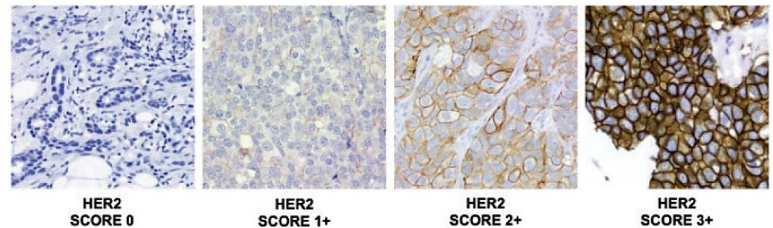
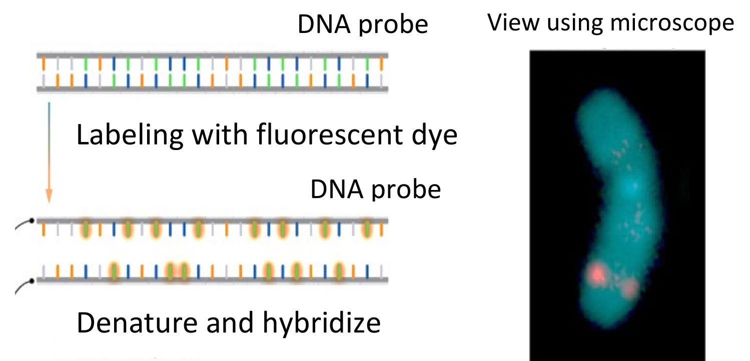
GENOMIC TESTING

Immunohistochemistry (IHC): Uses antibodies linked to dye to detect markers (antigens). Tissue is treated with florescent antibodies that bind with the antigen. The presence and distribution of the specific antigen can then be identified by examining the stained tissue.



Results are reported as +1, +2, +3. If > 50% are +3, it is considered a positive test. *Example HER2*.

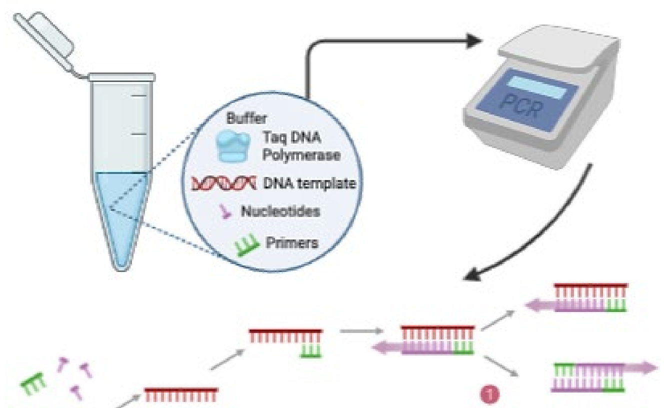
Fluorescence insitu hybridization (FISH): Detects and locates a specific DNA sequence on a chromosome. The technique exposes chromosomes to a small DNA sequence called a probe with a fluorescent molecule attached. The probe sequence binds to its corresponding sequence on the chromosome. It completes a single gene map, allowing visualization of amplification of gene alterations.



Results are either detected (+) or not detected (-) *Example:* BCR-ABL: Positive. If the test was performed on nuclei, the result would be reported with an ISCN description or nuc ish.

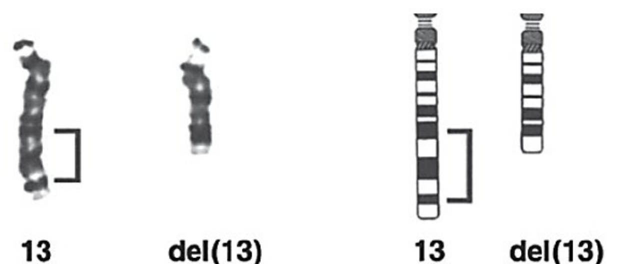
Example: *nuc ish* 3q27(BCL6X2)[200]

Polymerase Chain Reaction (PCR): This test amplifies small segments of DNA, creating millions of copies of a specific DNA sequence. PCR uses short synthetic DNA fragments called primers to select a segment of the genome to be amplified, and then multiple rounds of DNA synthesis to amplify that segment. *Example:* Microsatellite instability (MSI)



Results are positive (detected), Negative (not detected) or inconclusive or indeterminate. MSI may be reported as high, stable or low.

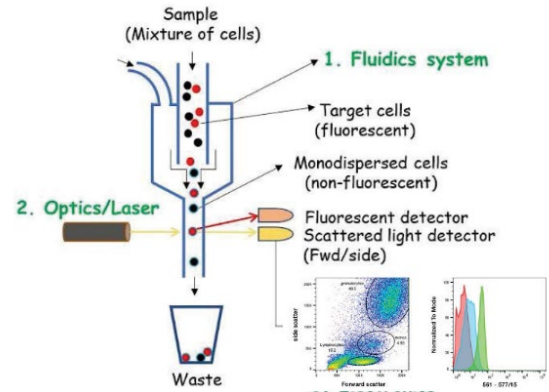
Karyotype: A laboratory technique that produces an image of an individual's chromosomes. The image is used to look for abnormal numbers or structures of chromosomes.



Results will look like a long string of numbers and letters that are specific to the genetic change that was identified.
Example: 46XY,del(17)(p13.1) Deletion of TP53

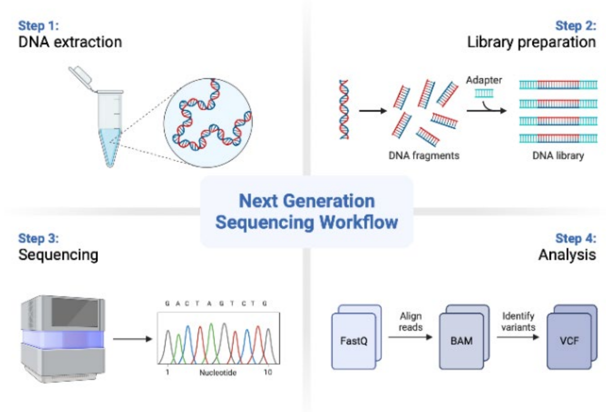
Flow Cytometry: This measures the number and characteristics of cells in a sample of blood, bone marrow or other tissue. Tumor markers on the cell surface are also measured. The cells are stained with a light sensitive dye, placed in a fluid and then passed one at a time through a beam of light. The results are based on how the stained cells react to the beam of light.

Results are reported as positive or negative, usually with the % of cells with the specific marker. *Examples*: CD20, receptors (e.g. VGFR), cytokines (e.g. IL2 and IFN)



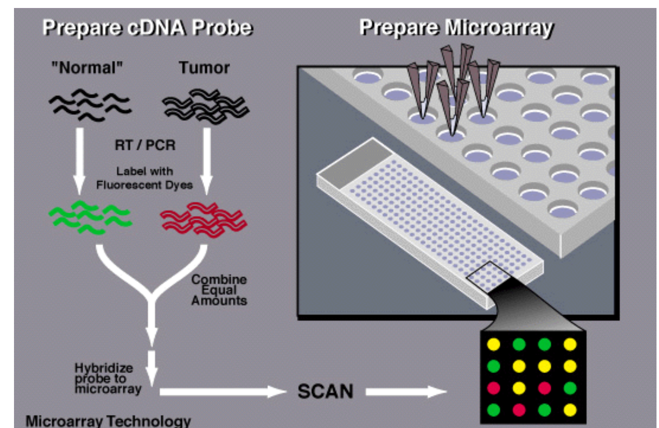
Next Generation Sequencing (NGS:) A method used to determine a portion of the nucleotide sequence of an individual's genome. Using DNA sequencing technology, it can process multiple DNA sequences simultaneously, allowing detection of multiple variants across targeted areas of the genome.

Results are generally reported as variants and what treatments are recommended. *Example*: EGFR, amplification; T790M. There are also variants of uncertain significance, meaning there is no current evidence for their therapeutic, prognostic or diagnostic utility.



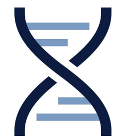
Microarray: Chromosomal microarray analysis (CMA) is used to detect genetic variations across the genome. A sample of DNA is obtained from tissue, blood or saliva as well as a control sample. The DNA is fragmented into smaller pieces. The patient's DNA is labeled with green dye and the control with red. These fragments are then attached to a microarray chip and allowed to bind. If there is no mutation, both the red and green samples bind to the sequences on the chip that represent the normal. If the mutation is present, the DNA will bind to the sequence on the chip that represents the mutated DNA.

Results indicate if a gene is over or under expressed, which can indicate a mutation. *Example: ATM & TP53*



Chromosome Region	Cytoband	Event
chr11:98,901,957-117,228,848	11q22.1 - q23.3	CN Loss
chr17:1-22,062,044	17p13.3 - p11.2	CN Loss

CYTOGENETIC TERMINOLOGY

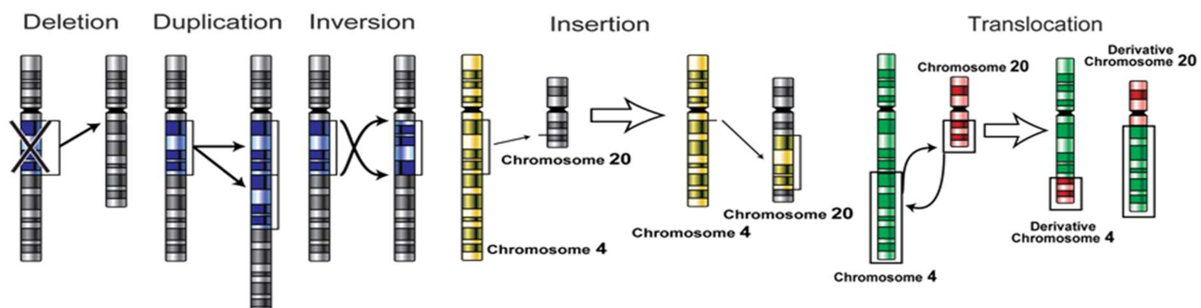


Common punctuation seen in a cytogenetic report.

Description	Symbol	Significance
Bracket, angle	<n >	Denotes ploidy level (the # of complete sets of chromosomes in a cell)
Bracket, square	[ ]	Denotes number of cells in a cell line/clone, # of metaphases examined
Colon, single	:	Chromosomal break in the detailed system
Colon, double	::	Chromosomal break and reunion in the detailed system & fusion genes
Comma	,	Separates chromosome numbers and chromosome abnormalities
Connected	con	Conjoined /fusion between 2 probes, which may be due to a translocation
Hyperdiploidy	+	Where a cell contains more than the normal 46 chromosomes
Minus sign	-	Loss or decrease in length
Multiply sign	X	Multiple copies of rearranged chromosomes or number of copies
Parenthesis	( )	Surround structurally altered chromosomes and breakpoints, or genes
Plus sign	+	Additional normal or abnormal chromosome, or increase in length
Semicolon	;	Separates altered chromosome and breakpoints in structural rearrangements involving more than one chromosome.
Slant	/	Separates cell lines, clones or contiguous probes
Greater than	>	Substitution

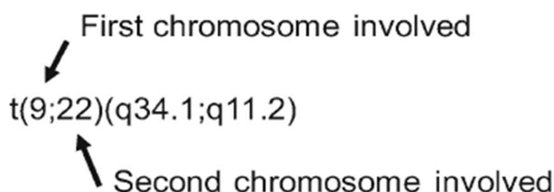
Common abbreviations seen on a cytogenetic report.

Aberration	Example	Description
add	add(1)(q21)	Additional material attached to a chromosome region or band.
del	del(7)(q22q31)	Deletion or loss of chromosome material.
der	der(5)inv(5)	A structurally rearranged chromosome from either a rearrangement involving 2 or more chromosome or by multiple aberrations within a single chromosome.
dic	45,XY,dic(13;14)	One chromosome replaces 2 normal chromosomes resulting in 2 centromeres. The 2 chromosome segments fuse, resulting in the loss of the acentric fragments.
dup	dup(1)(q21q32)	Part of a chromosome is repeated.
ins	ins(5)(p13q31q15)	Addition of material from another chromosome.
inv	inv(9)(p13q15)	Part of the chromosome is inverted within the chromosome.
rec	rec(6)	Recombinant chromosome due to meiotic crossing-over.
t	t(8;9;22)(p21;q34.1;q11.2)	Material between 2 different chromosomes is exchanged.



CYTOGENETIC TERMINOLOGY

First set of parentheses describes the chromosomes involved:



Second set of parentheses describes the chromosome bands involved:

Chromosome 9, long or q arm, region 3,band 4, sub-band 1

t(9;22)(q34.1;q11.2)

Chromosome 22, q arm, region 1, band 1, sub-band 2

GENE	Location	Testing	Description
TP53	17p13.1	FISH, NGS, PCR	Regulation of cell division, acts as a tumor suppressor.
IgVH	14q32.33	NGS, PCR	Involved in the production of antibodies by B cells.
DLEU2/MIR15A/MIR16-1 RB1	13q14	FISH,Microarray Karyotyping,PCR	Tumor suppressor Regulating cell cycle progression and genomic stability
Trisomy 12	+12	FISH, Karyotyping	There is an extra copy of chromosome 12; Cell adhesion and migration
ATM	11q22-q23	FISH,Microarray Karyotyping	DNA repair and cell cycle control
FGFR3	4p16.3	FISH,Microarray Karyotyping,PCR	t(4;14), associated with aggressive disease
CCND1	11q13	FISH,Microarray Karyotyping,PCR	Regulation of cell cycle. t(11;14) is a favorable prognosis
MAF	16q23	FISH,Microarray Karyotyping,PCR	t(14;16), linked to poor prognosis
MAFB	20q12	FISH,Microarray Karyotyping,PCR	t(14;20), linked to poor prognosis
BCR::ABL	t(9;22)	FISH, Karyotyping, PCR	Philadelphia chromosome encodes a protein with abnormal tyrosine kinase activity, which leads to uncontrolled cell division
MSH2, MSH6, MLH1, PMS2	3, 2 and 7	NGS, IHC, PCR	Mismatch repair (MMR) system, DNA repair mechanism that maintains genetic stability.
MYC	8q24.21	FISH, IHC, PCR, NGS	Cell cycle progression, apoptosis & cell transformation

This list is not all inclusive

Interpreting the NUC ISH

nuc ish: Nucleic Acid in situ hybridization, a technique used with ISH to detect specific DNA sequences within the nucleus of cells.

The probe for the chromosome being tested: Chromosome 12 and 17

nuc ish (D12Z3,MDM2)X2 [100],(TP53X1,D17Z1X2)[44/100]

The gene being evaluated: MDM2 is on Chromosome 12 & TP 53 gene is on the short arm (p) of Chromosome 17

The number of copies of the chromosome/gene:

- **The normal finding would be 2 copies of a gene, one from each parent.**
- **There are 2 copies of MDM2, this is normal X2**
- **There is 1 copy of TP53, this means one is missing. X1 (as there is a X2 for Chromosome 17, this means that only the section with the TP53 was lost (or the short arm p)**

The number of interphases with the aberration/the number of interphases total.

- **The first number in the brackets [ ] indicates how many cells have the aberration. [44/100]**
- **The second number in the brackets [ ] indicates how many cells were evaluated. [44/100]**
- **If it lists only 100, that means that all the cells that were evaluated were normal. [100]**