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# Cytogenetics and molecular genetics in oncology

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# Outline of the presentation

1. Differences in molecular genetics and cytogenetics of congenital disorders vs cancer
2. Application of molecular-genetic and cytogenetic findings in oncology
3. Material sources and material used
4. Methods used and practical examples

MG: molecular genetics
CG: cytogenetics



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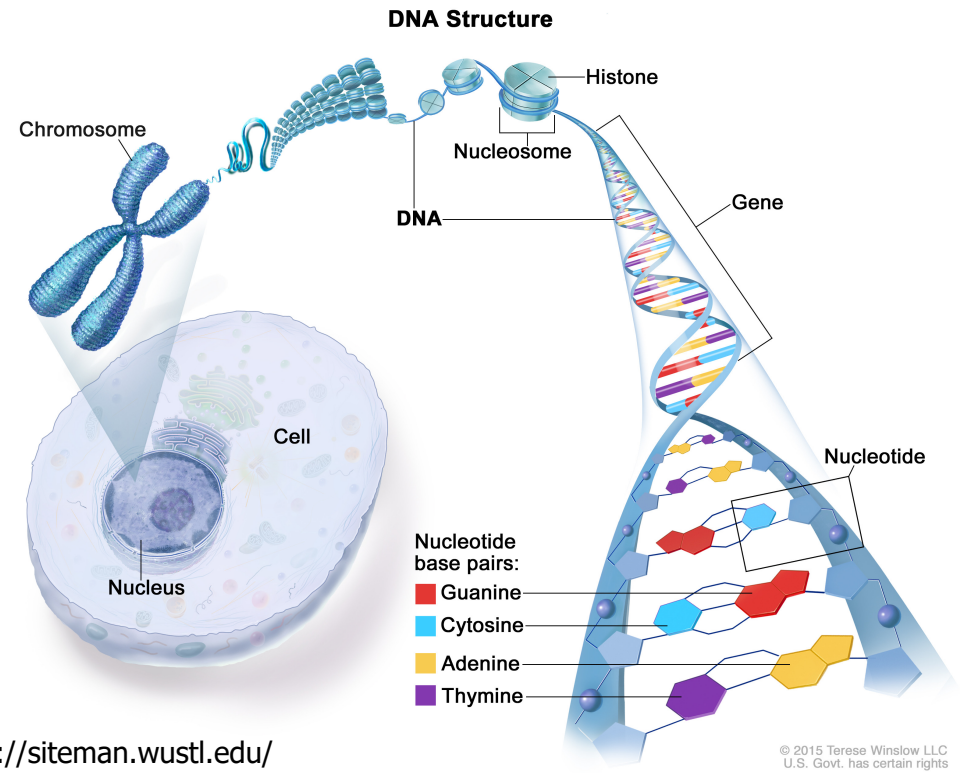
# Cancer as a genetic disease

Two levels: Cancer hereditary syndromes – germline mutations
Genetic alterations gained during a lifetime - somatic




https://siteman.wustl.edu/



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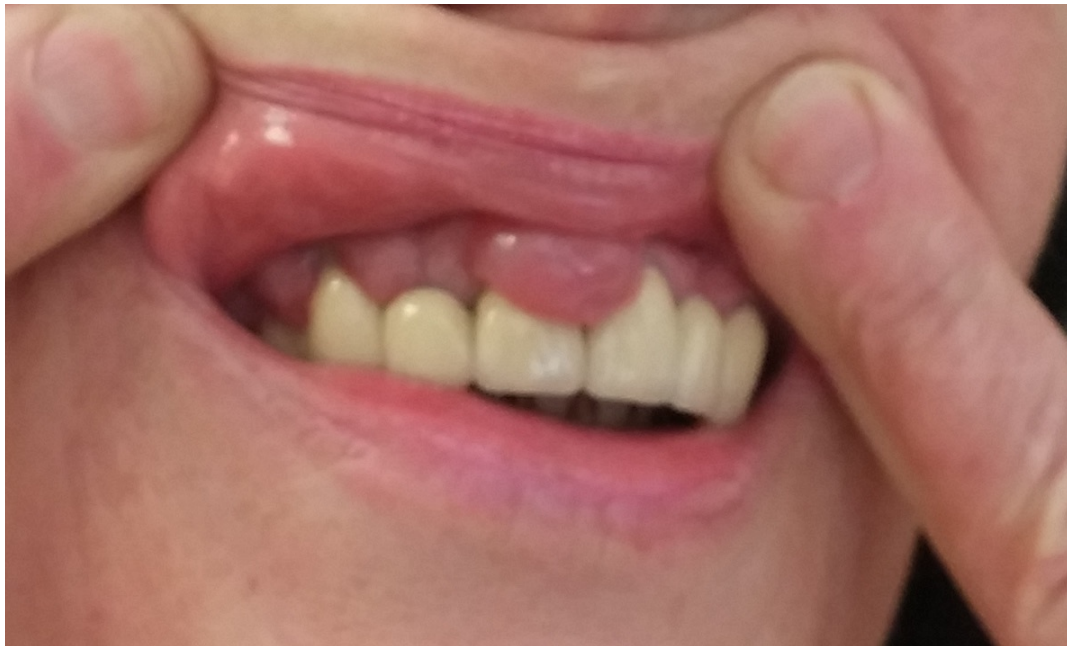
# Why molecular genetics and cytogenetics in oncology can be interesting for a dentist?



Acute myeloid leukemia (AML) manifesting by blast infiltration in gums.

# 1. Differences in MG and CG of congenital disorders vs cancer

| Characteristics | Congenital disorders | Cancer |
|---|---|---|
| Prevailing origin of genetic defects | Germline | Somatic |
| Extent of genetic abnormalities | Single or small number of changes | Variable, typically higher |
| Type of abnormalities | One/two types present per case | Combination of all types |
| Mosaicism | Rare | Common |



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# 2. Application of MG and CG findings in oncology

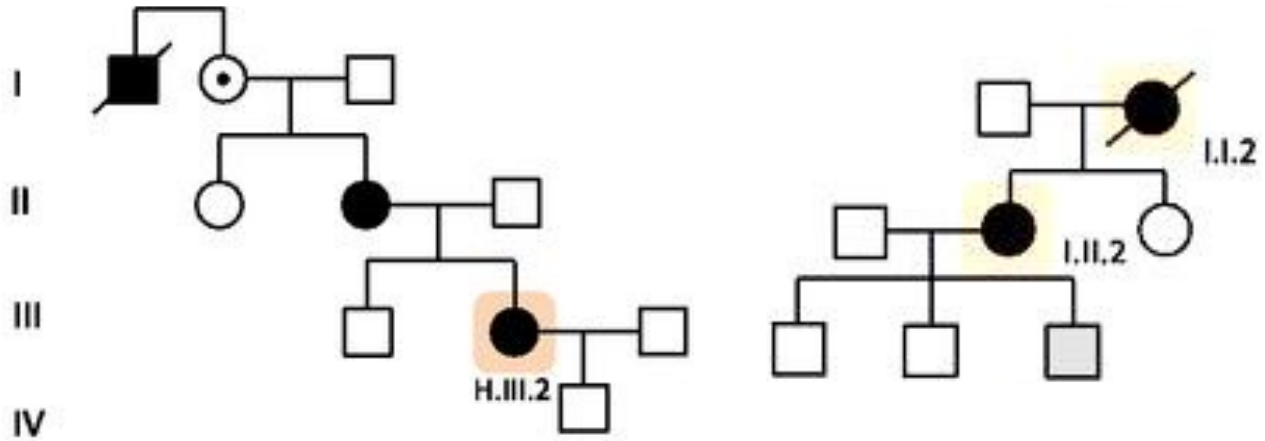
a. Hereditary predisposition assessment
b. Establishing and refining diagnosis
c. Disease prognostication
d. Treatment optimization
e. Disease activity monitoring
f. Disease complication diagnostics

# 2. Requirements on the techniques

- High specificity and high sensitivity, limit of detection
- Fast processing – range of few hour to few days
- Tools for data analysis (bioinformatics for NGS)
- Standardization and validation
- Availability of reference material (positive/negative controls), reference sequences
- Regular quality assessment
- Compliance with legislation regulations

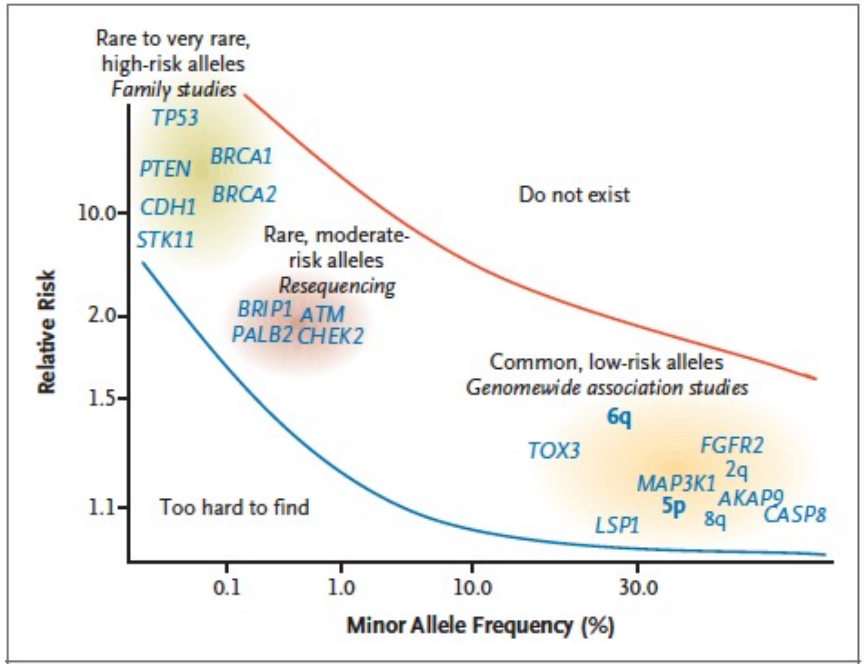
# a. Hereditary predisposition assessment

- Cases of cancer accumulated in families
- Autosomal dominant and recessive inheritance
- Typical onset at young age
- Genetic counseling
- Screening for causative variants




Tawana et al, Blood 2015

Breast-Cancer Susceptibility Loci and Genes.




Foulkes, NEJM 2008



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# a. Hereditary predisposition assessment

WHO classification of myeloid neoplasms with germ line predisposition and guide for molecular genetic diagnostics

| WHO classification |
|---|
| **Classification**[*] |
| Myeloid neoplasms with germ line predisposition without a preexisting disorder or organ dysfunction |
| AML with germ line *CEBPA* mutation |
| Myeloid neoplasms with germ line *DDX41* mutation[‡] |
| Myeloid neoplasms with germ line predisposition and preexisting platelet disorders |
| Myeloid neoplasms with germ line *RUNX1* mutation[‡] |
| Myeloid neoplasms with germ line *ANKRD26* mutation[‡] |
| Myeloid neoplasms with germ line *ETV6* mutation[‡] |
| Myeloid neoplasms with germ line predisposition and other organ dysfunction |
| Myeloid neoplasms with germ line *GATA2* mutation |
| Myeloid neoplasms associated with bone marrow failure syndromes |
| Juvenile myelomonocytic leukemia associated with neurofibromatosis, Noonan syndrome, or Noonan syndrome-like disorders |
| Myeloid neoplasms associated with Noonan syndrome |
| Myeloid neoplasms associated with Down syndrome[‡] |

Döhner et al, Blood 2017

**Table 2** - ACMG list of hereditary cancer syndromes, most with childhood onset, for reporting incidental findings.

| Syndrome | Gene | Inheritance |
|---|---|---|
| Li-Fraumeni | *TP53* | AD |
| Peutz-Jeghers | *STK11* | AD |
| Familial adenomatous polyposis | *APC* | AD |
| Von-Hippel Lindau | *VHL* | AD |
| Multiple endocrine neoplasia | *MEN1* (type 1); *RET* (type 2) | AD |
| Hamartomatosis | *PTEN* | AD |
| Retinoblastoma | *RB* | AD |
| Paraganglioma-pheochromocytoma | *SDHAF2, SDHB, SDHC, SDHD* | AD |
| Tuberous sclerosis complex | *TSC1, TSC2* | AD |
| Neurofibromatosis type 2 | *NF2* | AD |
| WT1-related Wilms tumor | *WT1* | AD |

AD, autosomal dominant.

Gomy & Diz, Genet Mol Biol 2016



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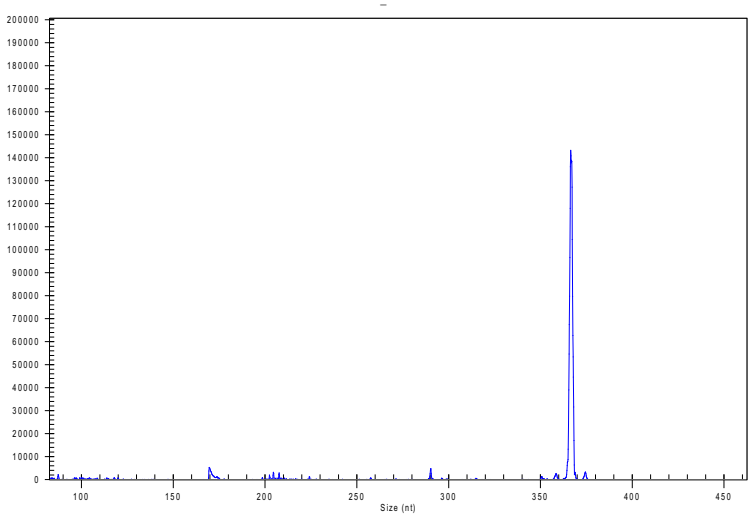
# b. Establishing and refining diagnosis

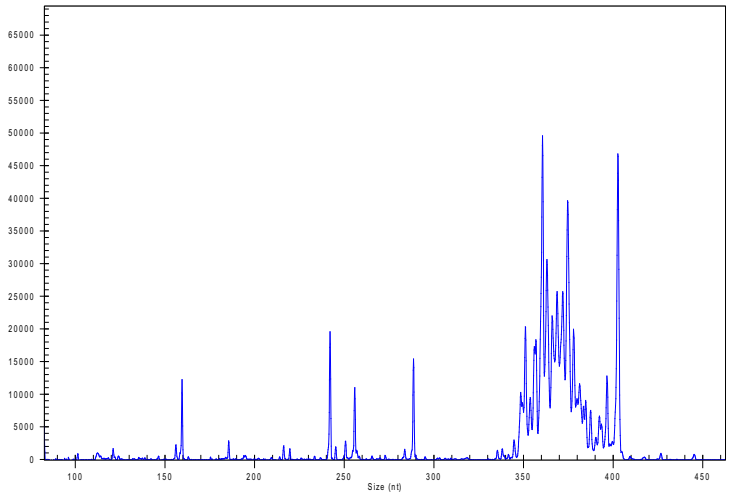
- Diagnosis confirmation based on detection of specific (marker) gene or chromosomal abnormalities
- Incorporation of genetic/cytogenetic markers in WHO classification
- Resolving ambiguous cases
- Markers specific for the whole diagnostic entity or only for a subset of a disease (implications for treatment)
- Examples
  - Mantle cell lymphoma – translocation t(11;14)
  - Hairy cell leukemia - BRAF V600E mutation

# b. Establishing and refining diagnosis

Clonality

- Typical characteristics of lymphoid (but also other) malignancies
- Analysis of antigen receptor rearrangements, translocations and gene mutations
- Monoclonal vs polyclonal picture – distiguishing of malignant vs reactive conditions
- Quantification of tumor load



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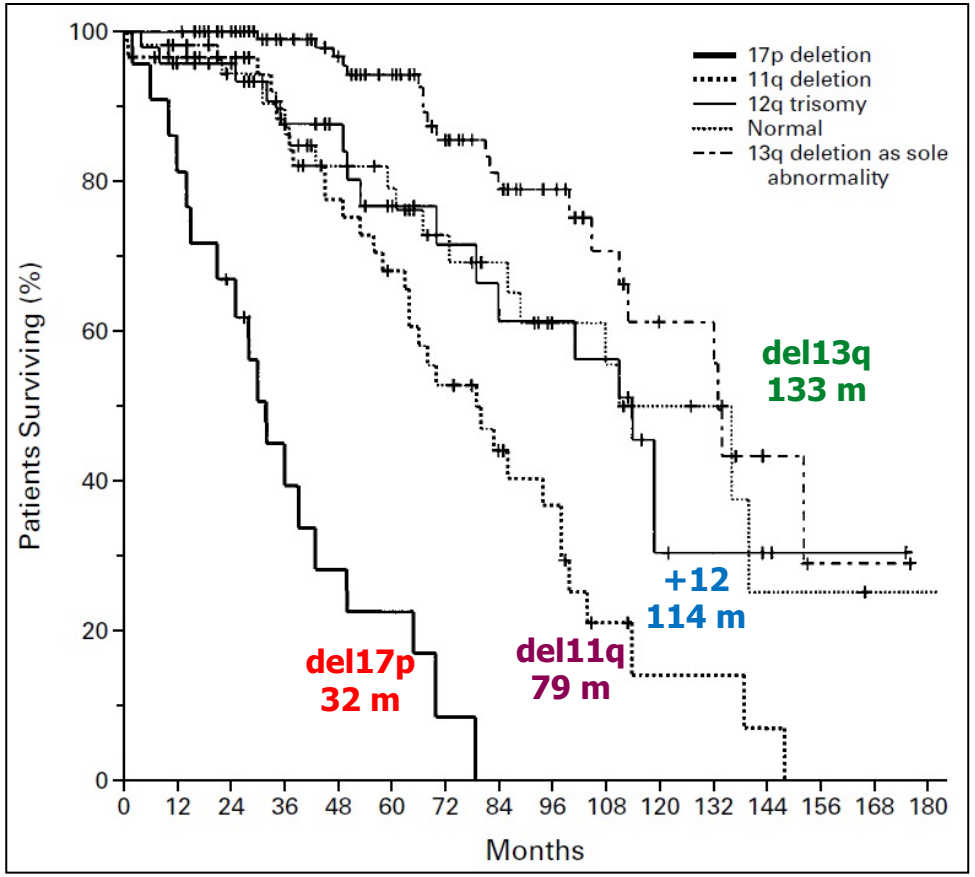
# c. Disease prognostication

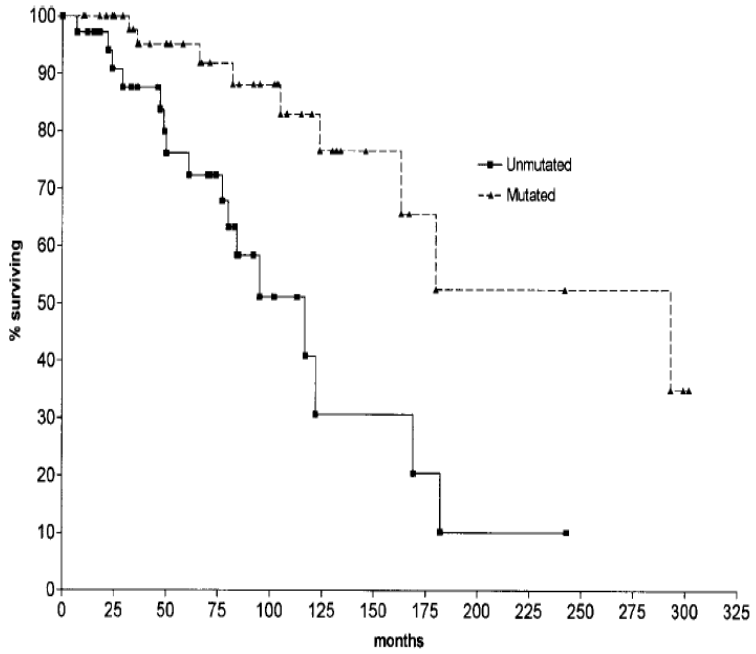
- Genetic and cytogenetic markers associated with certain disease features
- Risk assessment at time of diagnosis
- Genetic markers of various types – gene mutations, chromosomal abnormalities, a type of antigen receptor rearrangement, …
- Prognostic vs predictive markers




Döhner et al, Blood 2000

# c. Disease prognostication

CLL:




Hamblin et al, Blood 1999

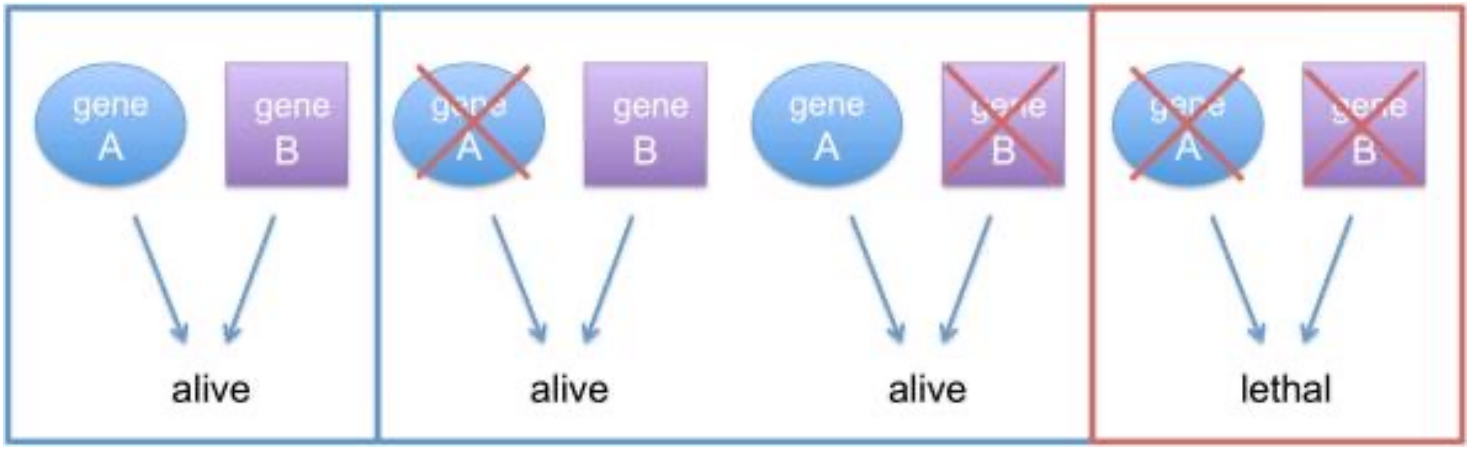
AML:

| Risk category | Cytogenetic or molecular genetic abnormality |
|---|---|
| Favourable | t(8,21)(q22;q22); *RUNX1–RUNX1T1* |
| | inv(16)(p13.1;q22) or t(16;16)(p13.1;q22); *CBFB–MYH11* |
| | t(15;17)(q24;q21) *PM–RARA* |
| | Mutated *NPM1* without *FLT3–ITD* (normal karyotype) |
| | Biallelic mutated *CEBPA* (normal karyotype) |
| Intermediate | Mutated *NPM1* and *FLT3–ITD* (normal karyotype) |
| | Wildtype *NPM1* and *FLT3–ITD* (normal karyotype) |
| | Wildtype *NPM1* without *FLT3–ITD* (normal karyotype) |
| | Normal karyotype not classified as favourable. |
| | t(9;11)(p22;q23); *MLLT3–KMT2A* |
| | Cytogenetic abnormalities not classified as favourable or poor |
| Poor | inv(3)(q21q26.2) or t(3;3)(q21;q26.2);*GATA2–MECOM* (*EVI1*) |
| | t(6;9)(p23;q34); *DEK–NUP214* |
| | t(v;11)(v;q23); KMT2A rearranged |
| | t(9;22)(q34.1;q11.2); *BCR–ABL1* |
| | −5 or del(5q); −7; −17/abn(17p) |
| | Complex karyotype (>3), monosomal karyotype |
| | Wild type *NPM1* and *FLT3–ITD* |
| | Mutated *RUNX1* |
| | Mutated *ASXL1* |
| | Mutated *TP53* |

Dohner et al, Blood 2017

# d. Treatment optimization

- Concept of personalized treatment – tailored for individual patients
- Treatment response related to genetic abnormalities detected in cancer cells
- Targeted treatment – blocking the growth and spread of cancer by interfering with specific molecules ("molecular targets")
- Synthetic lethality – blocking or inactivation of two genes leads to cell death




Nijman, FEBS Lett 2011



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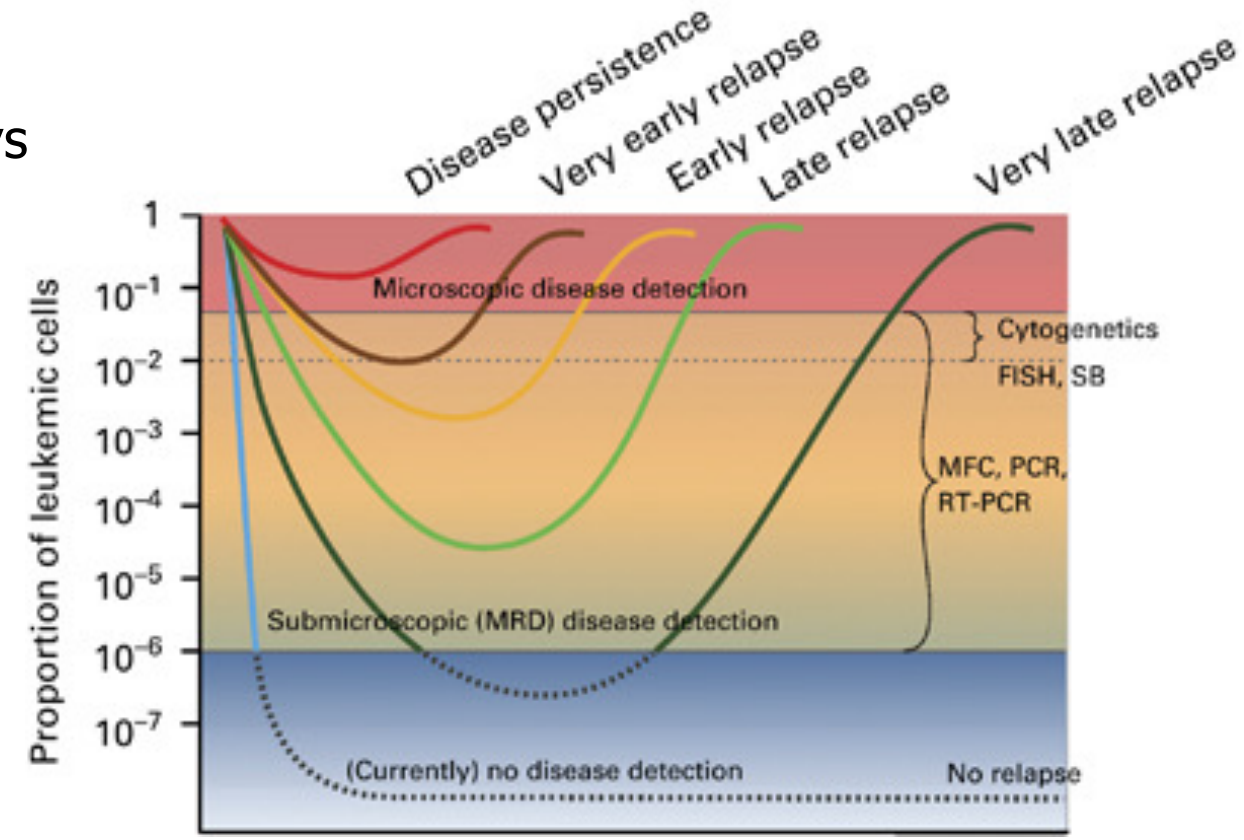
# d. Treatment optimization - examples

| Diagnosis | Genetic defect | Treatment option |
|---|---|---|
| Chronic myeloid leukemia | BCR/ABL fusion gene | Tyrosine kinase inhibitors (imatinib, dasatinib etc.) |
| Breast cancer | BRCA1 mutations | PARP inhibitor olaparib |
| Non-small cell lung cancer | EGFR mutations | EGFR inhibitors (erlotinib, afatinib etc.) |
| Non-small cell lung cancer | ALK gene rearrangements | ALK inhibitors (crizotinib, ceritinib etc.) |
| Melanoma<br>R/R hairy-cell leukemia | BRAF mutations | BRAF inhibitors (dabrafenib, vemurafenib etc.) |
| Colorectal cancer | KRAS mutations | Contraindication for targeting EGFR by cetuximab and panitumumab |
| Chronic lymphocytic leukemia | BTK mutations | Contraindication for ibrutinib administration |

# e. Disease activity monitoring

- Minimal residual disease (MRD) – cancer cells remaining after therapy
- Need for MRD marker identification before therapy
- Monitoring of MRD markers after therapy
- Design of patient-specific and sensitive assays
- Typical markers:
  - Gene rearrangements
  - Fusion genes
  - Gene mutations




Buckley SA, et al. Bone Marrow Transpl. 2013.

# f. Disease complication diagnostics

Infection complications related to cancer treatment

- Opportunistic infections – otherwise common pathogen causing severe symptoms
- Related to bone marrow (BM) and peripheral blood stem cell (PBSC) transplantation and other cancer-specific treatment (e.g. alemtuzumab)

Molecular diagnostics – typing of pathogens according to their DNA/RNA sequence

- (multiplex) PCR, real-time PCR, NGS
- Quantification and monitoring of pathogen load

Table 2.
Pathogen Frequency by Treatment Arm

| Organism | BM | PBSC |
|---|---|---|
| Bacterial infections | | |
| *Staphylococcus* (coagulase negative) | 123 (82) | 101 (67) |
| *Enterococcus* (all species) | 54 (42) | 49 (40) |
| *Clostridium difficile* | 69 (52) | 54 (41) |
| *Staphylococcus* (coagulase positive) | 10 (9) | 30 (20) |
| *Escherichia* (also *E. coli*) | 16 (15) | 23 (19) |
| Viral infections | | |
| CMV | 78 (61) | 81 (57) |
| Polyomavirus | 27 (25) | 27 (24) |
| Herpes simplex (HSV1, HSV2) | 16 (14) | 22 (17) |
| EBV | 15 (12) | 21 (15) |
| Influenza | 22 (19) | 13 (13) |
| Fungal/parasitic infections | | |
| Other (suspected) fungus | 12 (11) | 13 (12) |
| Yeast other than *Candida albicans* | 5 (4) | 12 (10) |
| *Candida albicans* | 6 (6) | 10 (8) |
| *Aspergillus fumigatus* | 5 (5) | 6 (5) |
| Mucormycosis (Zygomycetes, *Rhizopus*) | 5 (4) | 2 (2) |
| *Pneumocystis* | 1 (1) | 2 (2) |
| *Toxoplasma* | 1 (1) | 1 (1) |

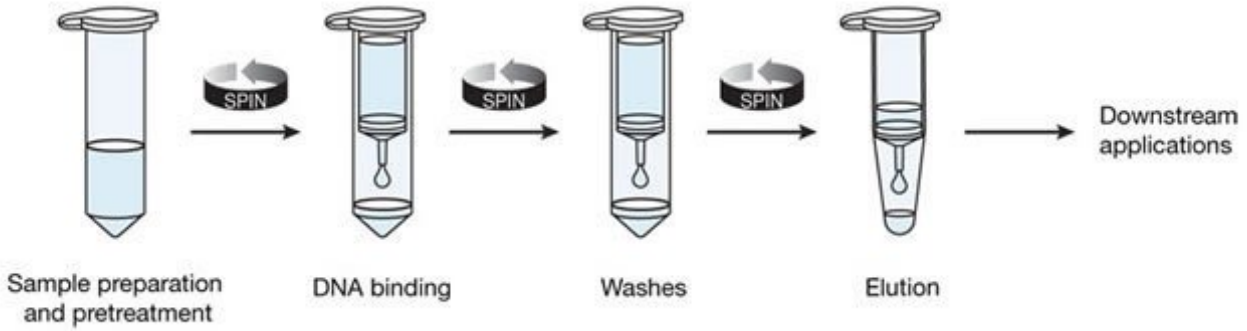
HSV indicates herpes simplex virus; CMV, cytomegalovirus; EBV, Epstein Barr virus.

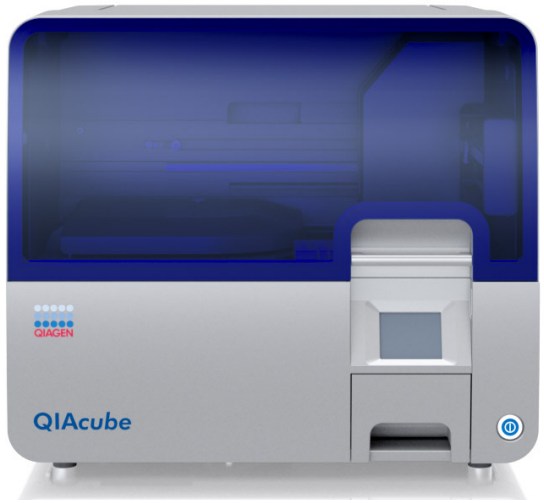
Only the top 5 organisms for each infection type are listed.

Young et al, Biol Blood Marrow Transplant 2016

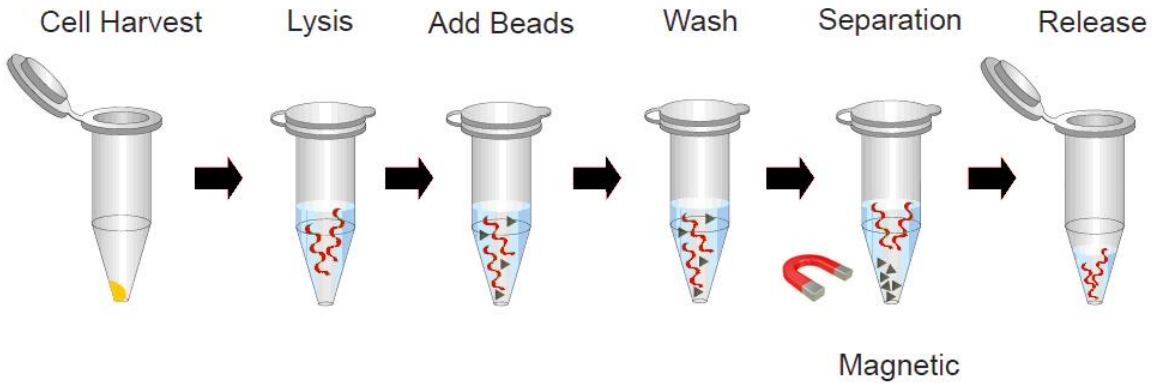
# 3. Materials used and material sources

- Types of samples
  - Cells
  - DNA
  - RNA
  - cfDNA







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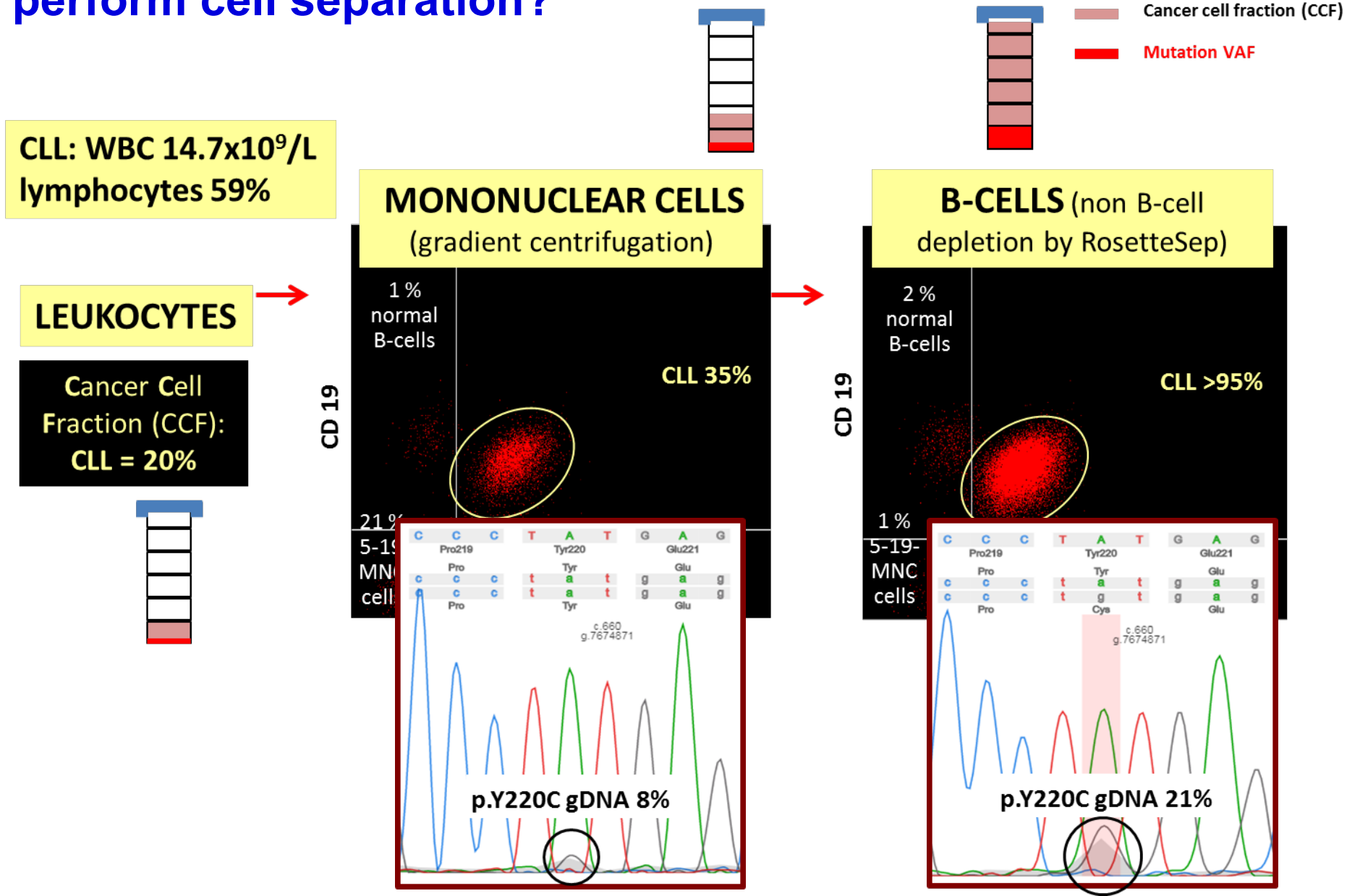
# 3. Materials used and material sources

- Sampling
  - Peripheral blood
  - Bone marrow
  - Liquid biopsies
  - Aspirates
  - Fine-needle biopsies
  - Fresh tissue
  - Formalin-fixed paraffin-embedded (FFPE) tissue
  - Swabs (buccal, NPh, …)

# Hematooncology – easy access to malignant cells

- Peripheral blood, bone marrow
- EDTA or heparin collection tubes
- Different cell population used according to the application
  - Leukocytes, Mononuclear cells, Granulocytes, Lymphocytes, Specific cell subpopulations
  - Utilization of separation methods

# Why to perform cell separation?



# Solid tumors – tissues

- Invasive
- Biopsies, fine-needle biopsies
- Fresh frozen vs FFPE tissue
- Decreased DNA and RNA quality (fragmented, chemically modified in case of FFPE material)

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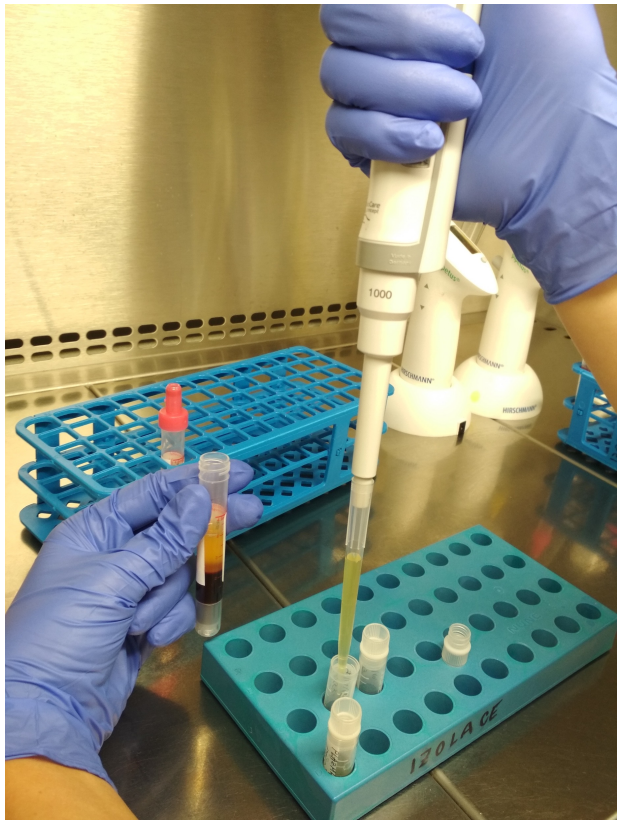
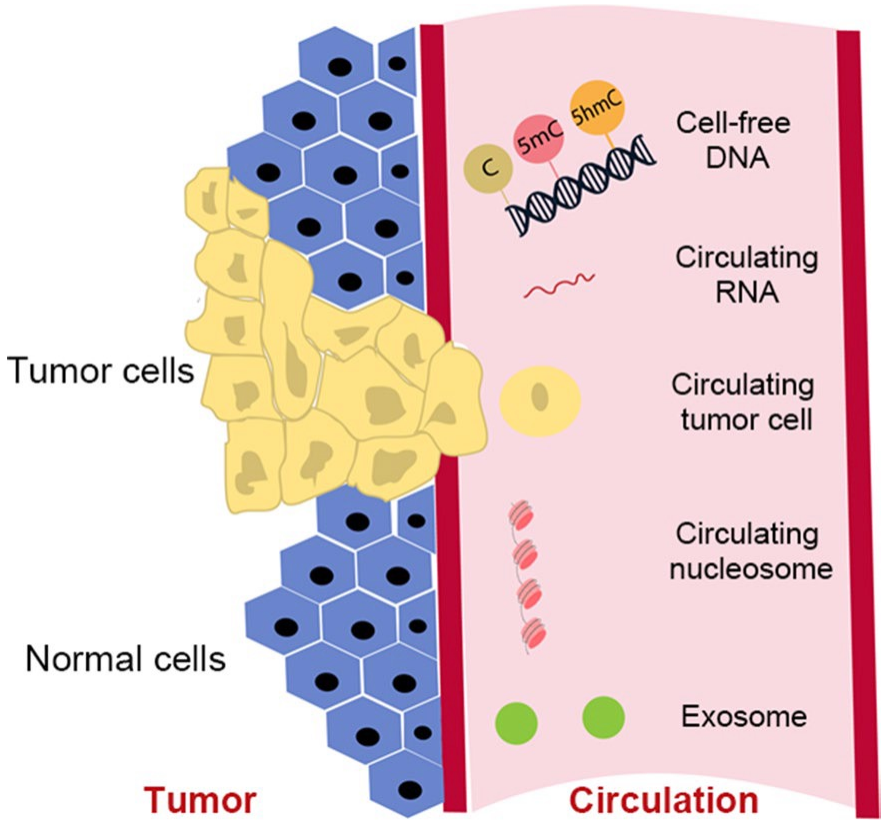
# Liquid biopsies

Very low amount of material

Sources:

- Plasma / serum
- Urine
- Joint fluid
- Cerebrospinal fluid
- …






Zeng et al, Cancer Comm 2019



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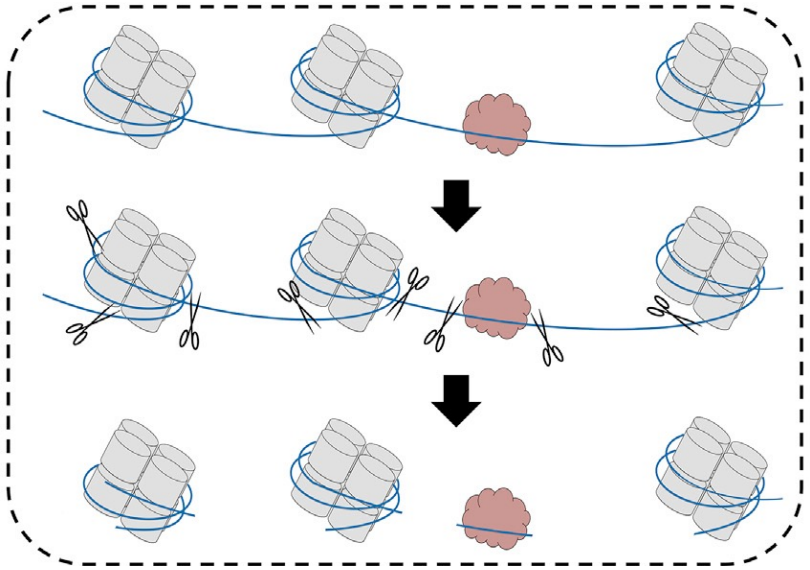
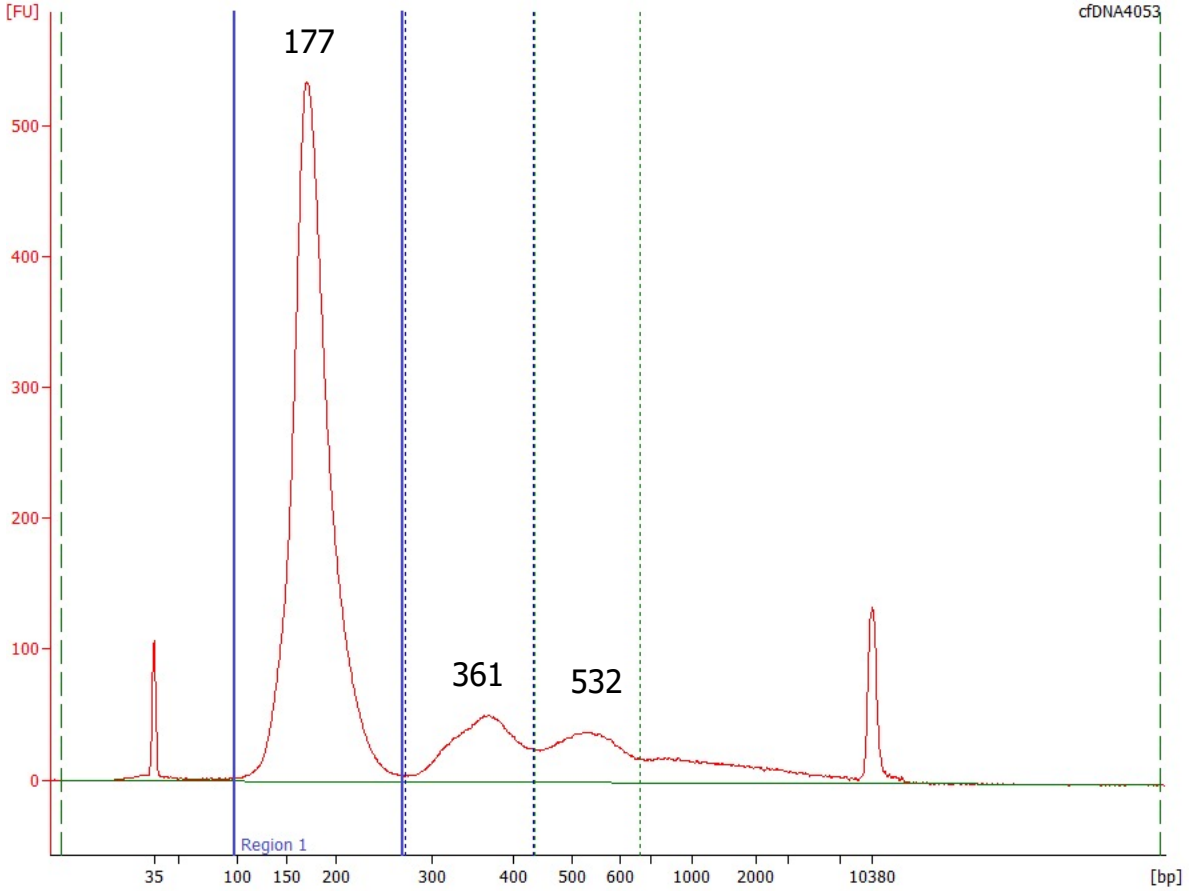
# cfDNA



Snyder et al, Cell 2016



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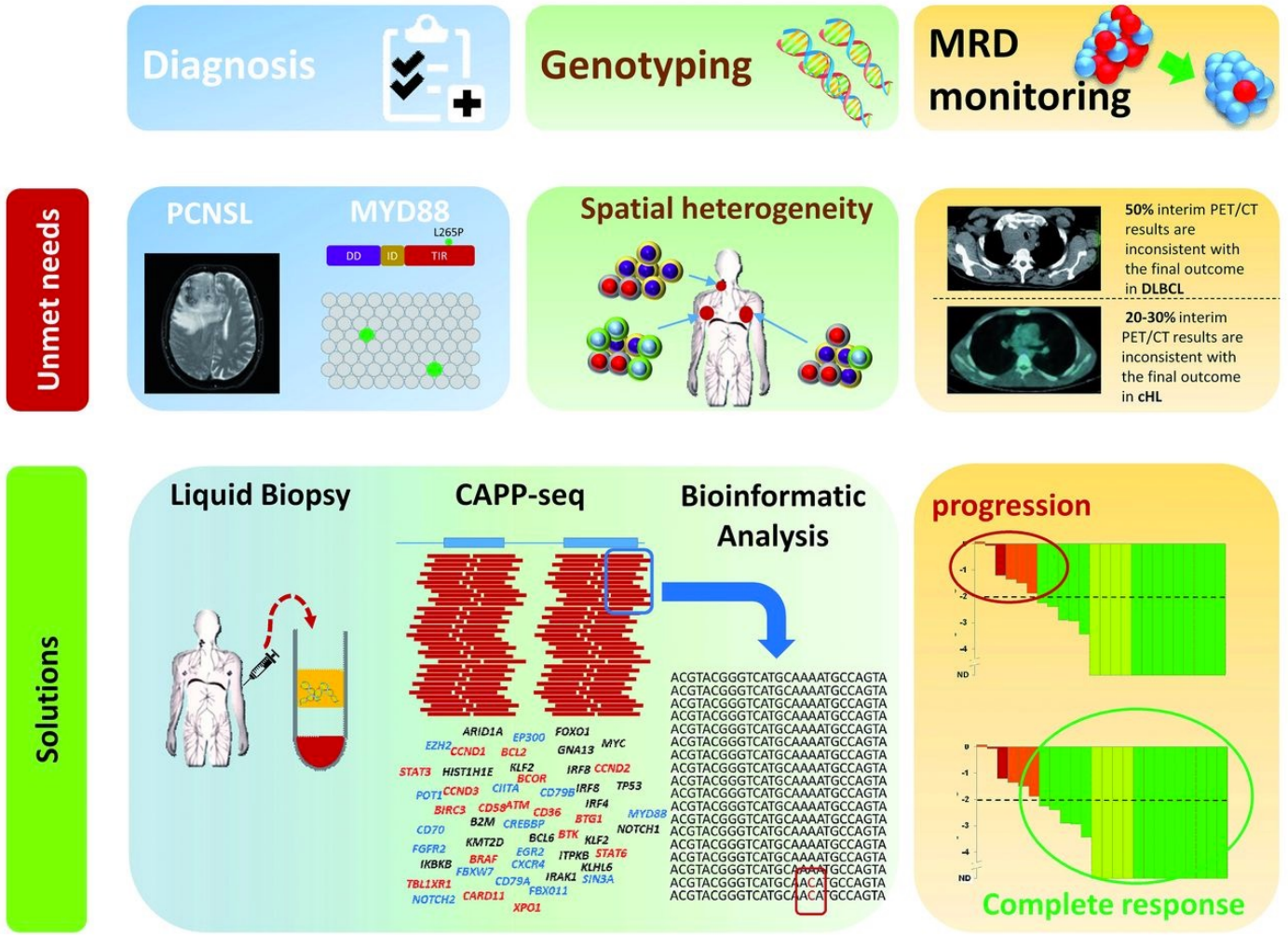
# cfDNA

Applications

- Diagnosis, early detection
- Genotyping
- Disease risk stratification
- Treatment selection
- Treatment response assessment
- Disease monitoring

FDA approved assays for gene mutation detection

Solid tumors and hematological malignancies




cHL, classical Hodgkin lymphoma; DLBCL, diffuse large B-cell lymphoma; MRD, minimal residual disease; PCNSL, primary nervous system lymphoma.

Rossi et al, Haematologica 2019

# 4. Methods used and practical examples

**a. Cytogenomics methods**

- Chromosome banding techniques
- Fluorescence *in situ* hybridization
- Genomic arrays

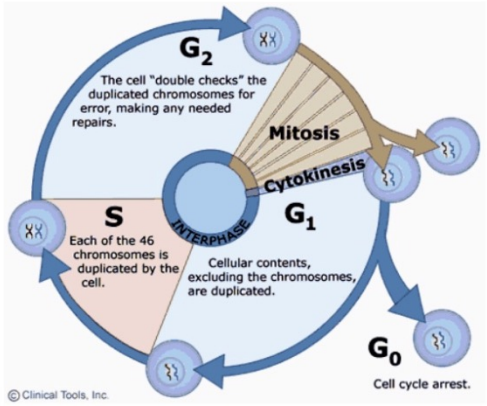
**b. Amplification-based methods**

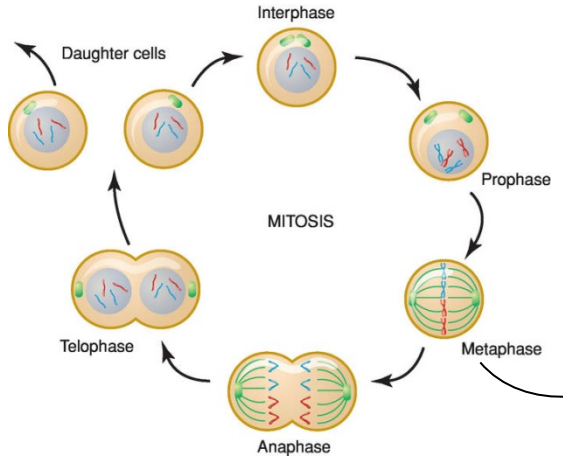
- PCR and real-time PCR
- Droplet digital PCR

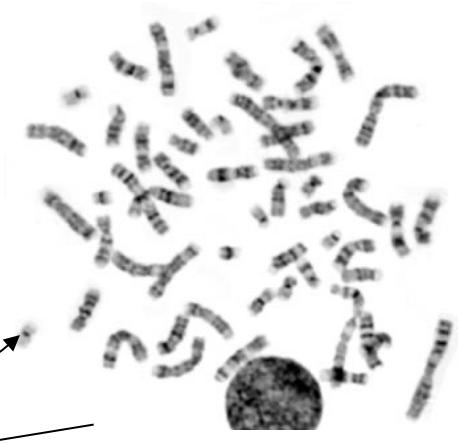
**c. Sequencing techniques**

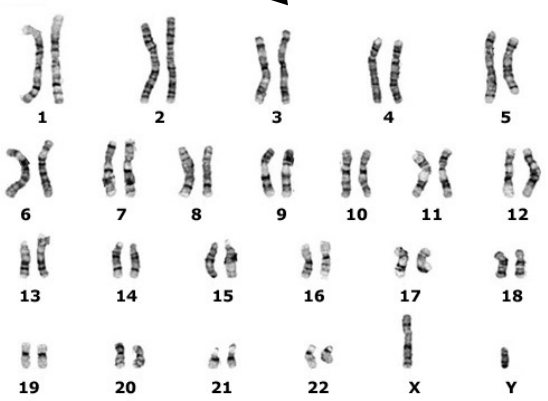
- Sanger sequencing
- Next-generation sequencing
  - Amplicon sequencing
  - Panel sequencing
  - Whole exome sequencing
  - Whole genome sequencing

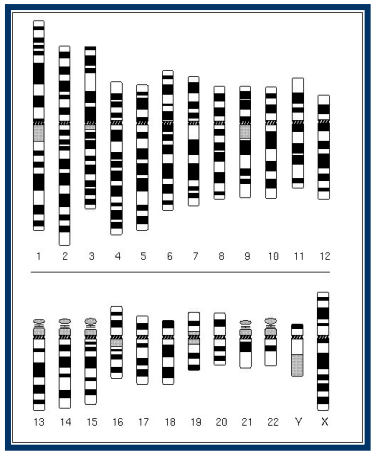
# a. Classical cytogenetics – chromosome banding techniques









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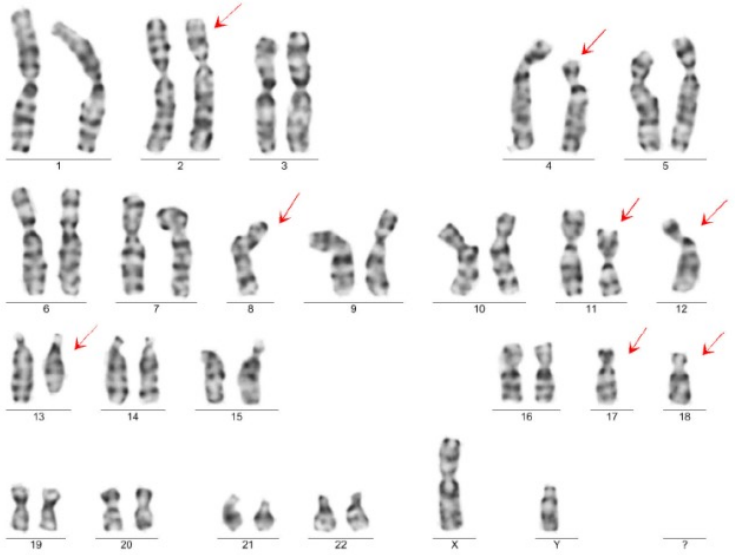
# a. Classical cytogenetics – chromosome banding techniques

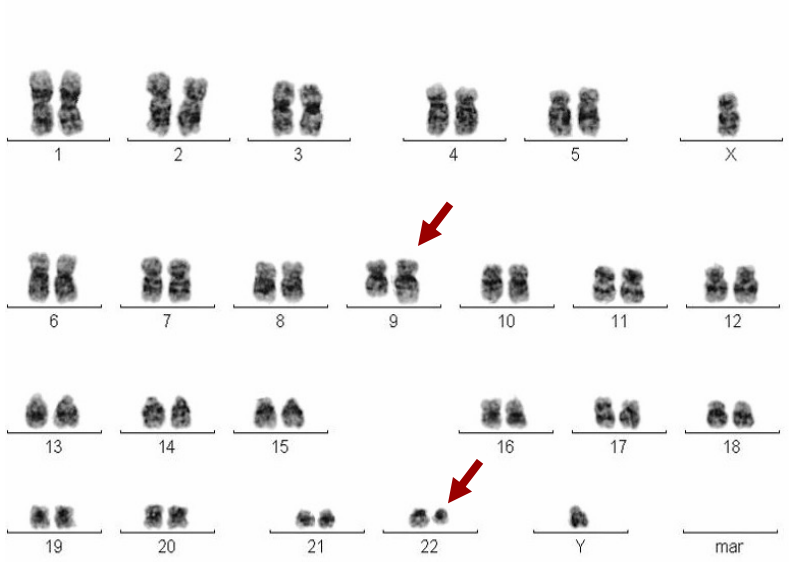
Resolution >10 Mbp

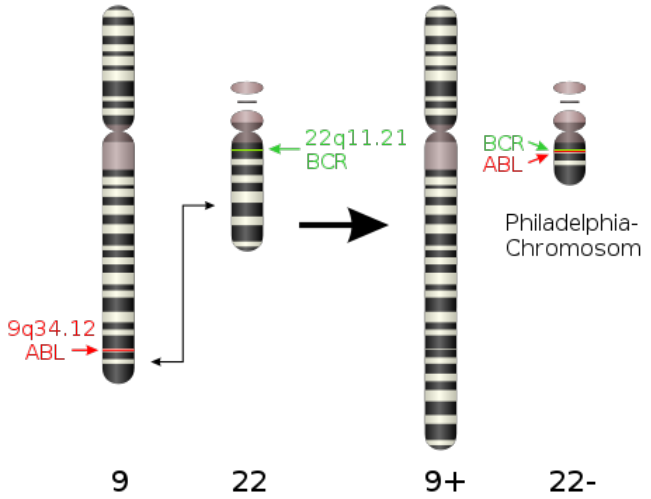
Applications

- Detection of typical abnormalities
- Complex karyotype assessment

  (≥3 or ≥5 abnormalities)





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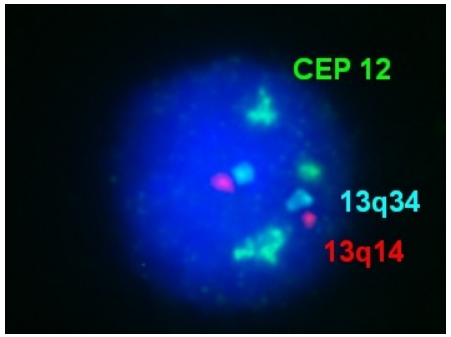
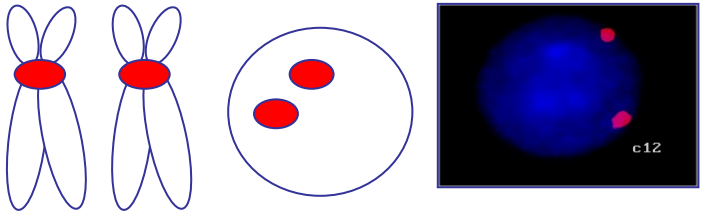
# a. Molecular cytogenetics

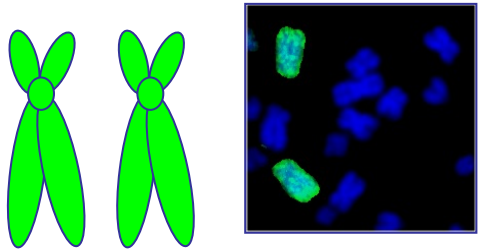
- Fluorescent in situ hybridization (FISH)
- Targets specific regions based on DNA sequence
- Detection of chromosomal abnormalities with diagnostic, prognostic and predictive value



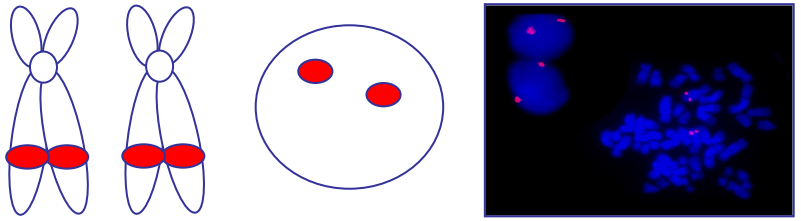

Probe types:




centromeric



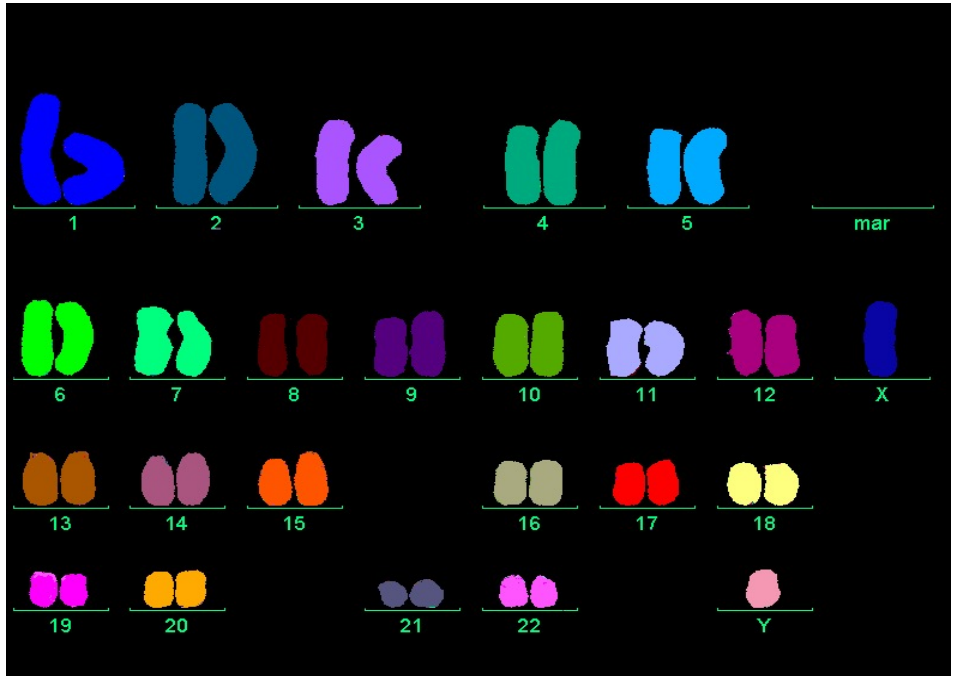
whole chromosome



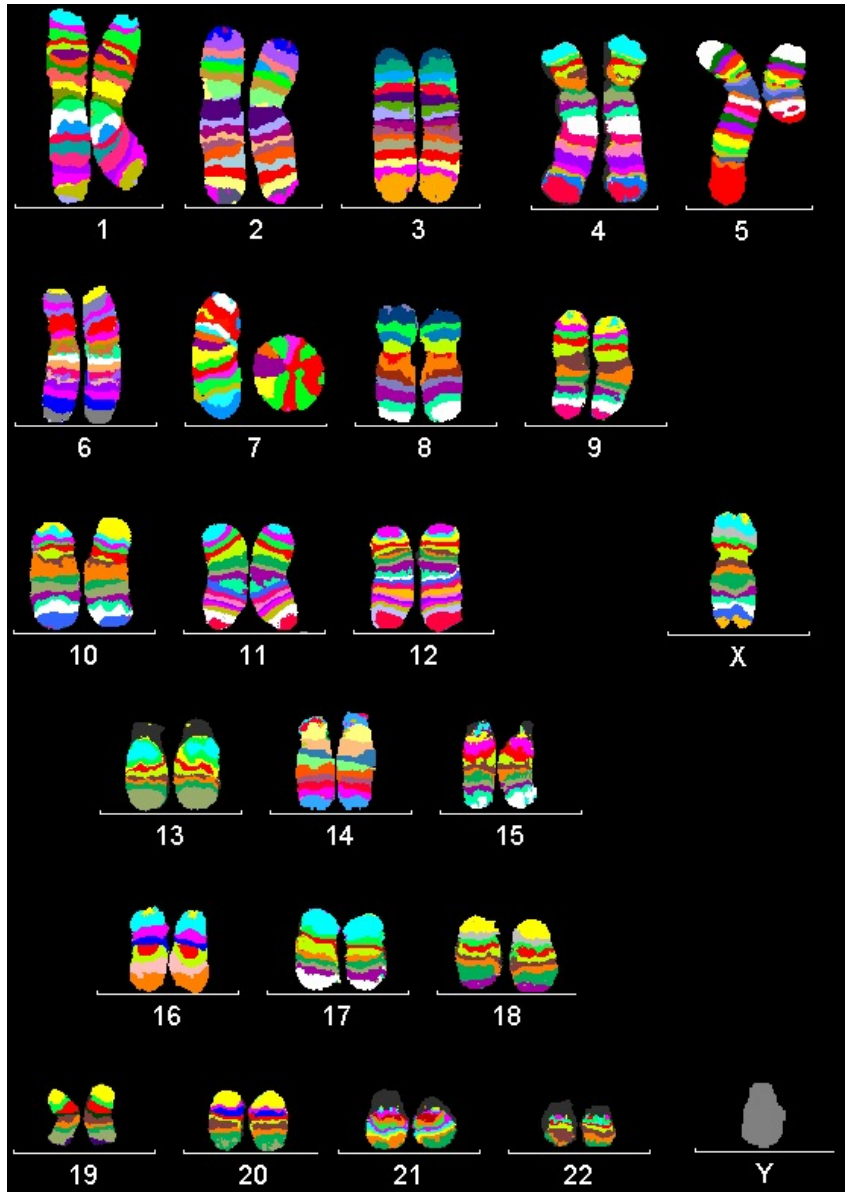
gene

# a. Molecular cytogenetics

– FISH methods for genome-wide analysis




mFISH

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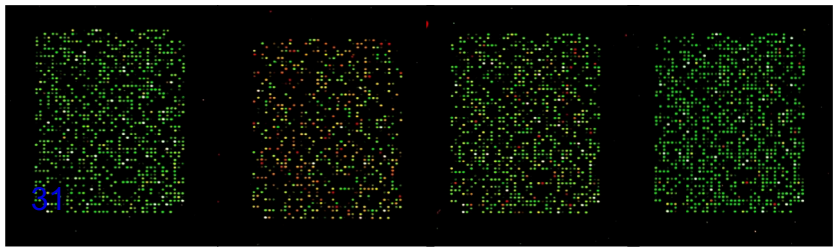
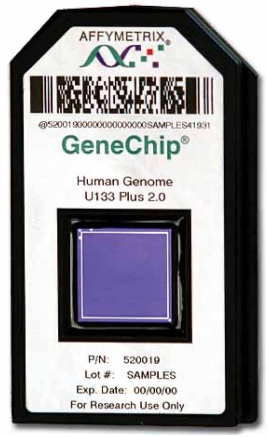
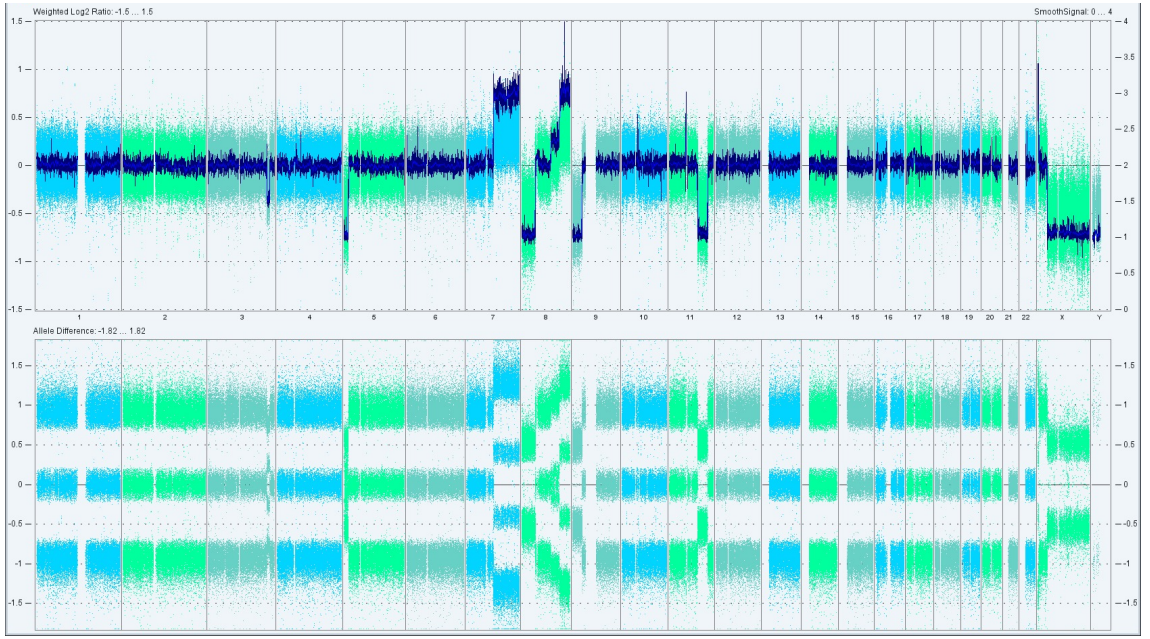
# a. Genomic arrays

- Molecular cytogenetic technique for detection of genomic gains and losses
- Detection of copy-neutral loss of heterozygosity
- Not possible to detect balanced rearrangements
- Precise breakpoint localization, identification of affected genes
- High resolution, genome-wide
- No need for viable cells

arrayCGH & SNP array





# a. Comparison of sensitivity of cytogenetic techniques

| | Aneu-ploidy | CNA | Poly-ploidy | Clonal heterogeneity | Focal amplification | Balanced rearrangements | Unbalanced rearrangements | cn-LOH |
|---|---|---|---|---|---|---|---|---|
| Classical cytogenetics | +++ | + | +++ | +++ | ++ | +++ | +++ | - |
| Interphase FISH | +++ | ++ | + | +++ | +++ | +++ | ++ | - |
| ArrayCGH | +++ | ++ | - | + | +++ | - | ++ | - |
| CGH+SNP array | +++ | +++ | + | + | +++ | - | ++ | ++ |
| SNP array | +++ | +++ | ++ | ++ | +++ | - | ++ | +++ |

**CNA** – copy number alteration, gains or lossess of genetic material
**cn-LOH** – copy neutral loss of heterozygozity

Schoumans J et al, 2016



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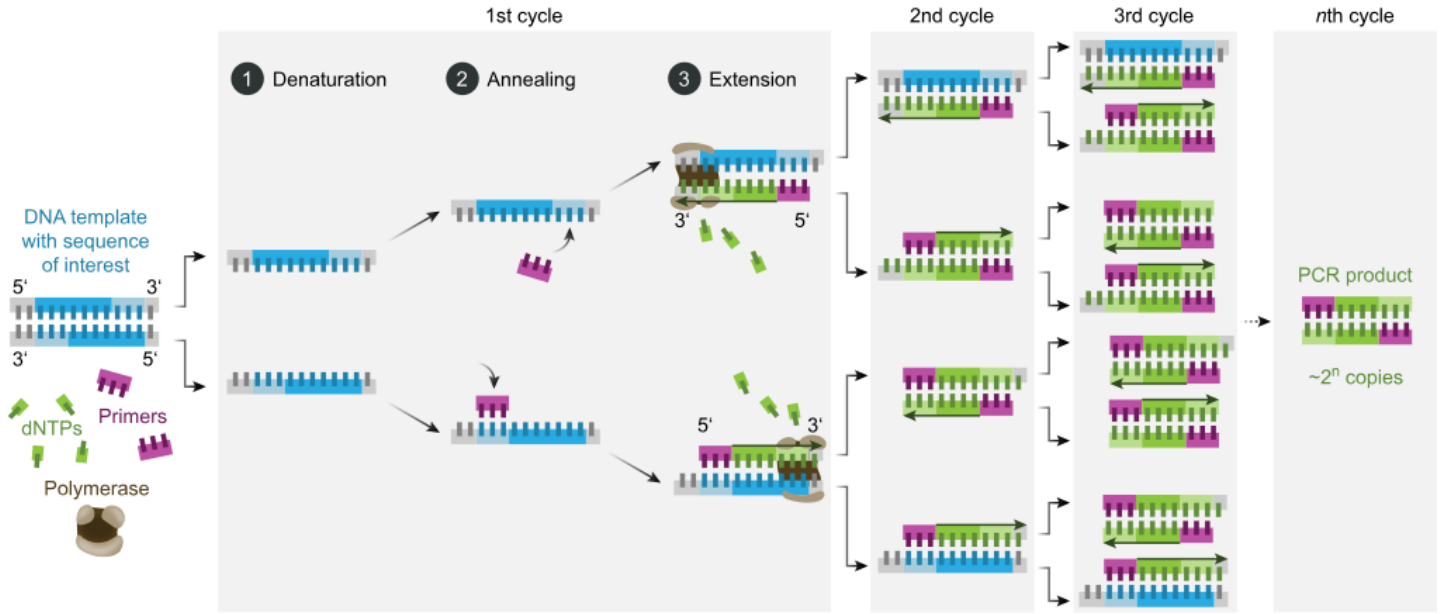
# b. Polymerase Chain Reaction (PCR)

- Amplification of region of interest using specific primers
- Cycling reaction condition
- Used for marker quantification
- Input for further analyses (restriction, Sanger sequencing, fragment analysis, NGS, …)



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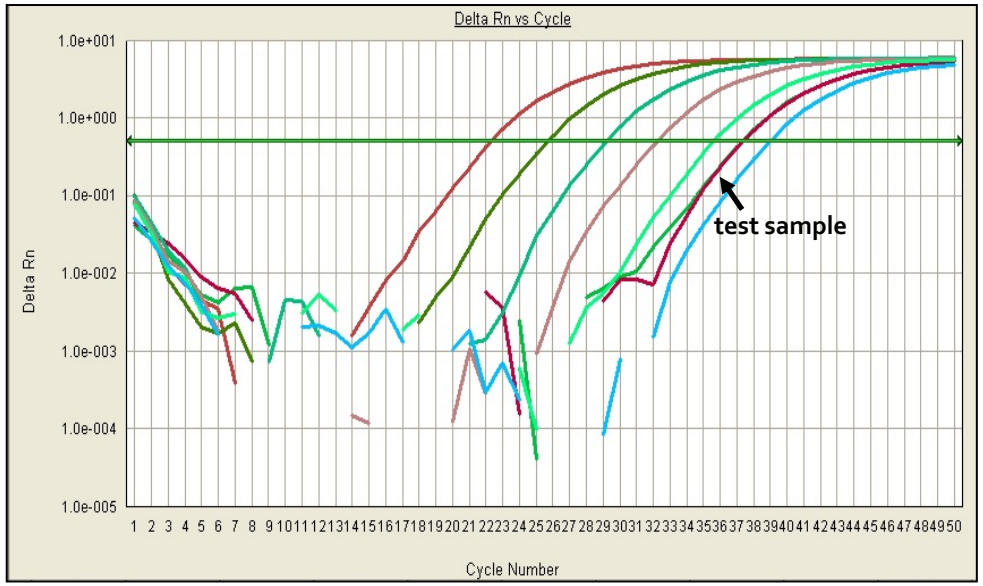
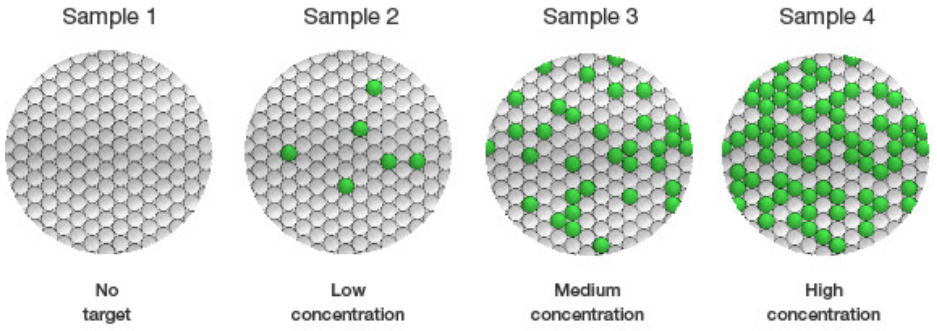
# b. PCR-based quantification methods

Real-time PCR
- Quantitative method – fluorescent detection of generated products
- Need for specific primers and probes
- Relative vs absolute quantification




Droplet digital PCR (ddPCR)
- Alternative method for marker absolute quantification
- Highly precise
- Need for specific instrumentation




www.bio-rad.com



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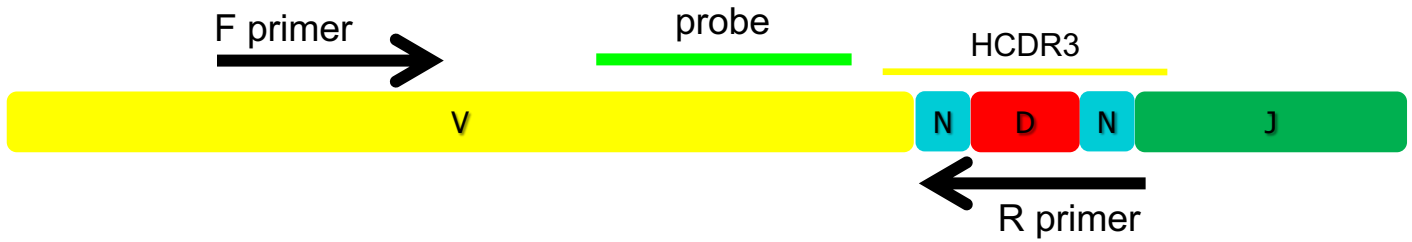
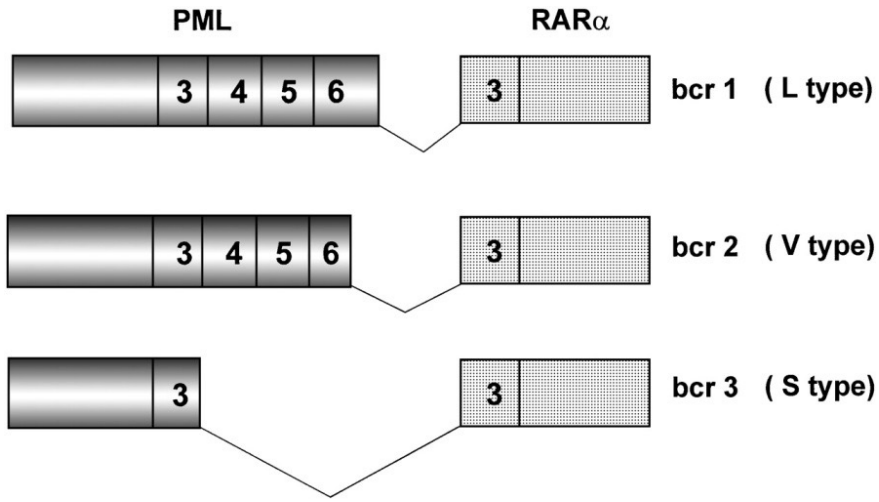
# b. Real-time PCR applications

- Quantification of minimal residual disease after therapy – detection of tumor specific markers (fusion genes, antigen receptor rearrangements etc.)
- Gene expression analysis
- Pathogen detection and pathogen load quantification



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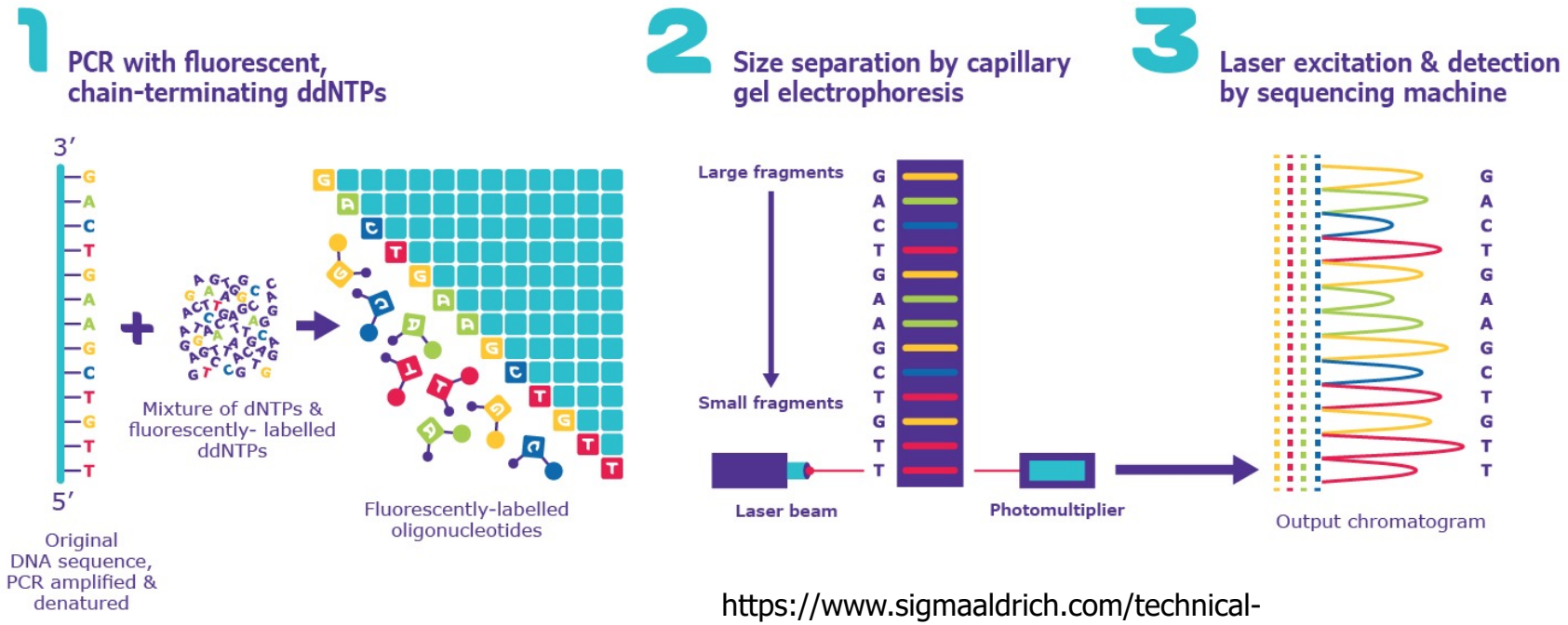
# c. Sanger sequencing

Modification of PCR
- single primer extension
- Incorporation of dNTPs and ddNTPs

Applications
- Basic method for sequence variant detection (mutations, breakpoint localization)
- Fragment analysis – modification of the method for detection of fragment length variation




https://www.sigmaaldrich.com/technical-documents/articles/biology/sanger-sequencing.html



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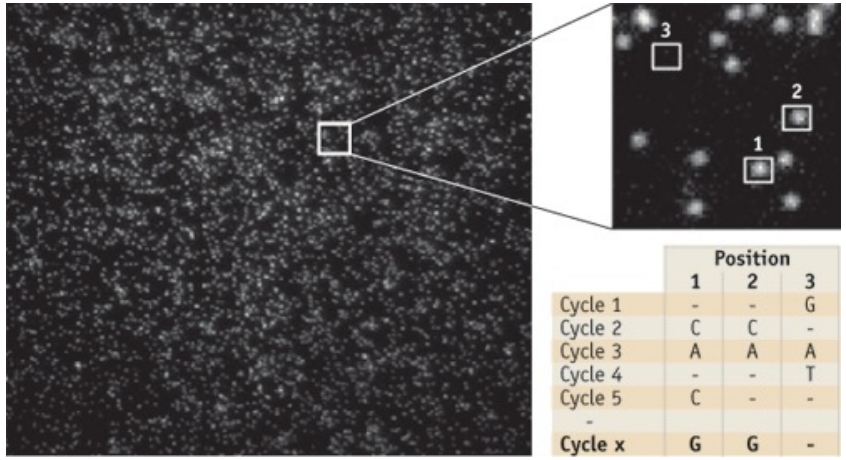
# c. NGS - principles and targeted regions

Next-generation sequencing (NGS) ~ masively parallel sequencing (MPS)

- PCR amplification of DNA fragments
  or direct sequencing of individual fragments (single molecule sequencing)
- The most common approach –
  sequencing by synthesis (Illumina sequencers)
- Milions of fragments are amplified simultaneously
  (vs capillary sequencer max 96 reactions)
- Short reads (tens to hundreds basepairs)



# c. NGS - principles and targeted regions

Illumina machines and their capacity

NovaSeq

48 genomes/run, 6 TB/run

HiSeq 4000

12 genomes/run, 1.5 TB/run

NextSeq 500

1 genome/run, 120 GB/run

MiSeq

0.15 genome/run, 15 GB/run

MiniSeq

0.07 genome/run, 7.5 GB/run

iSeq

0.01 genome/run, 1.2 GB/run

# c. NGS – regions of interest

genome

3 200 000 000 bp
30 x read depth

exome

20 000 genes
100 x read depth

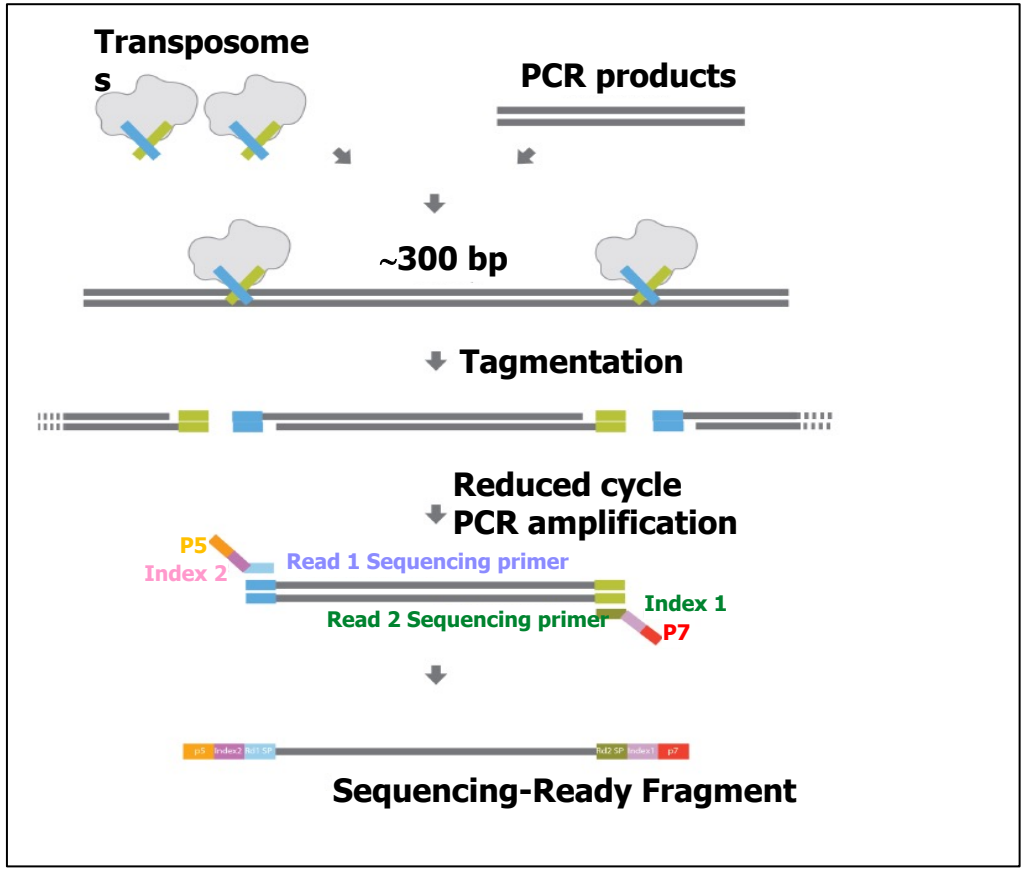
selected genes or loci

< 100 genes
≥ 1000 x read depth

# c. Amplicon sequencing

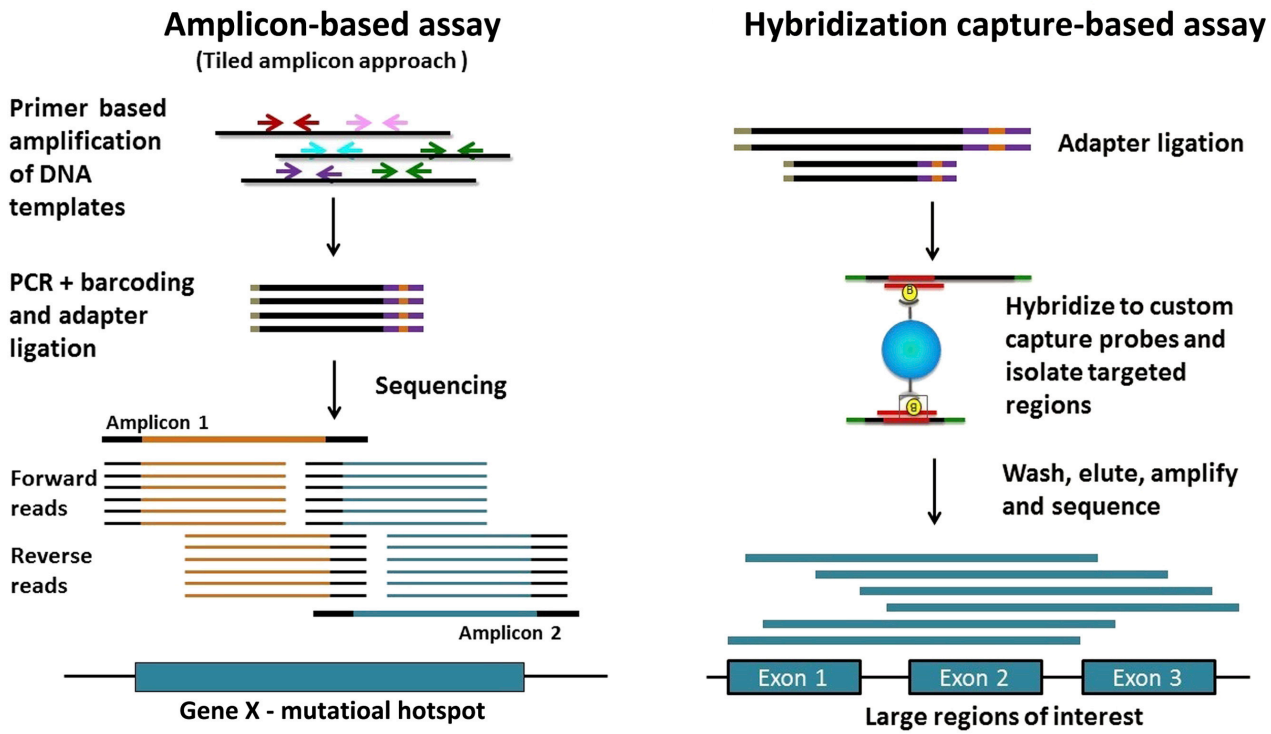
Application: TP53 mutation analysis

- NGS with high coverage (limit of detection 0.1 % of variant allele)
- treatment response prediction



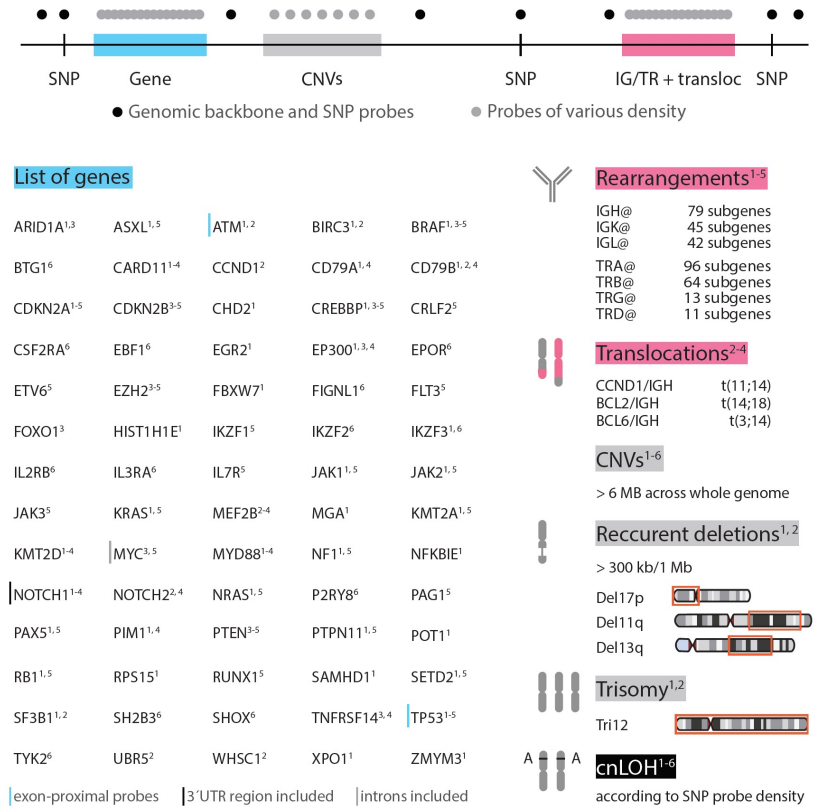
# c. Panel sequencing

- Sets of selected regions of interest
- Target enrichment by amplification or hybridization




Application: LYNX panel

- diagnostics of molecular markers in lymphoid malignancies

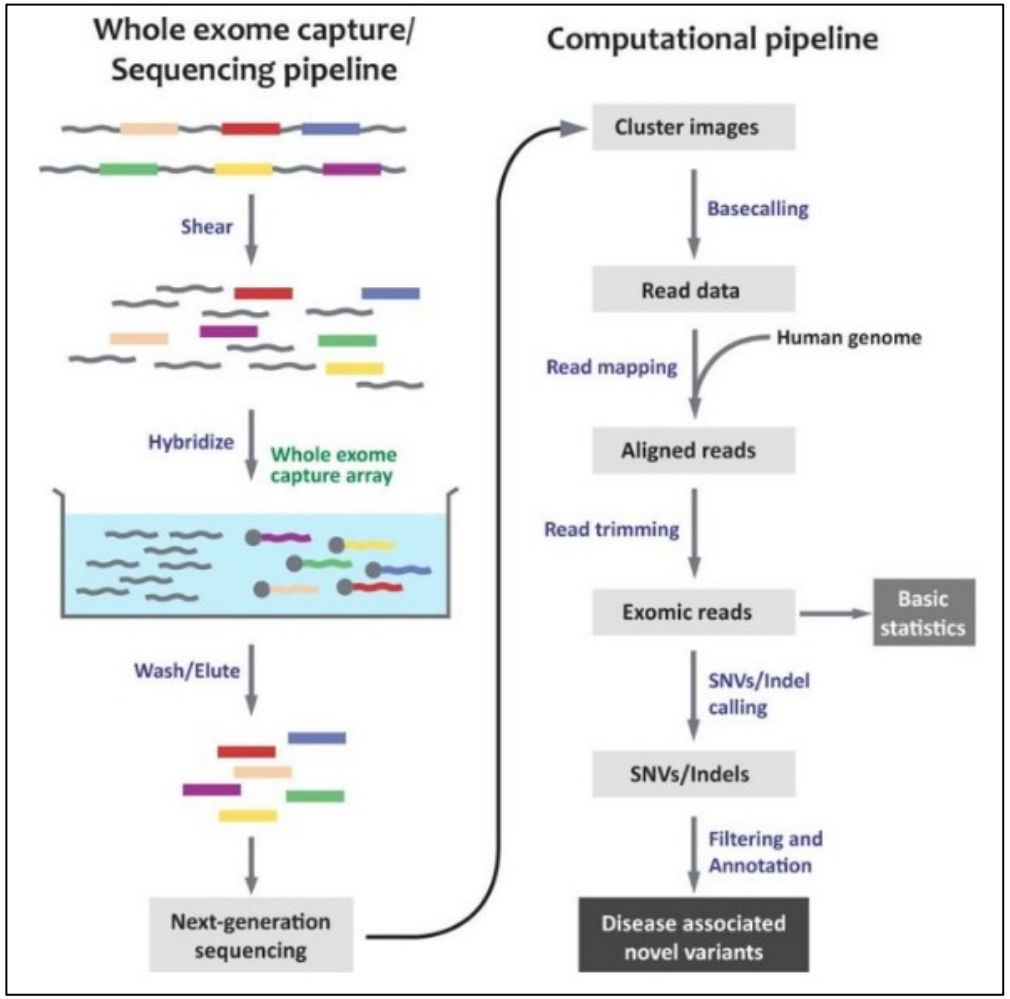





Jennings LJ et al. J Mol Diagn. 2017

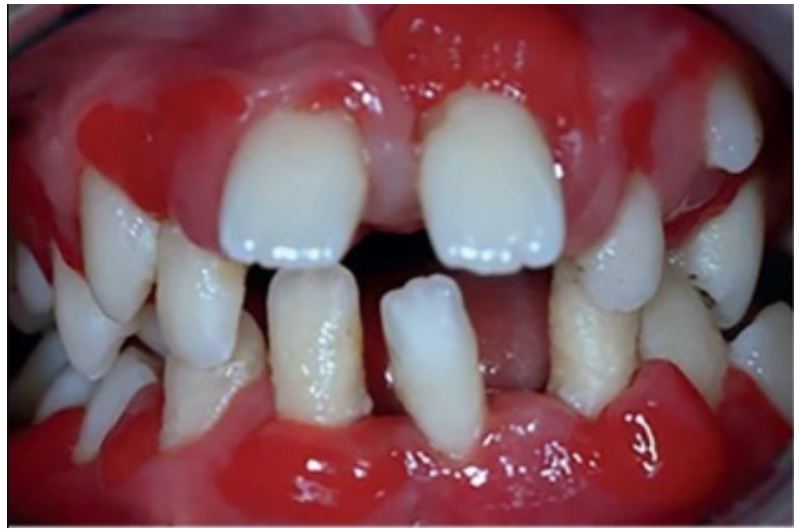
# c. Whole exome sequencing (WES)

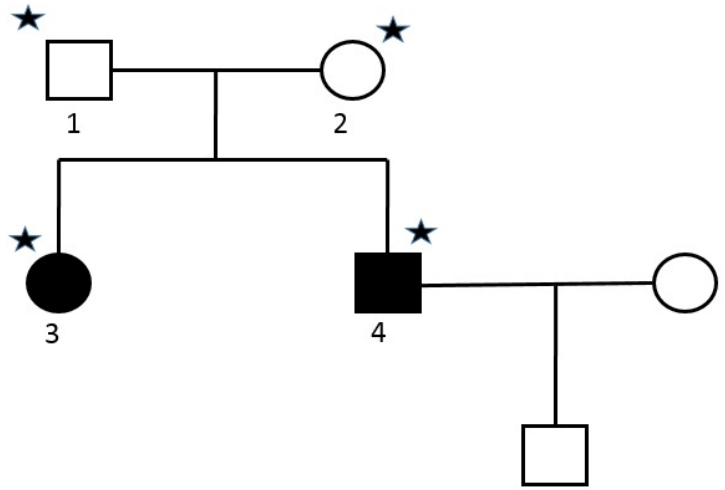
- Mainly experimental approach for exploring unknown variants
- Used in
  - genetic counseling for identification of causative variants
  - discovery of novel genetic markers
  - searching for treatment targets



# c. WES – case report of Shwachman-Diamond syndrome

- a multisystem autosomal recessive disorder
- clinical features: pancreatic exocrine insufficiency, hematologic dysfunction, and skeletal abnormalities
- haematological malignancies (e.g. myelodysplastic syndrome and acute myeloid leukemia) occur in one third of patients
- homozygous or compound heterozygous variations in *SBDS* gene





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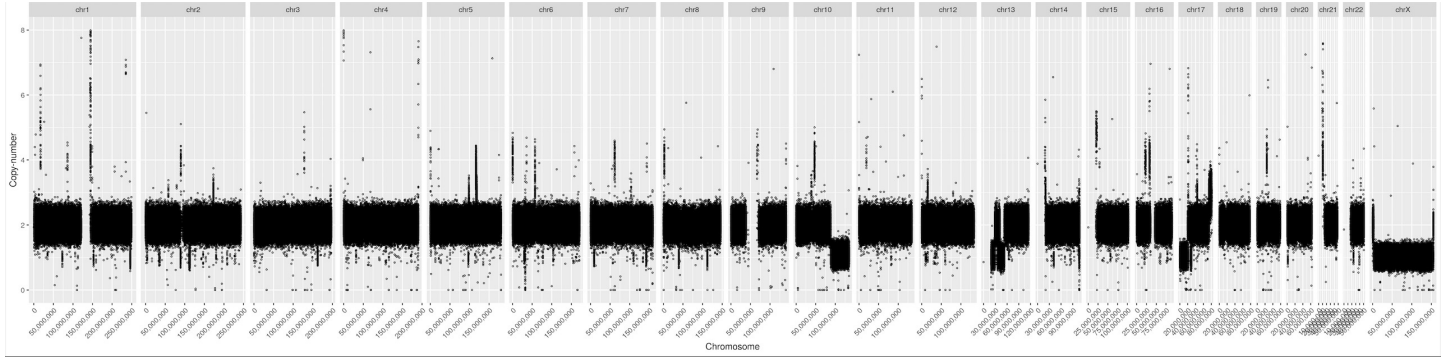
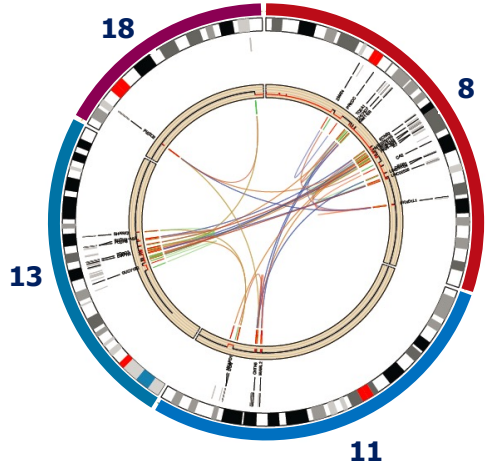
# c. Whole genome sequencing (WGS)

- Mainly experimental method for exploring unknown variants
- Applications similar to WES, additional information about non-coding regions and chromosomal abnormalities
- Typicall sequencing coverage ~ 30–100x – detection of clonal or germline mutations
- Shallow sequencing (~ 0.5-10x coverage) – genome-wide detection of chromosomal abnormalities, low yield of mutation detection
- In clinical practise a potential benefit of combination of shallow and panel sequencing



# The end…

Contact:
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# Thank you for your attention!



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