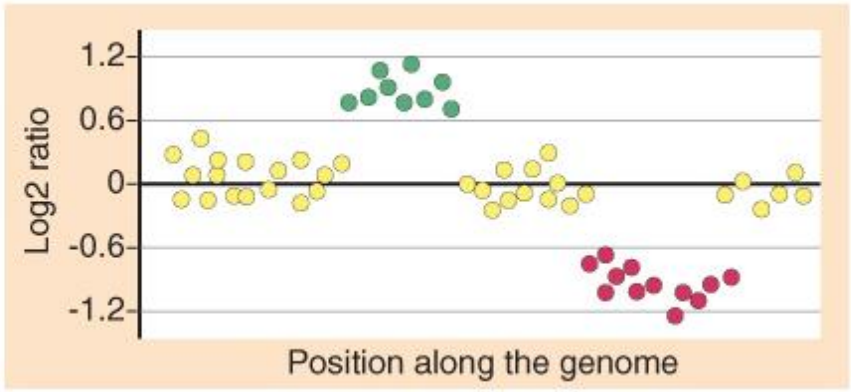
- Test DNA- has fluorescent tag (Green in example)
  - Derived from patient being tested
- Control DNA-also has fluorescent tag (Red)
  - Mix of male and female normal controls
- Sample and control DNA are mixed and then allowed to hybridize to microarray.
- Equal amounts of DNA hybridization from each sample to a target sequence will give **yellow** signal.
- Deletion of a region in test DNA allows more control DNA hybridization. Results in red colored dot (red arrows)
- Duplication of a region in test sample allows more test DNA hybridization. Results in green colored dot (green arrow)

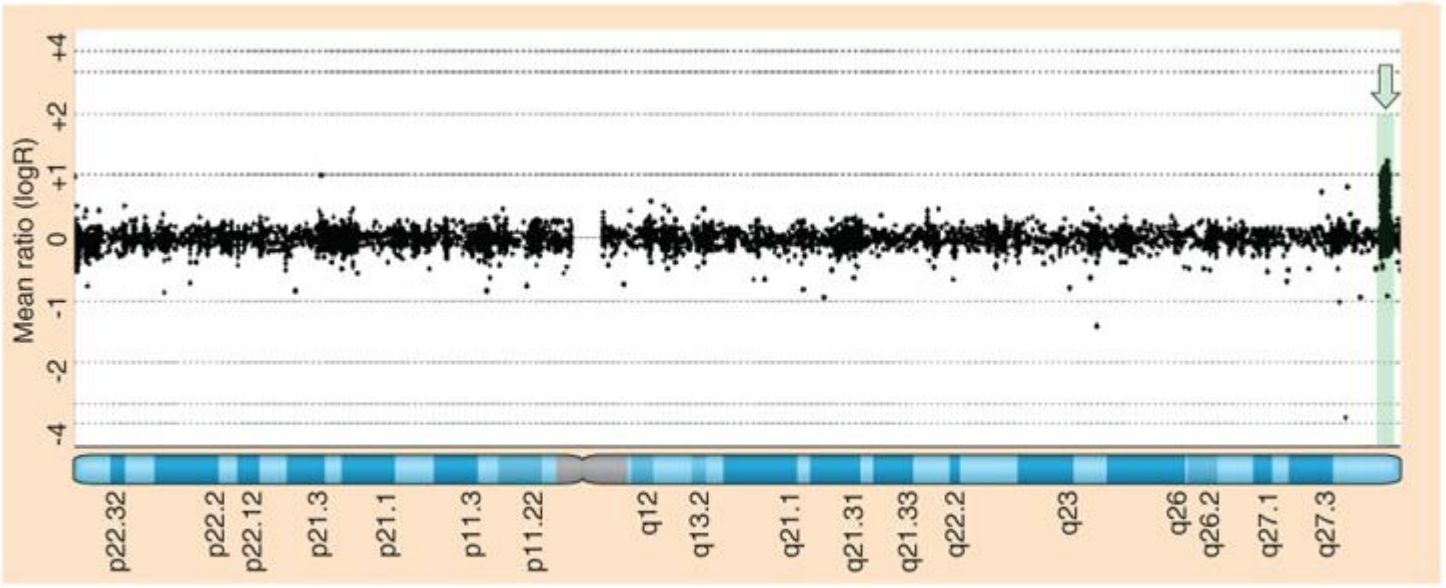


Molecular Karyotyping by CGH

Comparative Genome Hybridization

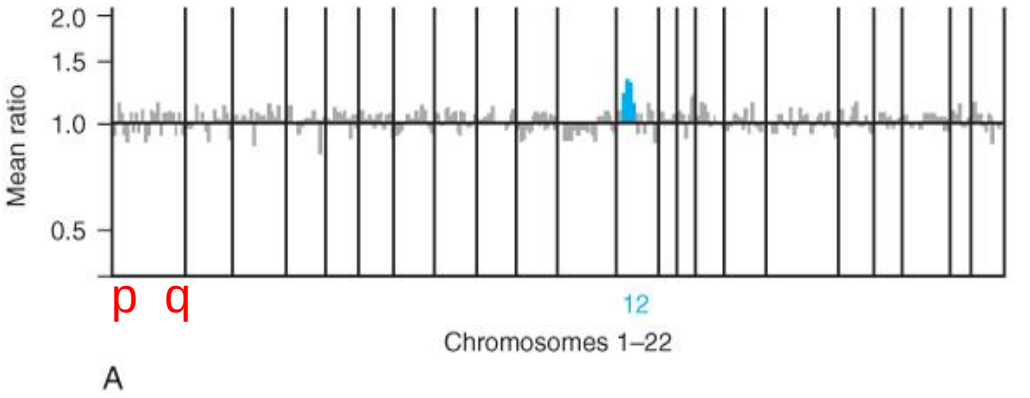






X q28
duplication

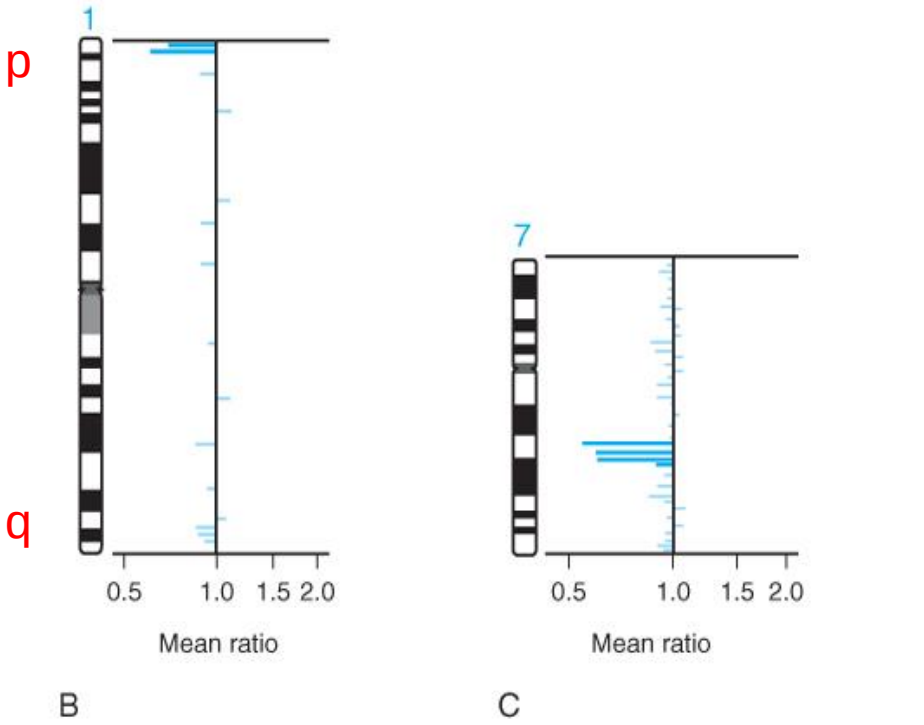
Molecular Karyotyping



A. Partial duplication p arm of chromosome #12

B. Deletion 1p term.

C. Deletion 7q.



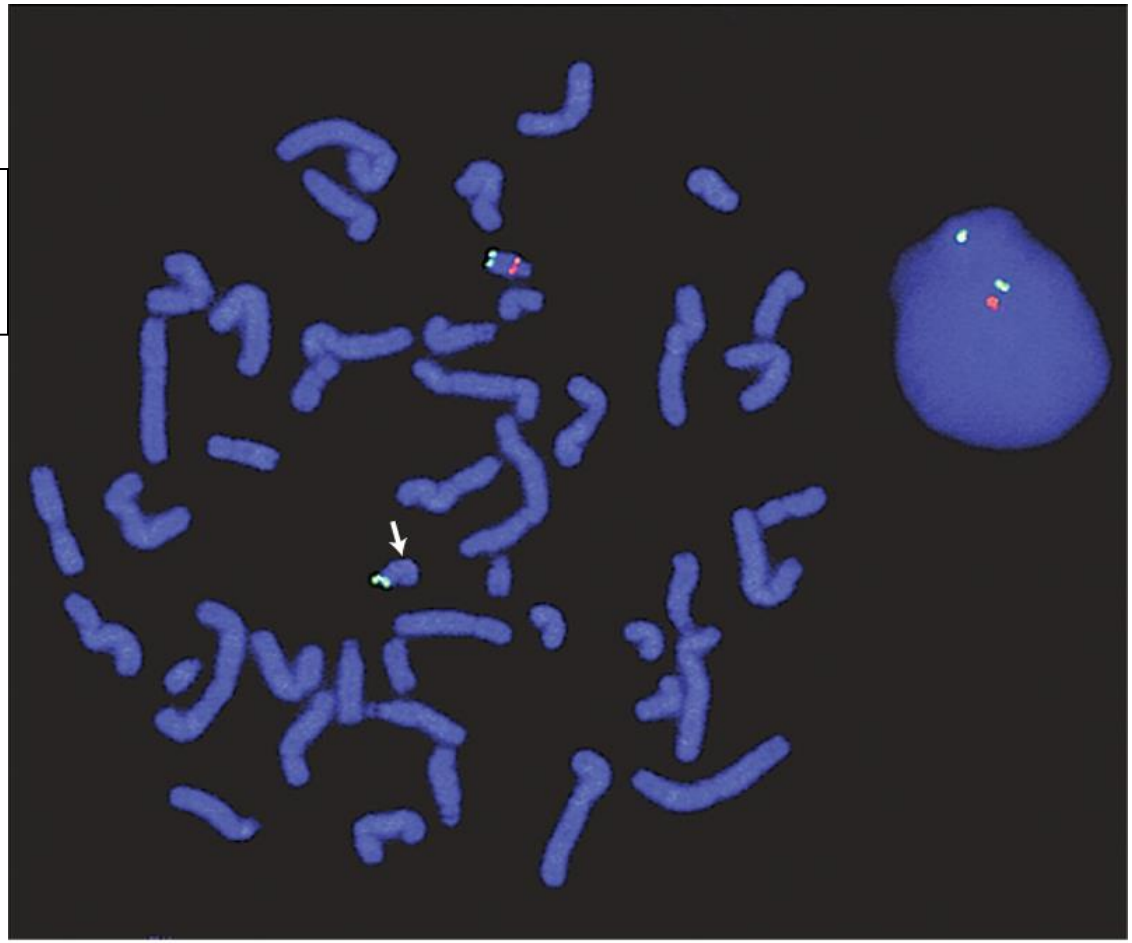
FISH Analysis

- DiGeorge Syndrome Diagnosis
  - Deletion 22q11.2

Control Probe for 22p: green

q11.2 probe: red

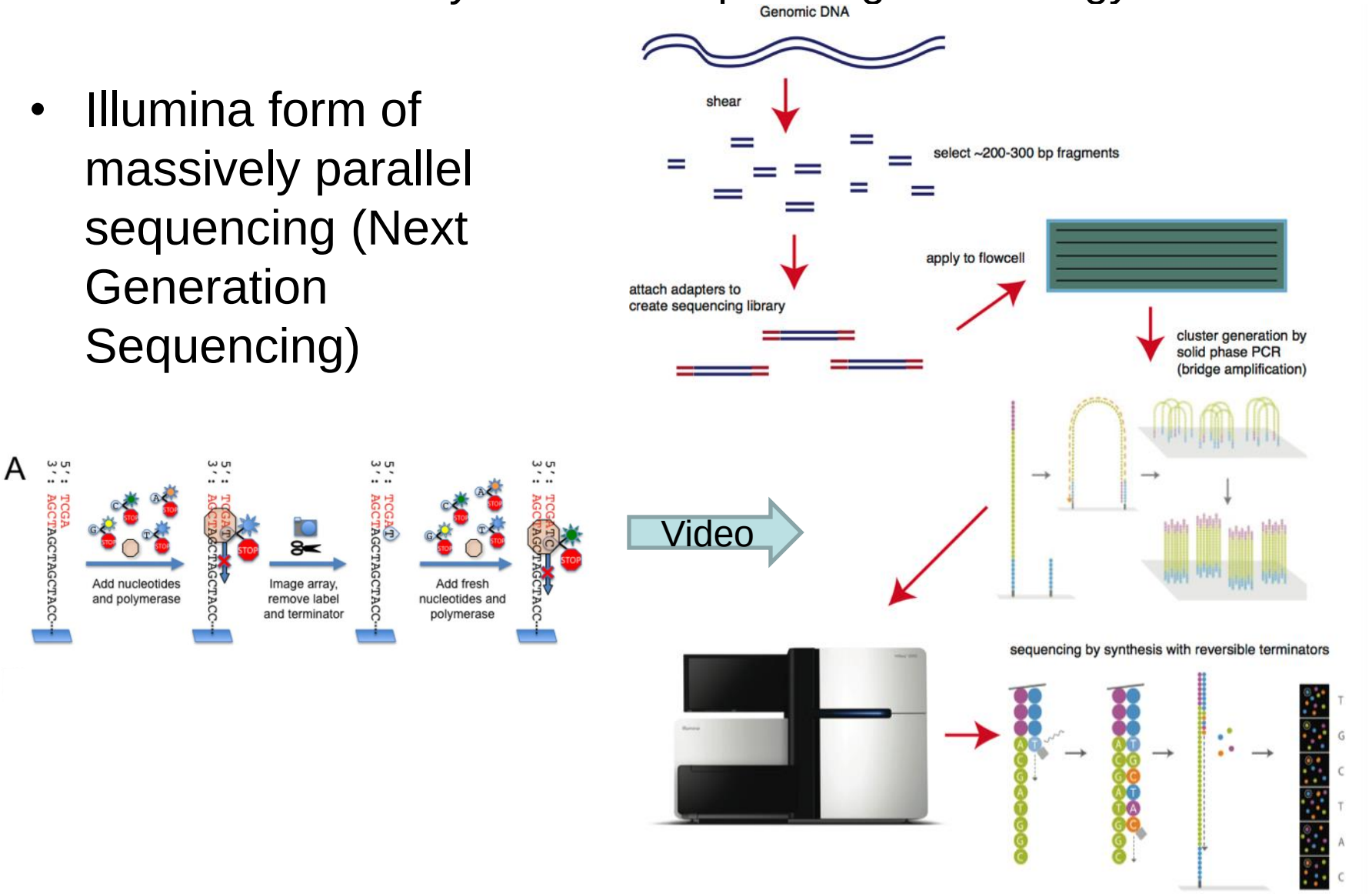
Deletion too small to identify by G-banding karyotype.



Whole Genome Sequencing

Massively Parallel Sequencing Technology

- Illumina form of massively parallel sequencing (Next Generation Sequencing)

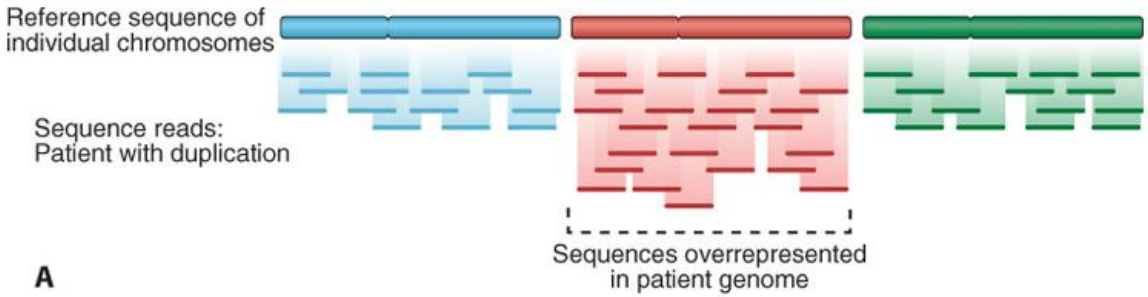


Whole Genome Sequencing

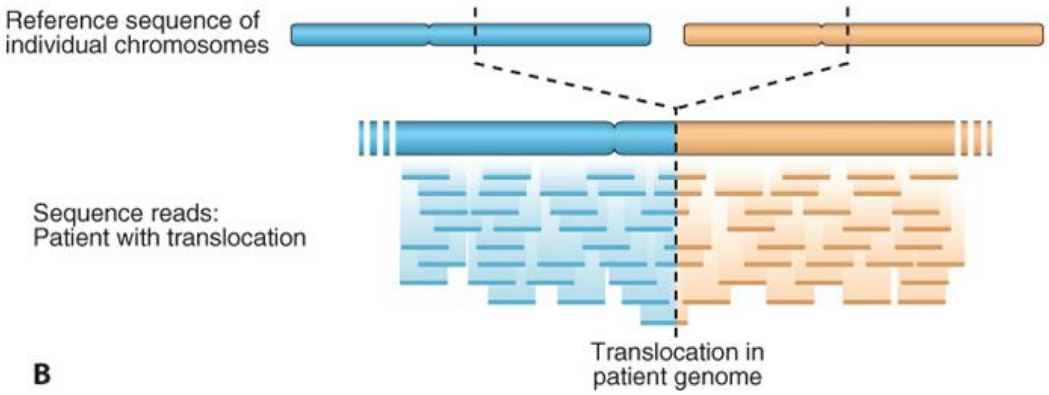
Massively Parallel Sequencing Technology

- Sequencing can provide two types of information related to chromosomal analysis.

Increased or decreased read number for duplications and deletions respectively.

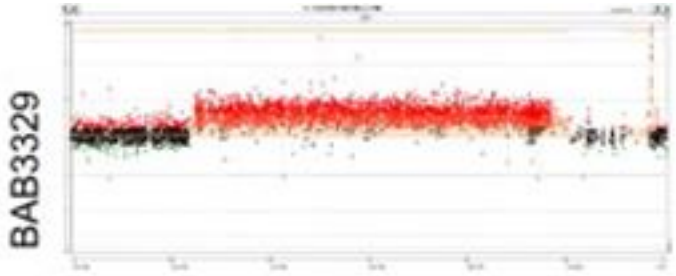


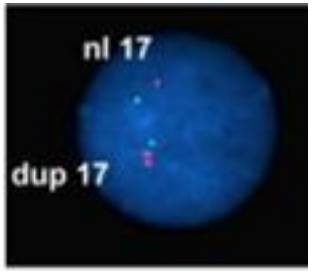
Continuous reads for distant sequences.
Can identify specific site of translocation or deletion.



Case Application

- Case
  - Female with progressive distal weakness
  - Distal muscle wasting
  - Hyporeflexia, footdrop when walking
  - Low nerve conduction rate
  - Biopsy revealed segmental demyelination and myelin sheath hypertrophy.
- Symptoms consistent with Charcot-Marie Tooth Disease 1A
- Most common cause is 1.5 Mb duplication of chromosome 17p11.2
  - Duplication caused by misaligned recombination between two flanking highly similar sequences. Extra copy of PMP22 gene.





Important Terms

- Acrocentric Chromosome
- Balanced and unbalanced genetic changes
- CGH
- CMT1A
- Karyotype abbreviations (see table 5-1)
- Homologous Recombination
- Isochromosome
- Mobile Genetic Elements
- Non-homologous end joining
- Peracentric rearrangement
- Pericentric rearrangement
- Ring Chromosome
- Robertsonian translocation