# Cytogenetics and molecular genetics in oncology

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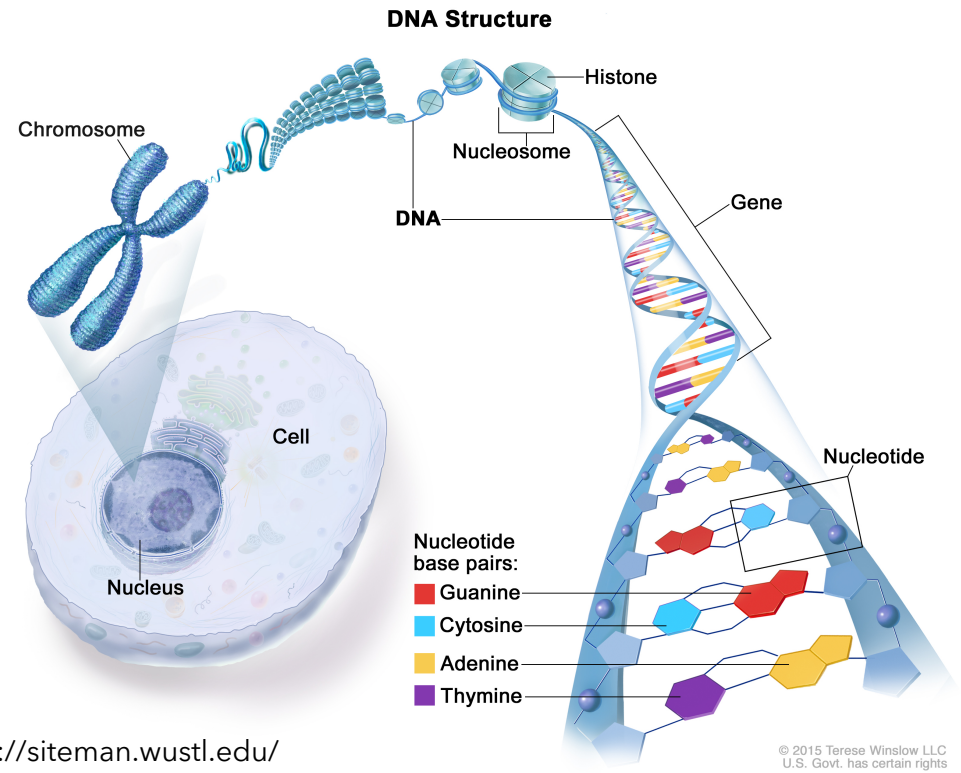
# Outline of the presentation

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

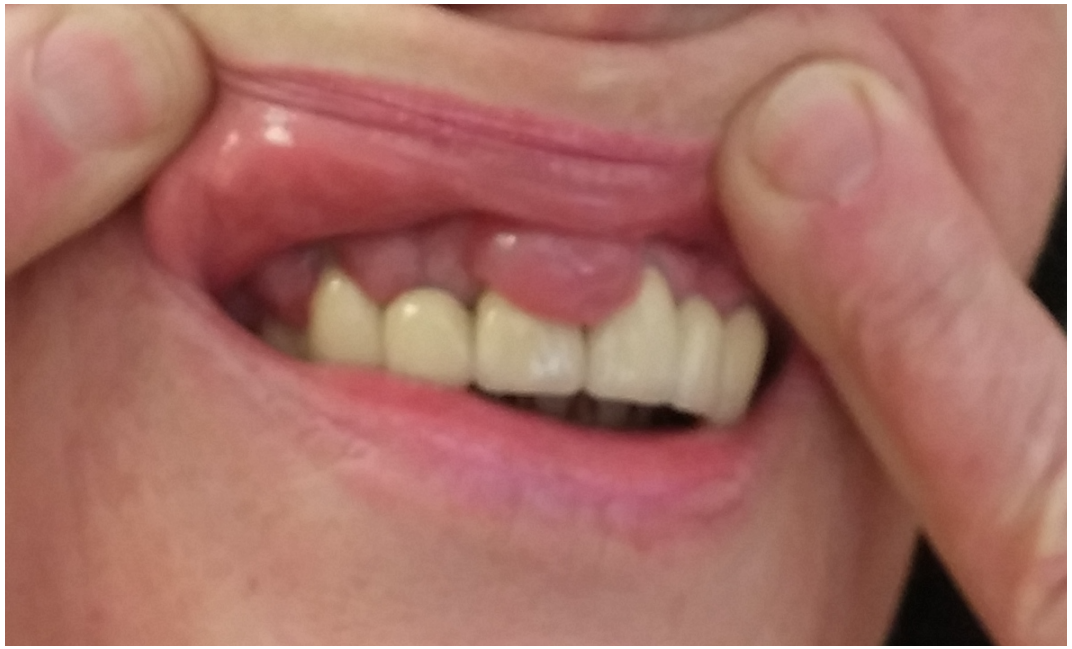
# Cancer as a genetic disease

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




https://siteman.wustl.edu/

# 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 |

# 2. Application of MG and CG findings in oncology

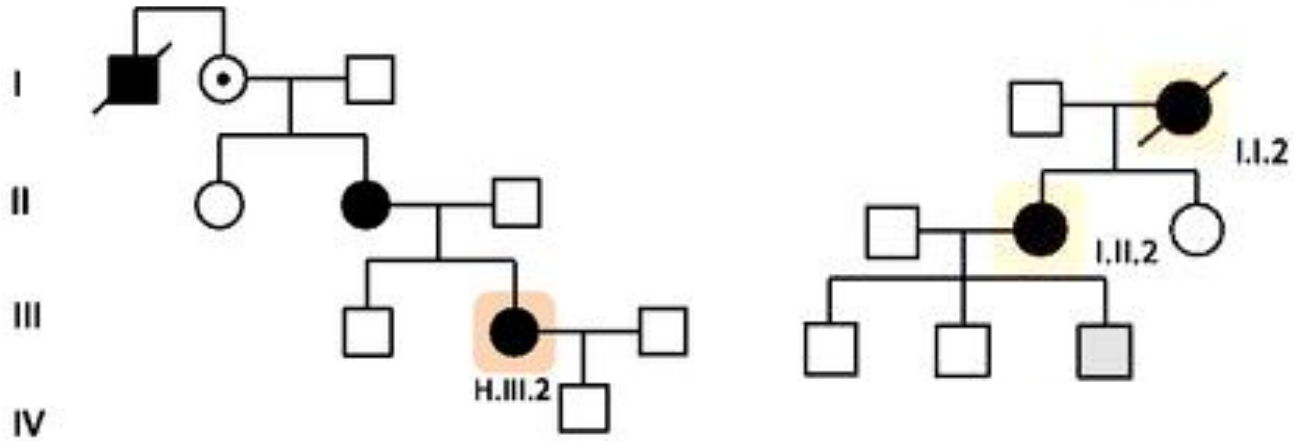
- Hereditary predisposition assessment
- Establishing and refining diagnosis
- Disease prognostication
- Treatment optimization
- Disease activity monitoring
- Disease complication diagnostics

# 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

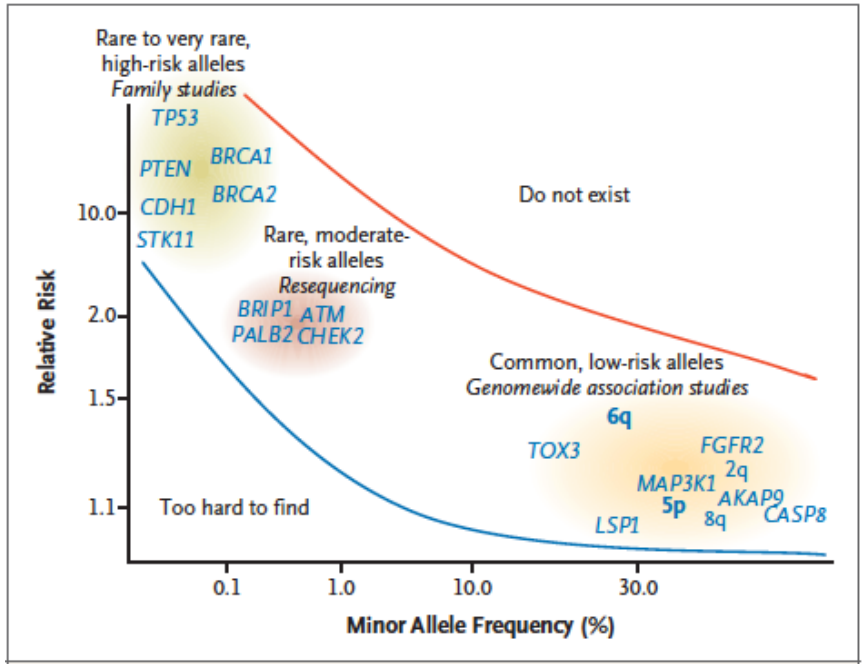
# Hereditary predisposition assessment

- 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

# 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

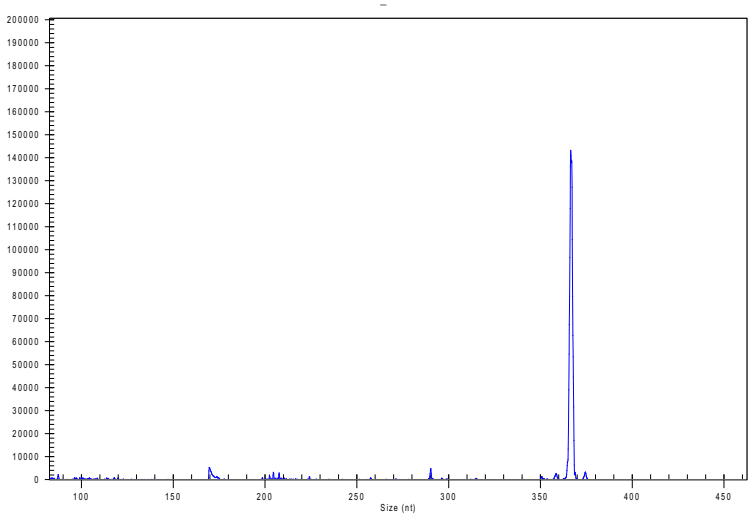
# Establishing and refining diagnosis

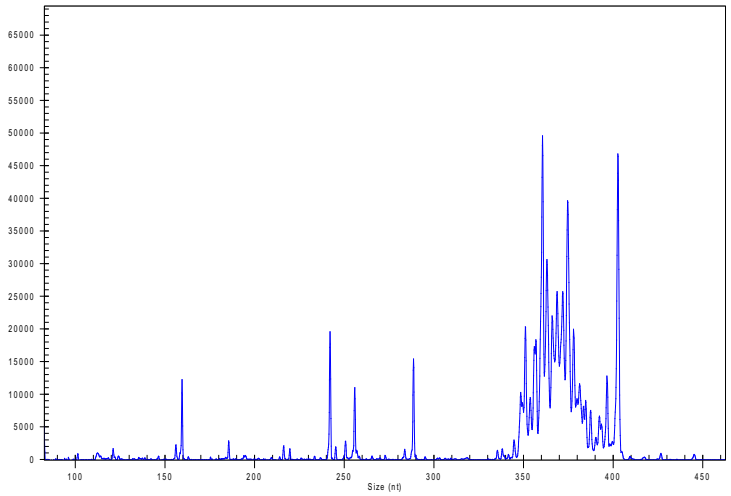
- Diagnosis confirmation based on detection of specific (marker) gene or chromosomal abnormalities
- Incorporation of genetic/cytogenetic markers in WHO classification
- Resolving ambiguous cases
- Markers specific for the whole diagnostic entity or only for a subset of a disease (implications for treatment)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

# Establishing and refining diagnosis

Clonality

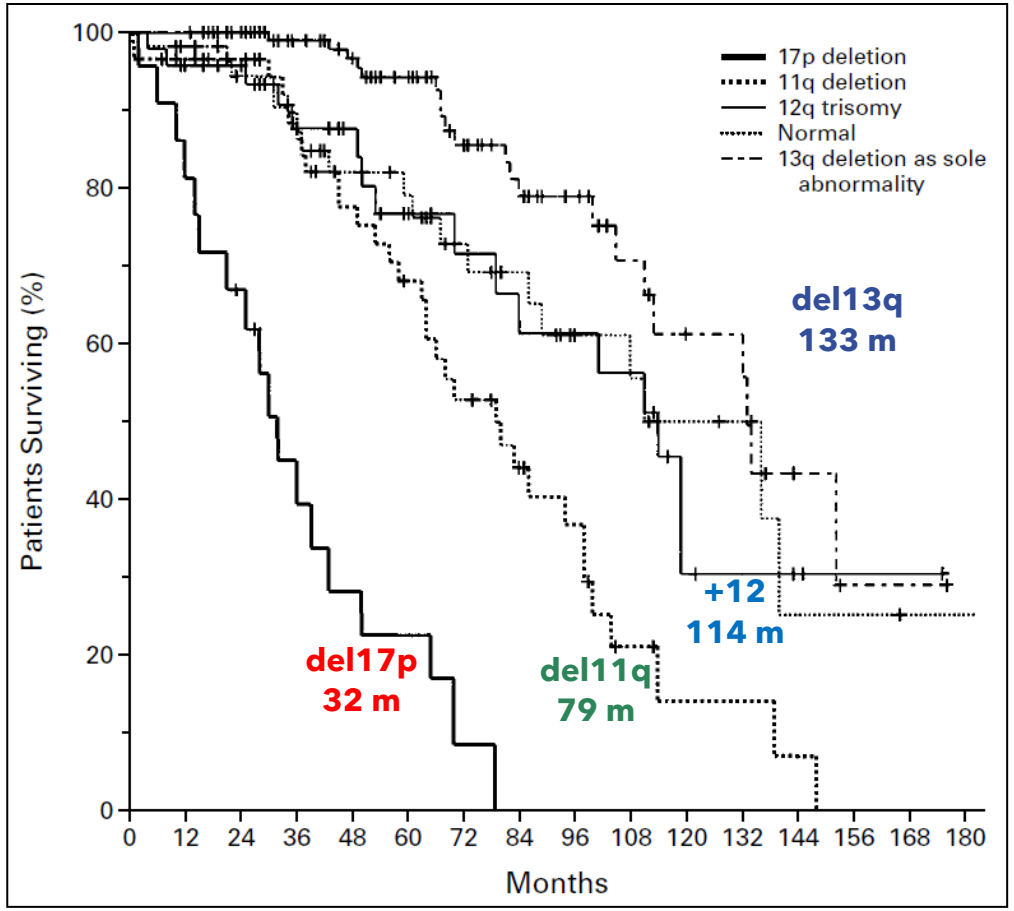
- Typical characteristics of lymphoid (but also other) malignancies
- Analysis of antigen receptor rearrangements, translocations and gene mutations
- Monoclonal vs polyclonal picture – distiguishing of malignant vs reactive conditions
- Quantification of tumor load



# Disease prognostication

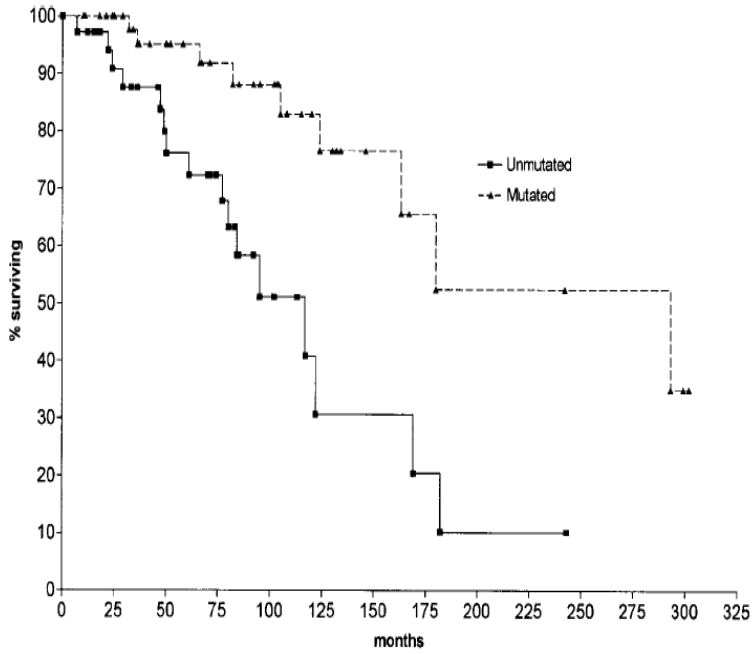
- Genetic and cytogenetic markers associated with certain disease features
- Risk assessment at time of diagnosis
- Genetic markers of various types – gene mutations, chromosomal abnormalities, a type of antigen receptor rearrangement, …
- Prognostic vs predictive markers




Döhner et al, Blood 2000

# Disease prognostication

CLL:




Hamblin et al, Blood 1999

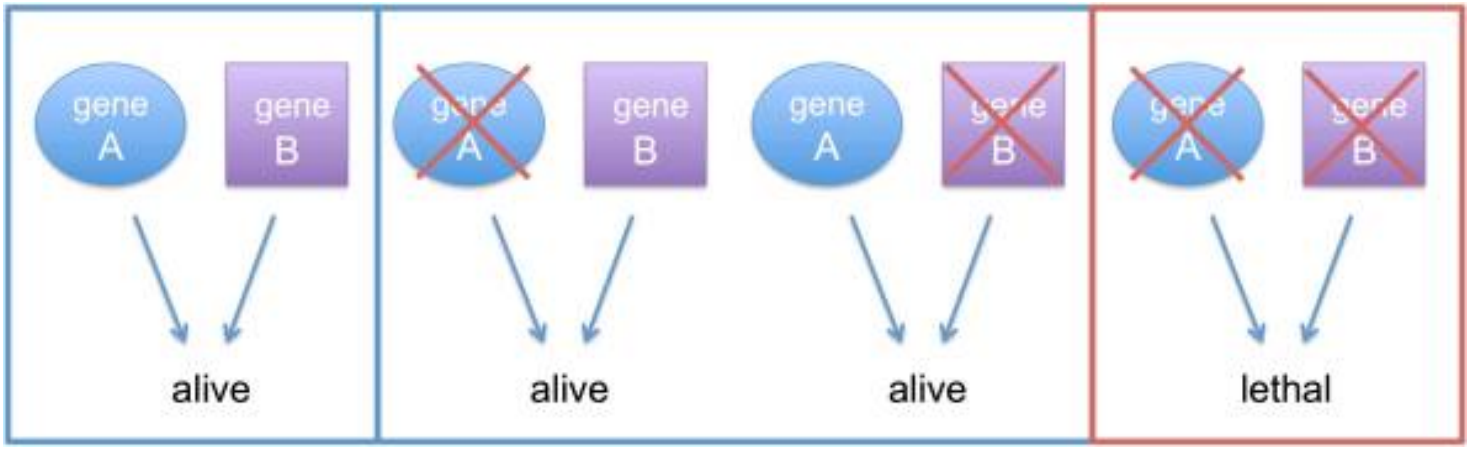
AML:

| 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

# 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



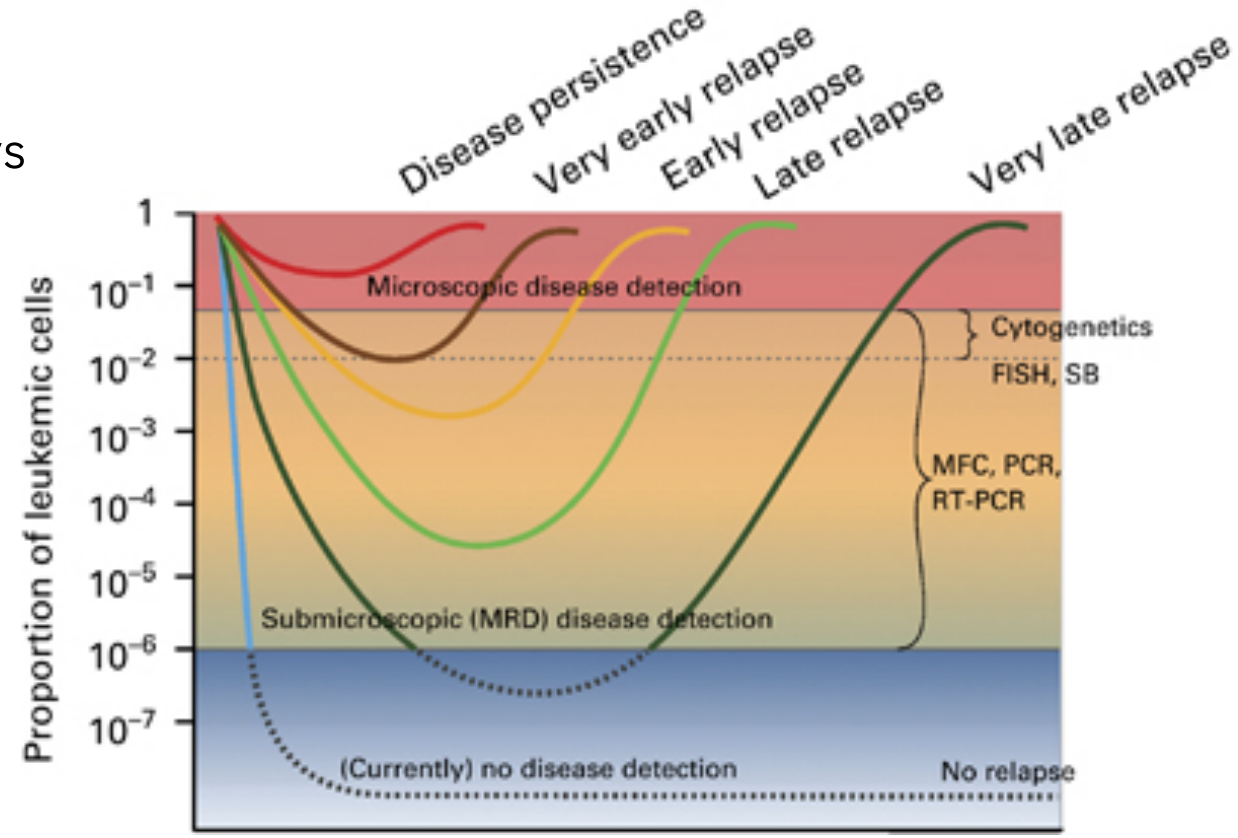

Nijman, FEBS Lett 2011

# 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 | BRAF mutations | BRAF inhibitors (dabrafenib, vemurafenib etc.) |
| Colorectal cancer | KRAS mutations | Contraindication for targeting EGFR by cetuximab and panitumumab |

# 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.

# 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) |

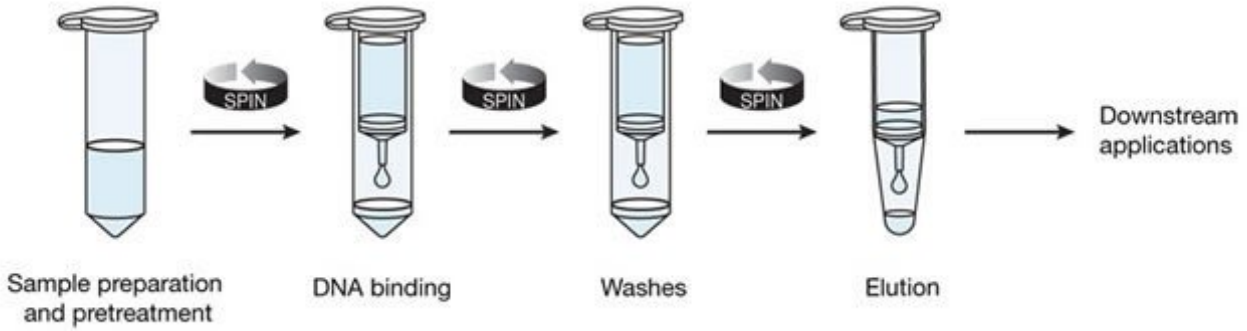
HSV indicates herpes simplex virus; CMV, cytomegalovirus; EBV, Epstein Barr virus.

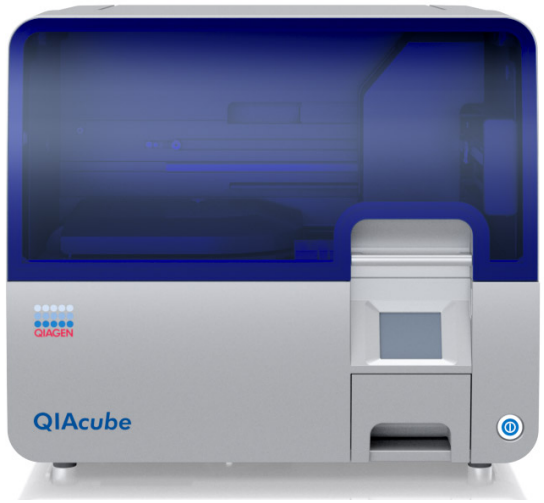
Only the top 5 organisms for each infection type are listed.

Young et al, Biol Blood Marrow Transplant 2016

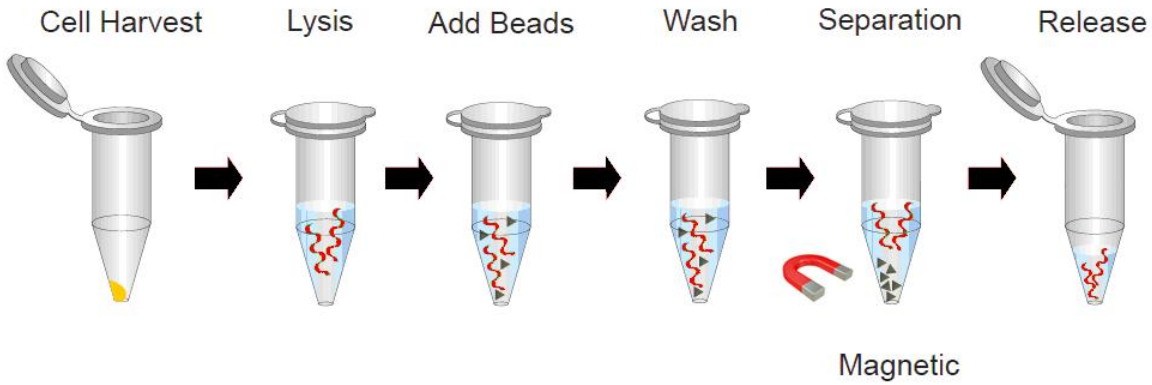
# 3. Materials used and material sources

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





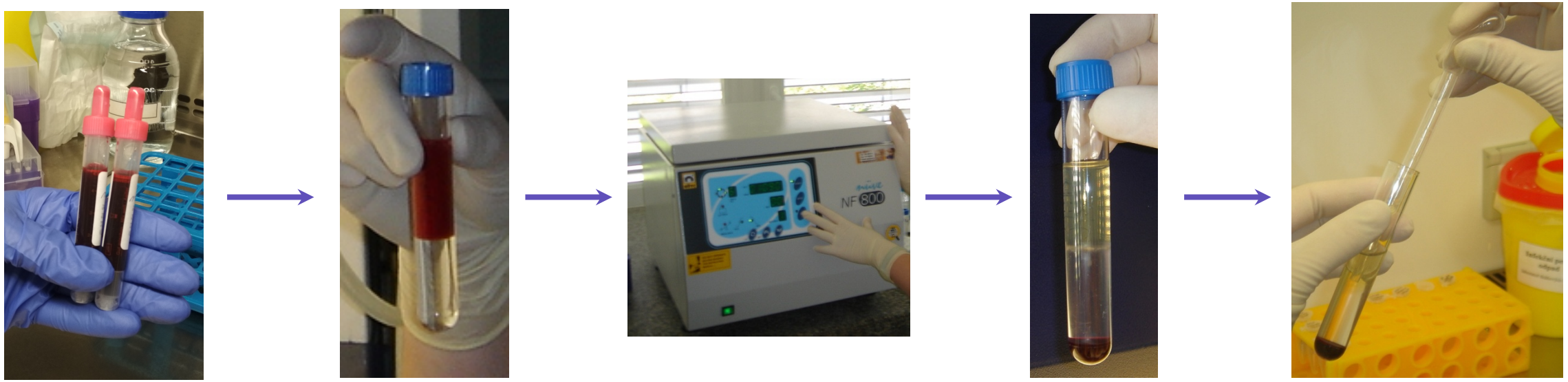


# Materials used and material sources

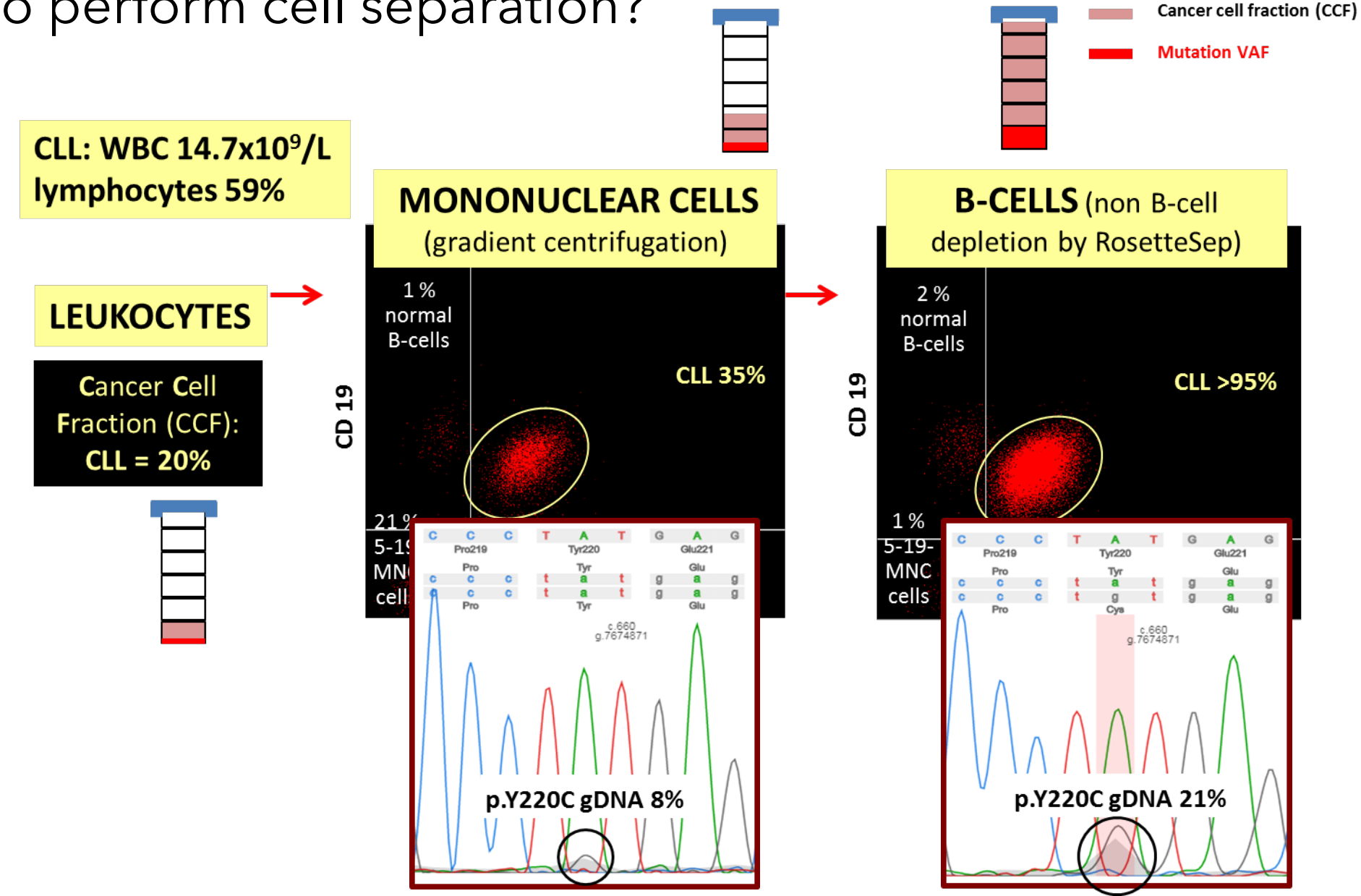
- Sampling
  - Peripheral blood
  - Bone marrow
  - Liquid biopsies
  - Aspirates
  - Fine-needle biopsies
  - Fresh tissue
  - Formalin-fixed paraffin-embedded (FFPE) tissue
  - Swabs (buccal, NPh, …)

# Peripheral blood

- EDTA or heparin collection tubes
- Different cell population used according to the application:
  - Leukocytes
  - Mononuclear cells
  - Granulocytes
  - Lymphocytes
  - Specific cell subpopulations



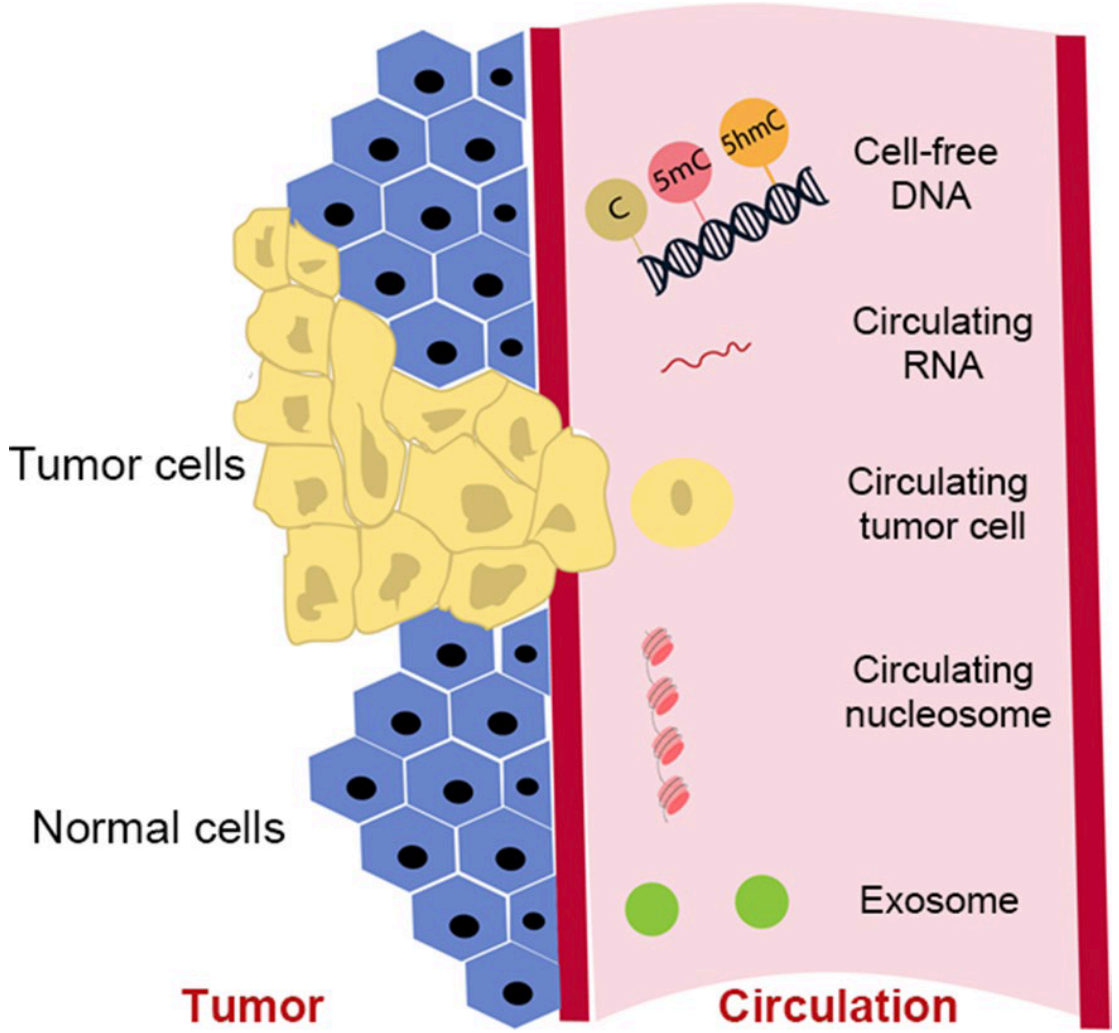
# Why to perform cell separation?



# Liquid biopsies

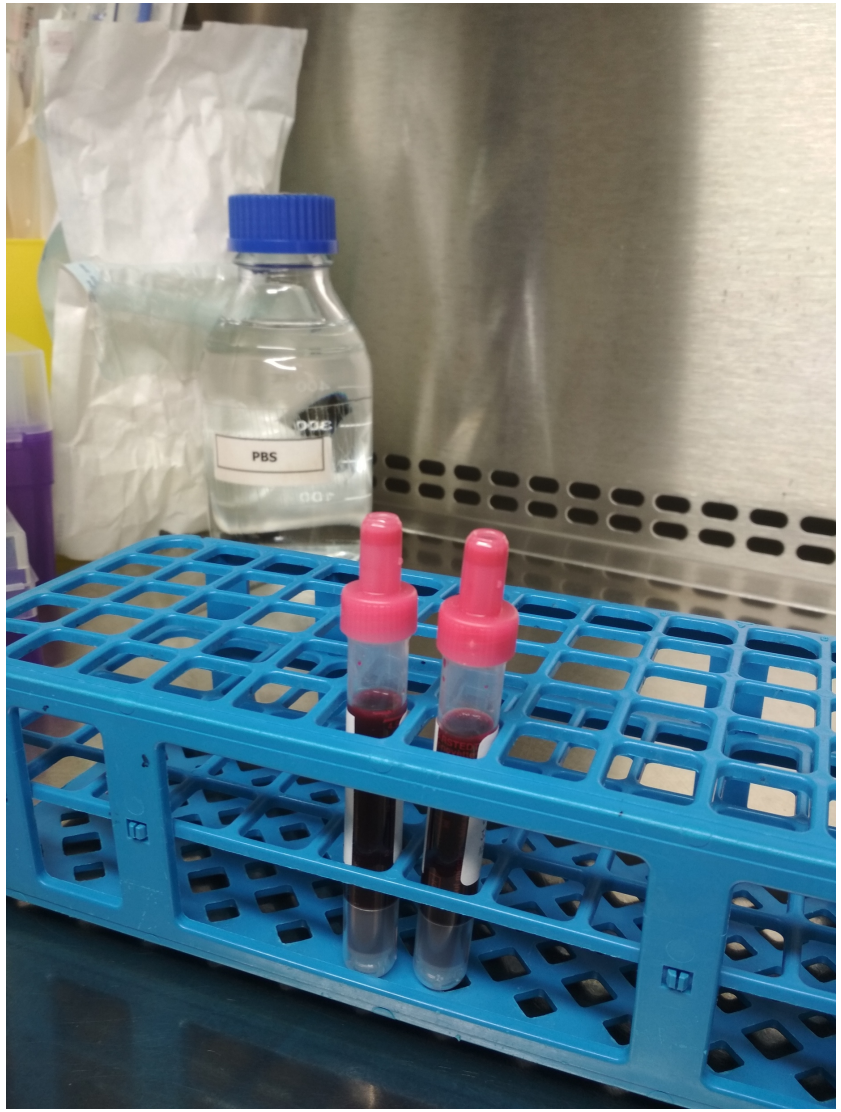
Very low amount of material

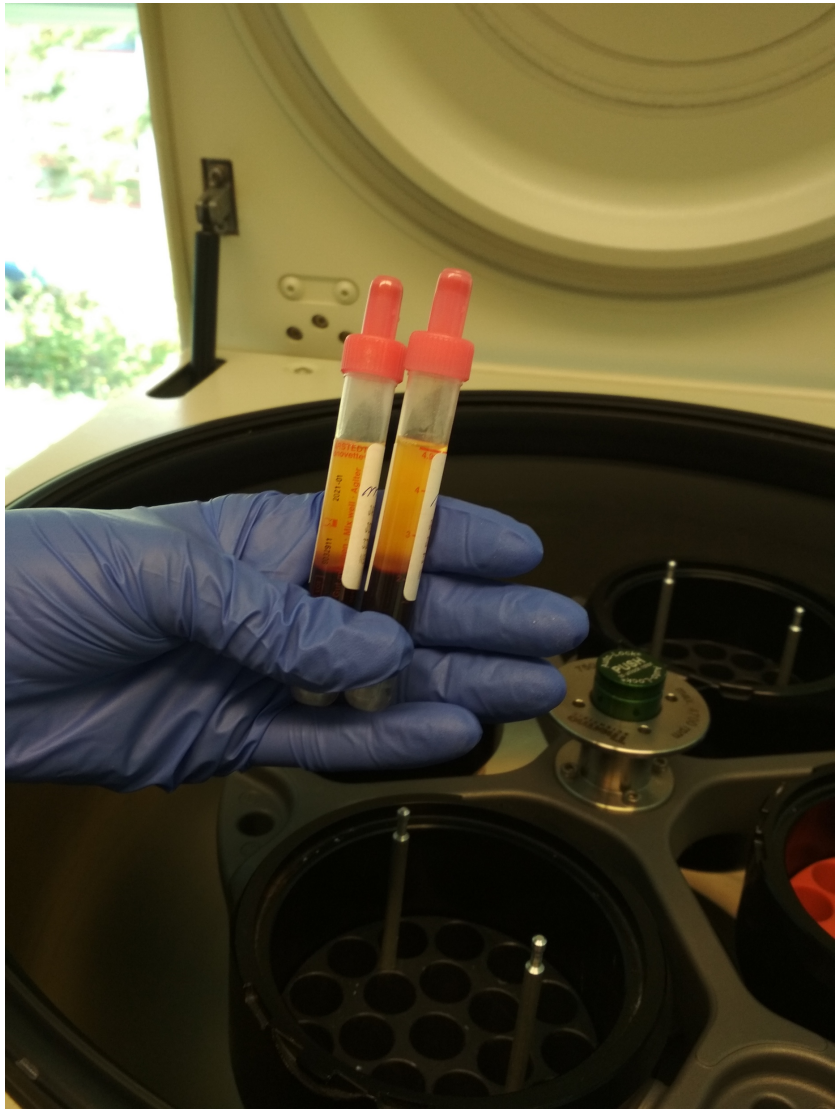
- Plasma / serum
- Urine
- Joint fluid
- Cerebrospinal fluid
- …

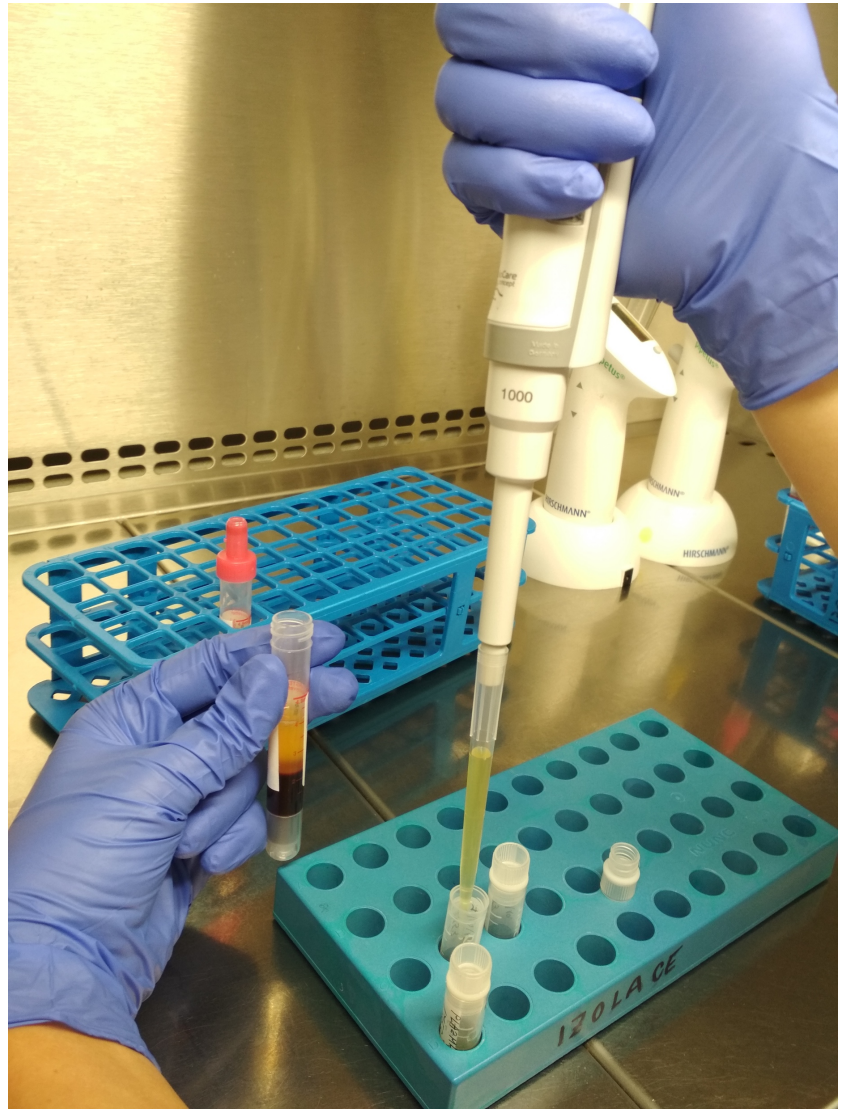



Zeng et al, Cancer Comm 2019

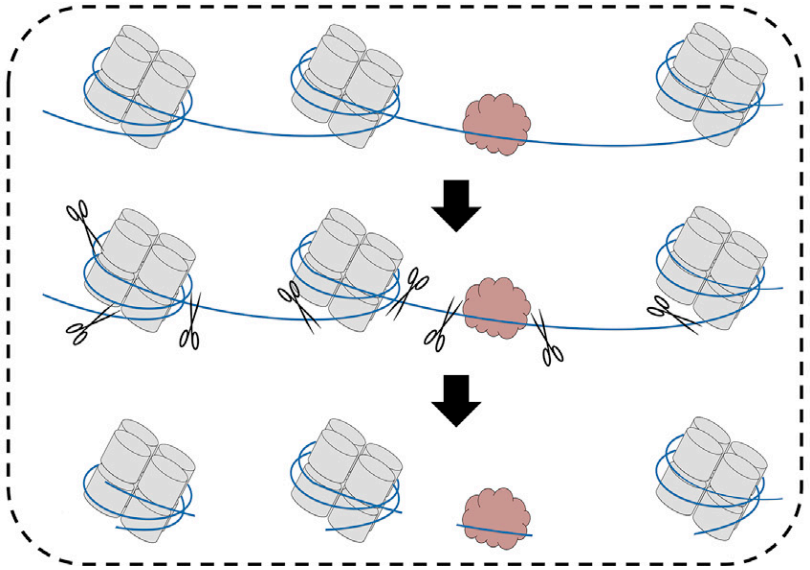
# Liquid biopsies – material collection



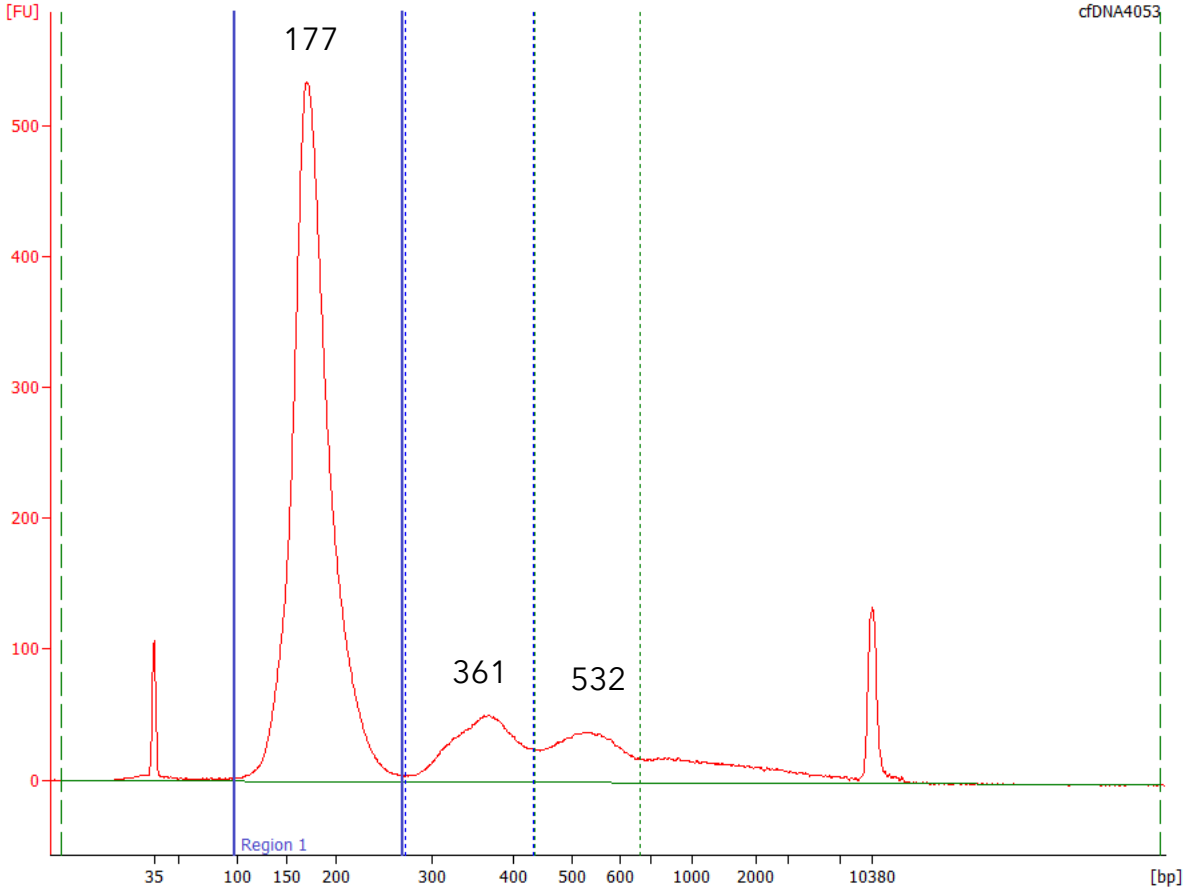





# cfDNA



Snyder et al, Cell 2016

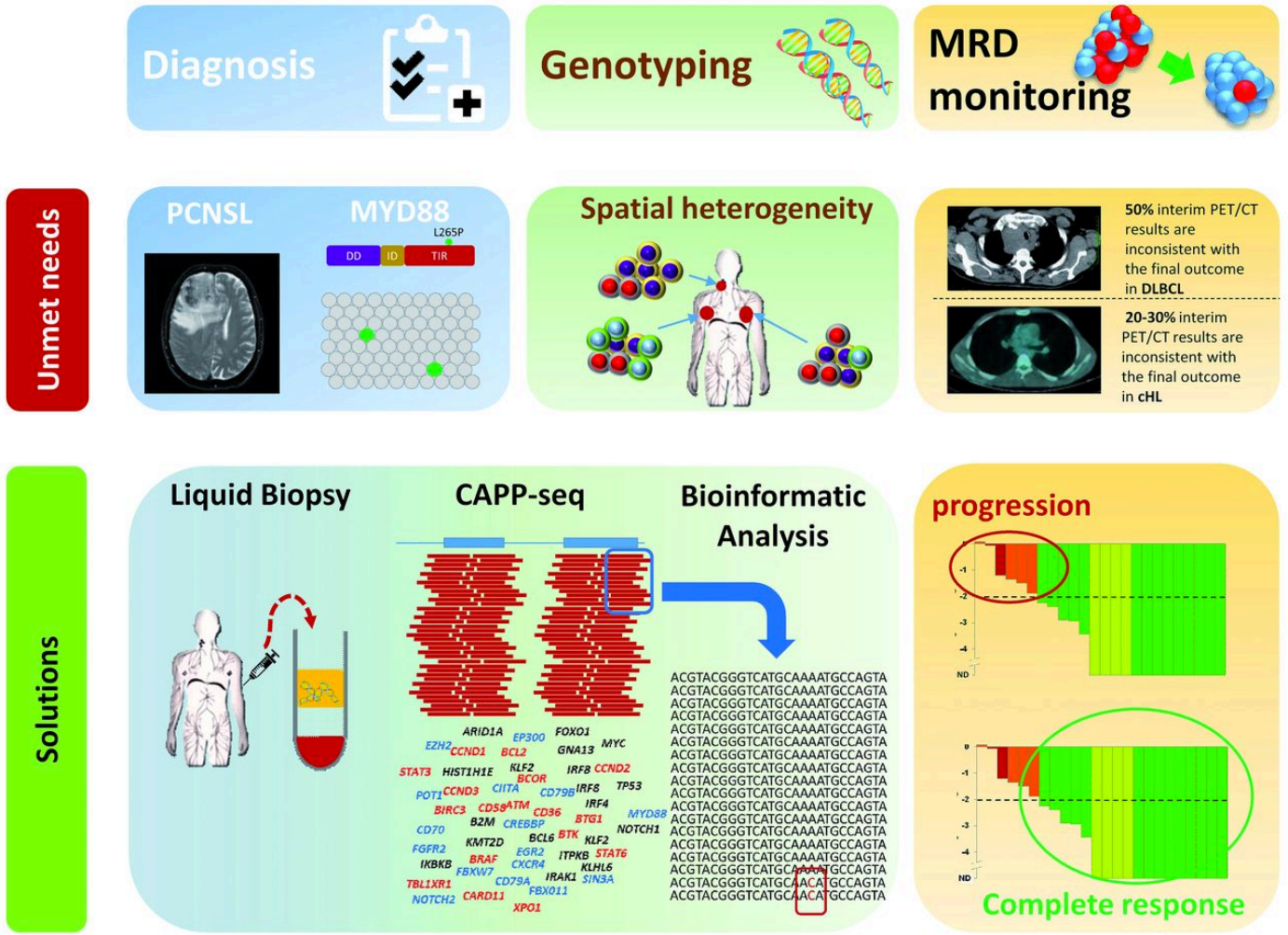


# cfDNA

Applications

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

Solid tumors and hematological malignancies




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

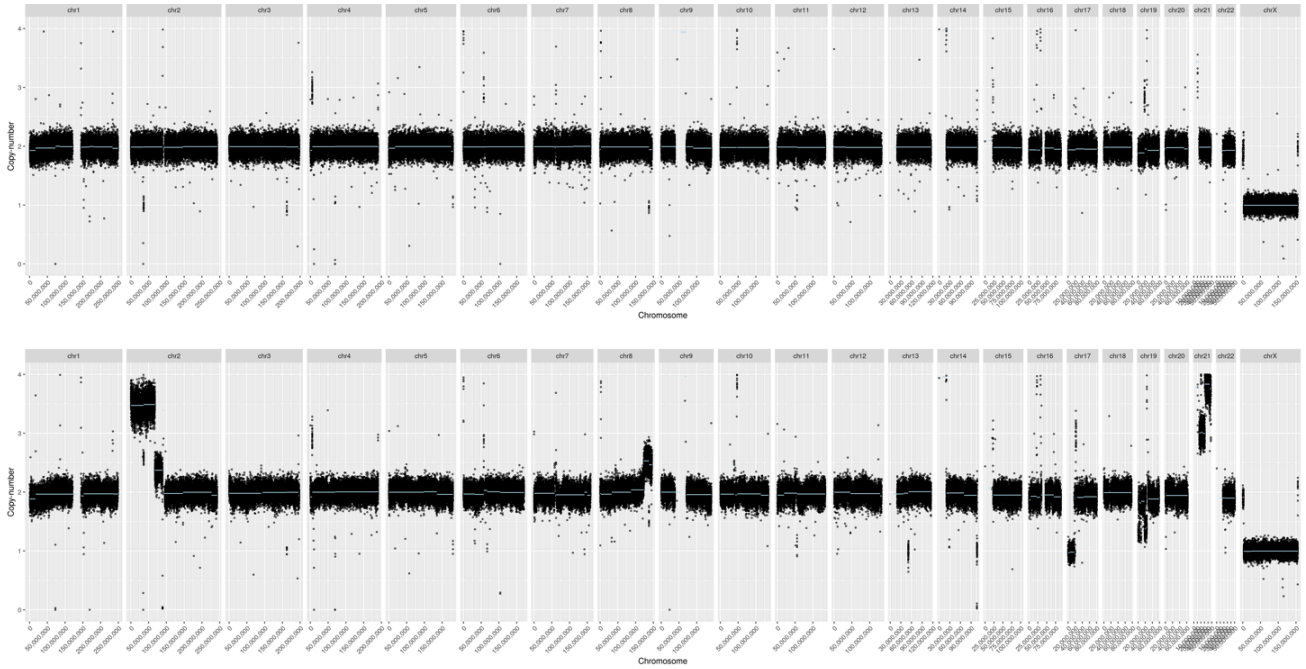
Rossi et al, Haematologica 2019

# cfDNA

Methods

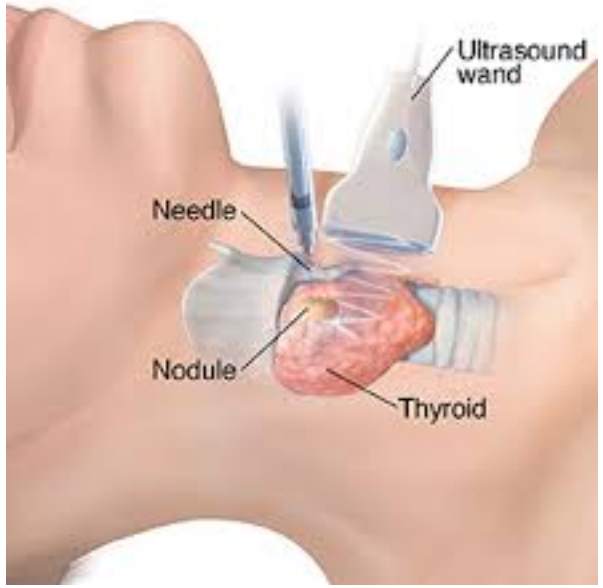
- NGS – targeted amplicons, gene panels, WGS
- real-time PCR

- FDA approved assays for gene mutation detection



# Tissues

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



# Swabs

Different applications

- Collection of germline material - buccal swabs
- Pathogen analyses – e.g. nasopharyngeal swabs

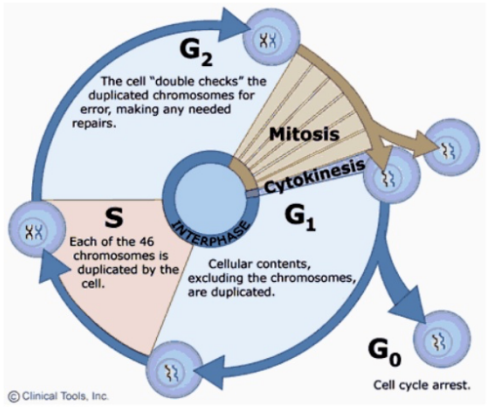
# 4. Methods used and practical examples

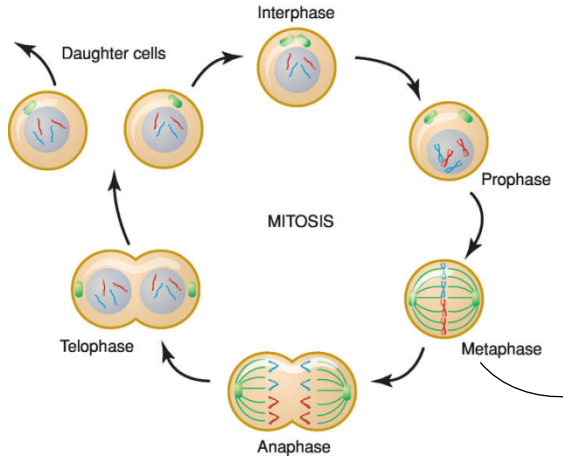
- Chromosome banding techniques
- Fluorescence *in situ* hybridization
- Genomic arrays

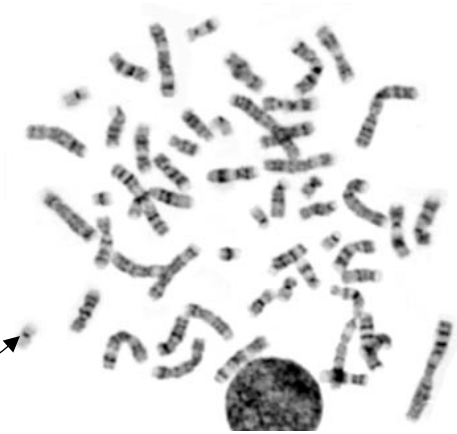
- PCR and real-time PCR
- Droplet digital PCR

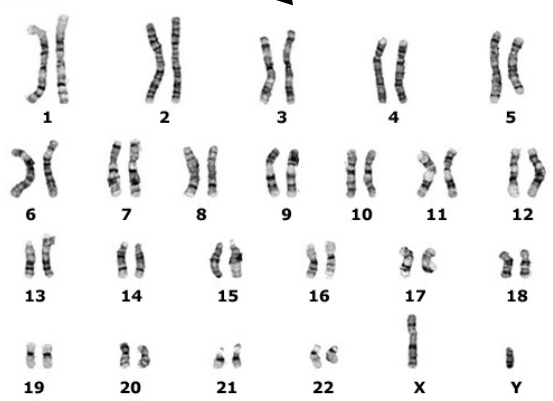
- Sanger sequencing
- Next-generation sequencing
  - Amplicon sequencing
  - Panel sequencing
  - Whole exome sequencing
  - Whole genome sequencing

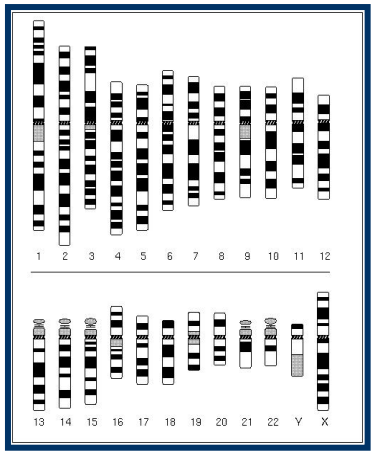
# Classical cytogenetics – chromosome banding techniques

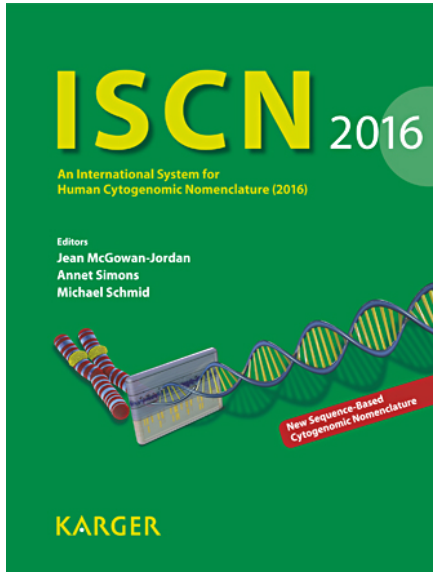




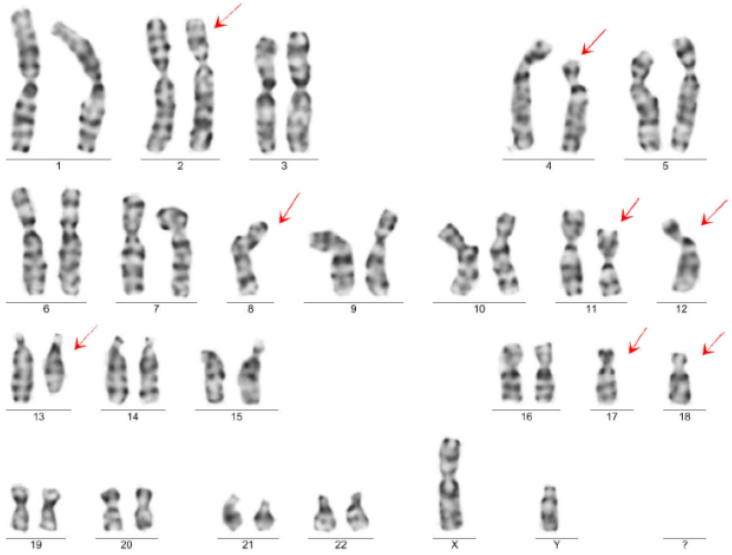
# Classical cytogenetics – chromosome banding techniques

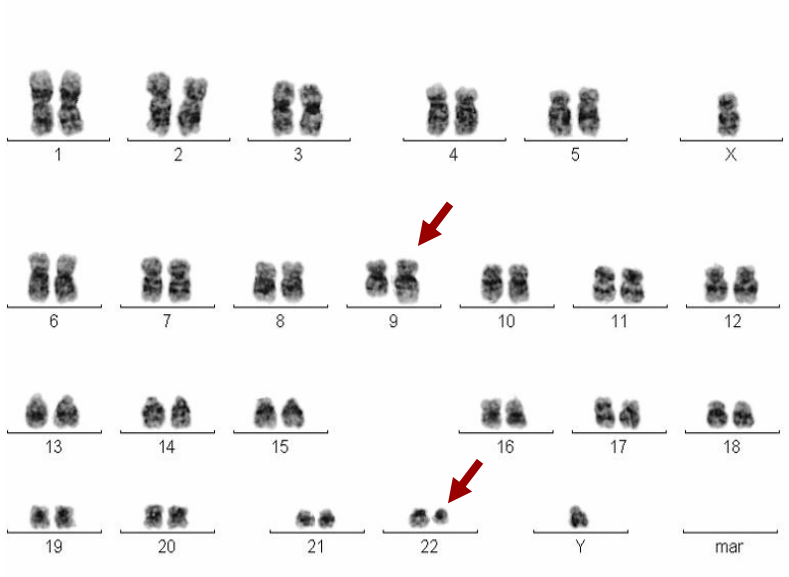
Resolution >10 Mbp

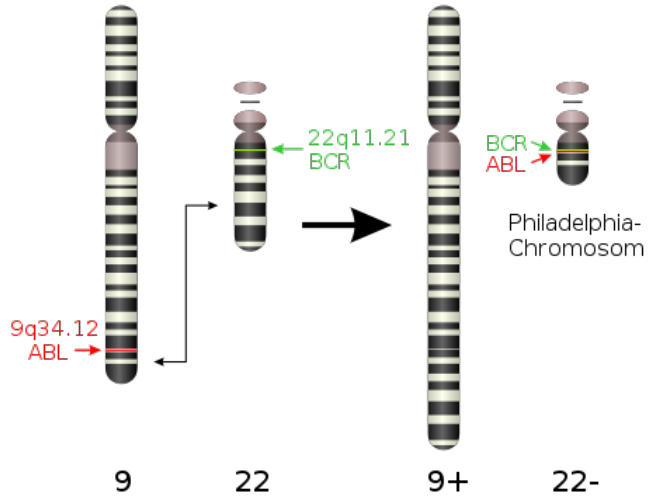
Applications

- Detection of typical abnormalities
- Complex karyotype assessment

  (≥3 or ≥5 abnormalities)

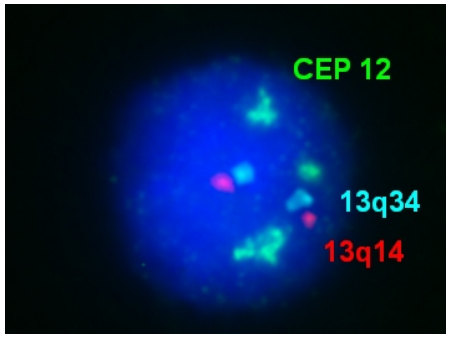




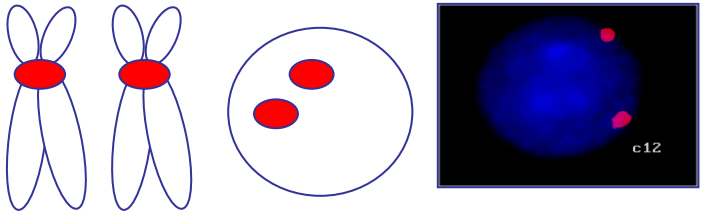



# Molecular cytogenetics

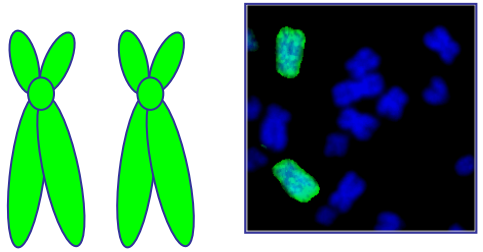
- Fluorescent in situ hybridization (FISH)
- Targets specific regions based on DNA sequence
- Detection of chromosomal abnormalities with diagnostic, prognostic and predictive value



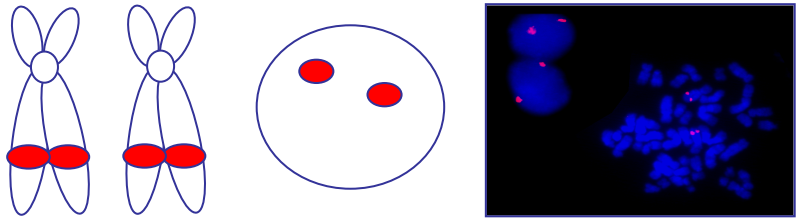

Probe types:




centromeric



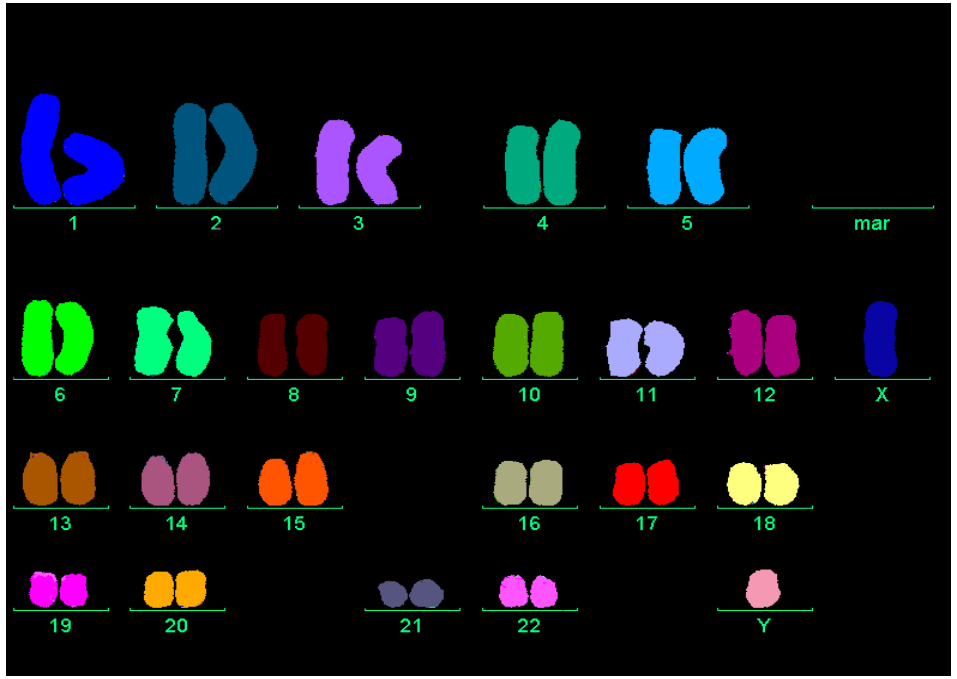
whole chromosome



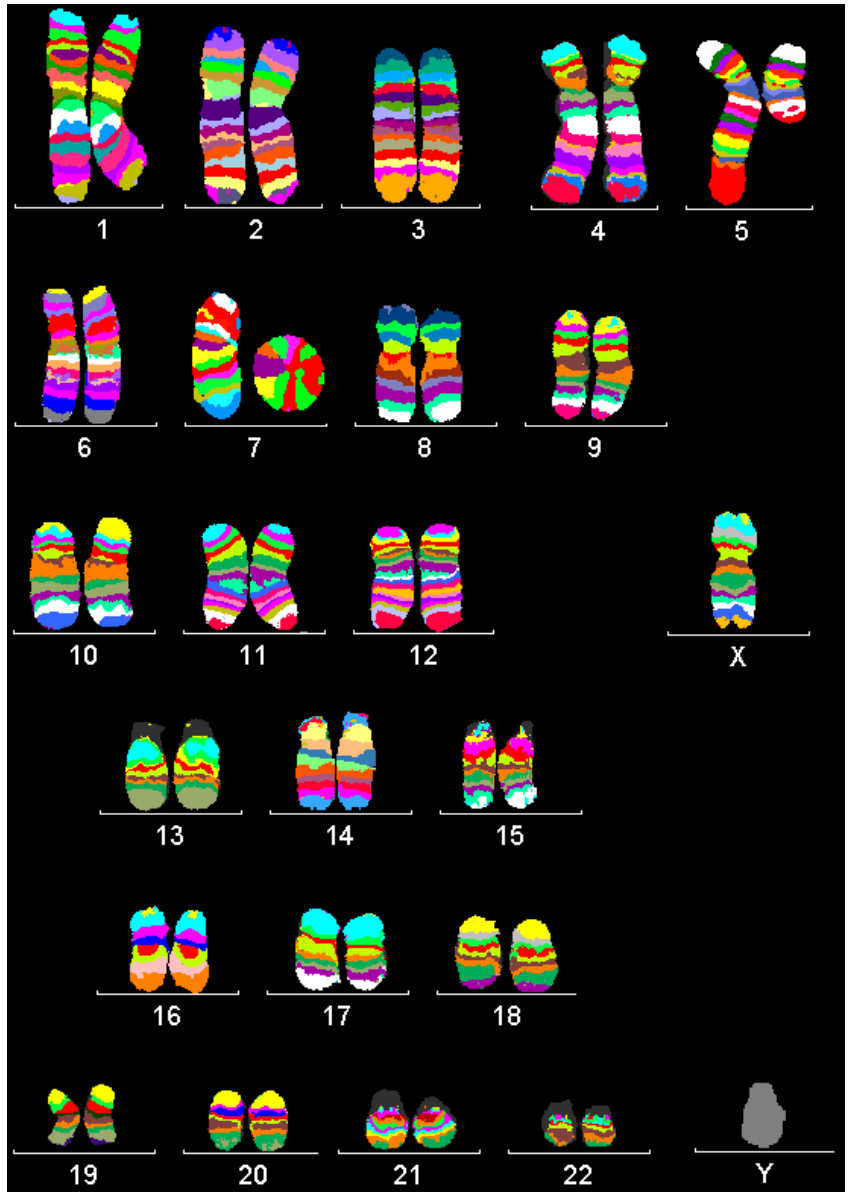
gene

# Molecular cytogenetics

- FISH methods for genome-wide analysis




mFISH

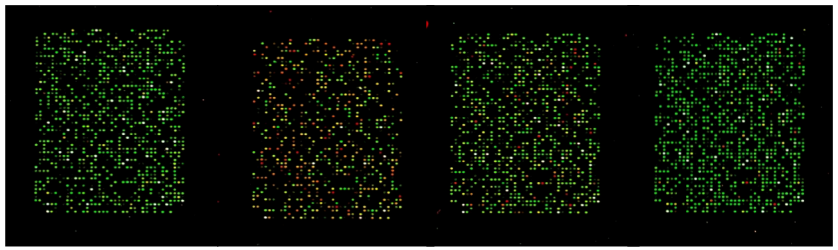


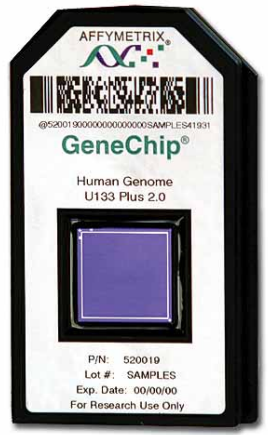

mBAND

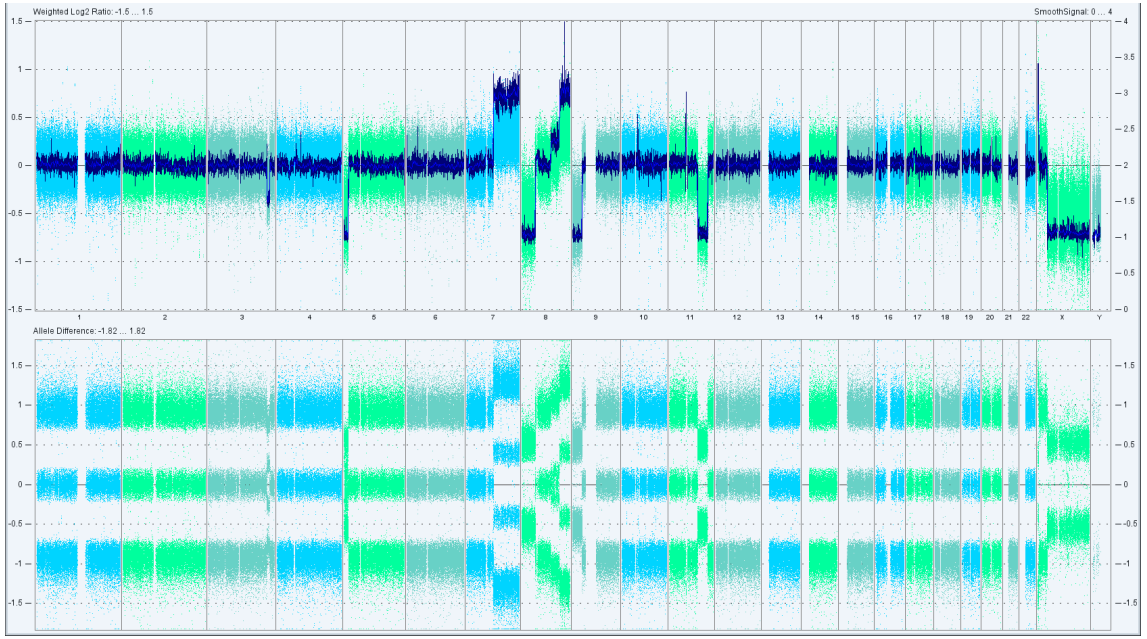
# Genomic arrays

- 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





# 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

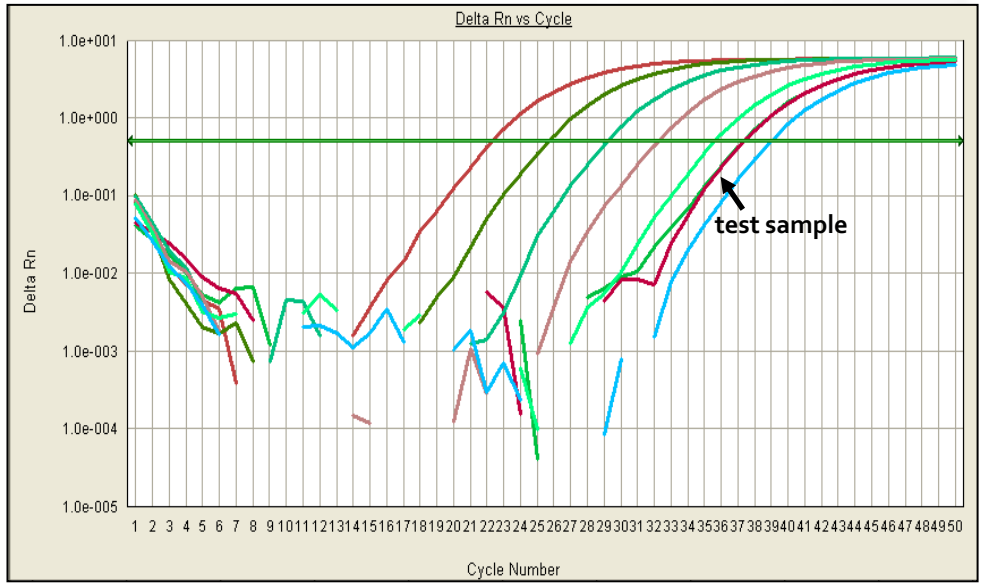
Schoumans J et al, 2016

# Polymerase Chain Reaction (PCR) and real-time PCR

PCR
- Amplification of region of interest using specific primers
- Cycling reaction condition
- Product of reaction serves as an input for further analyses (Sanger sequencing, fragment analysis, NGS, …)

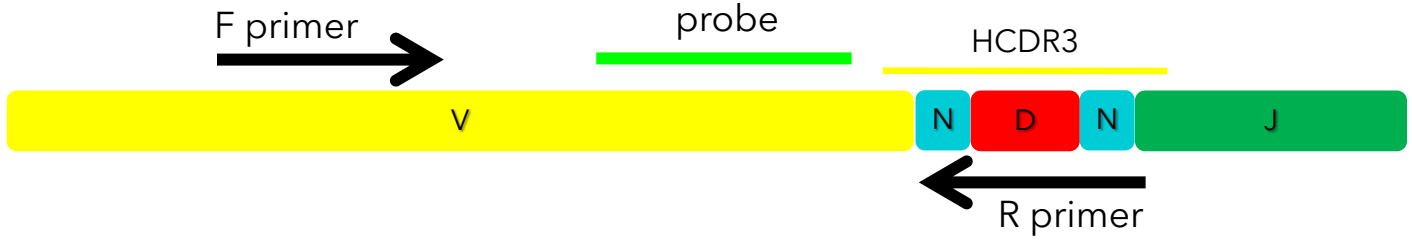
Real-time PCR
- Quantitative method – fluorescent detection of generated products
- Need for specific primers and probes
- Relative vs absolute quantification

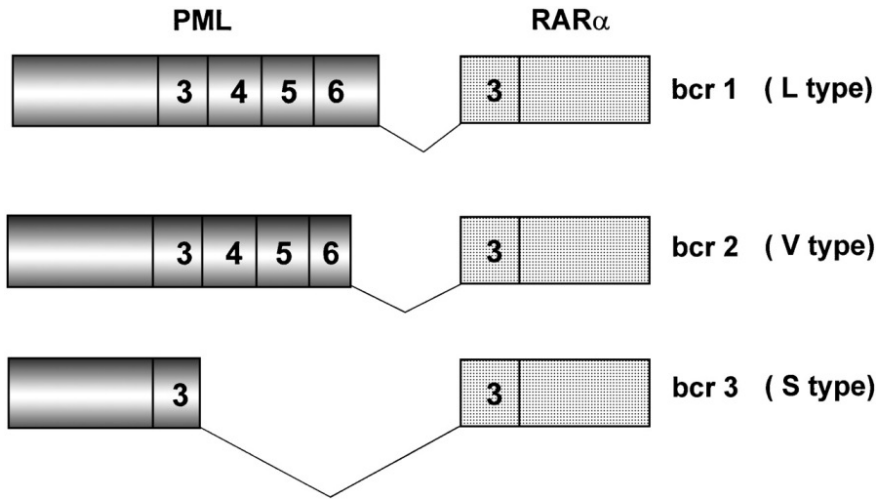
ABI 7300



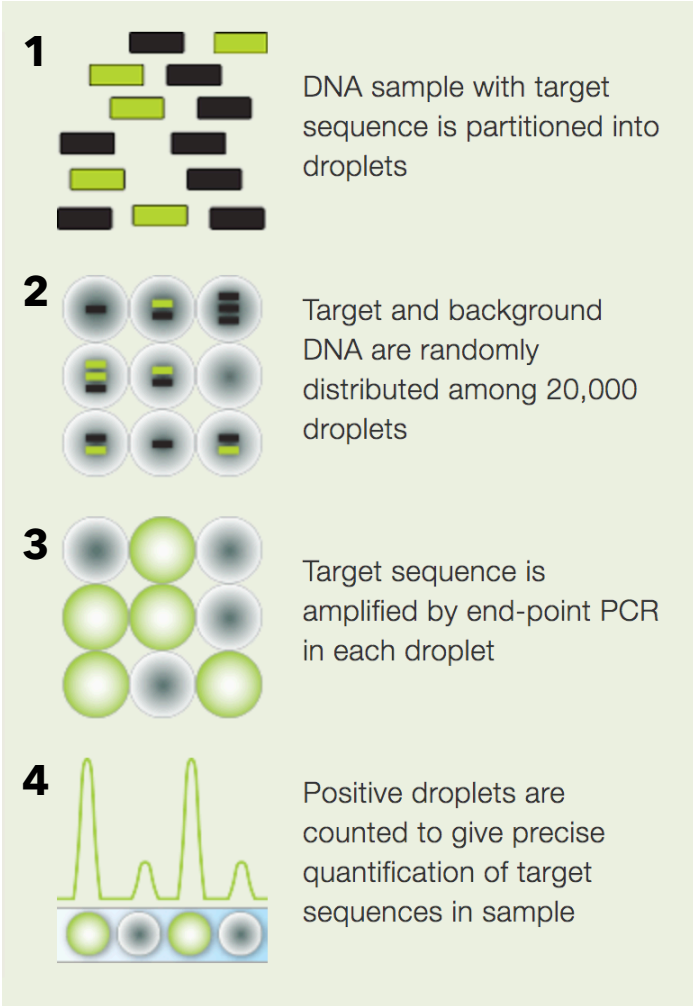
# Real-time PCR applications

- 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

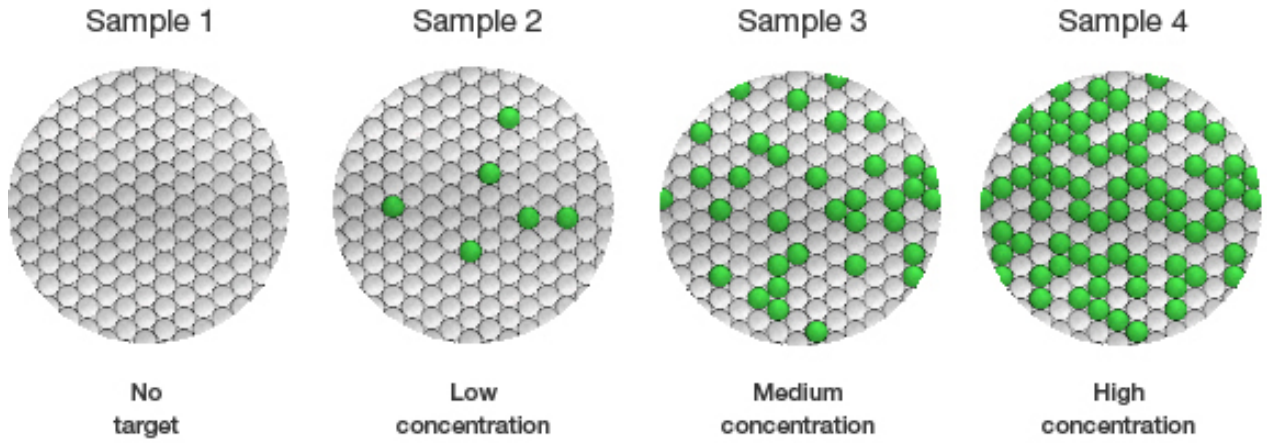


# Droplet digital PCR (ddPCR)




- Alternative method for marker absolute quantification
- Highly precise
- Need for specific instrumentation



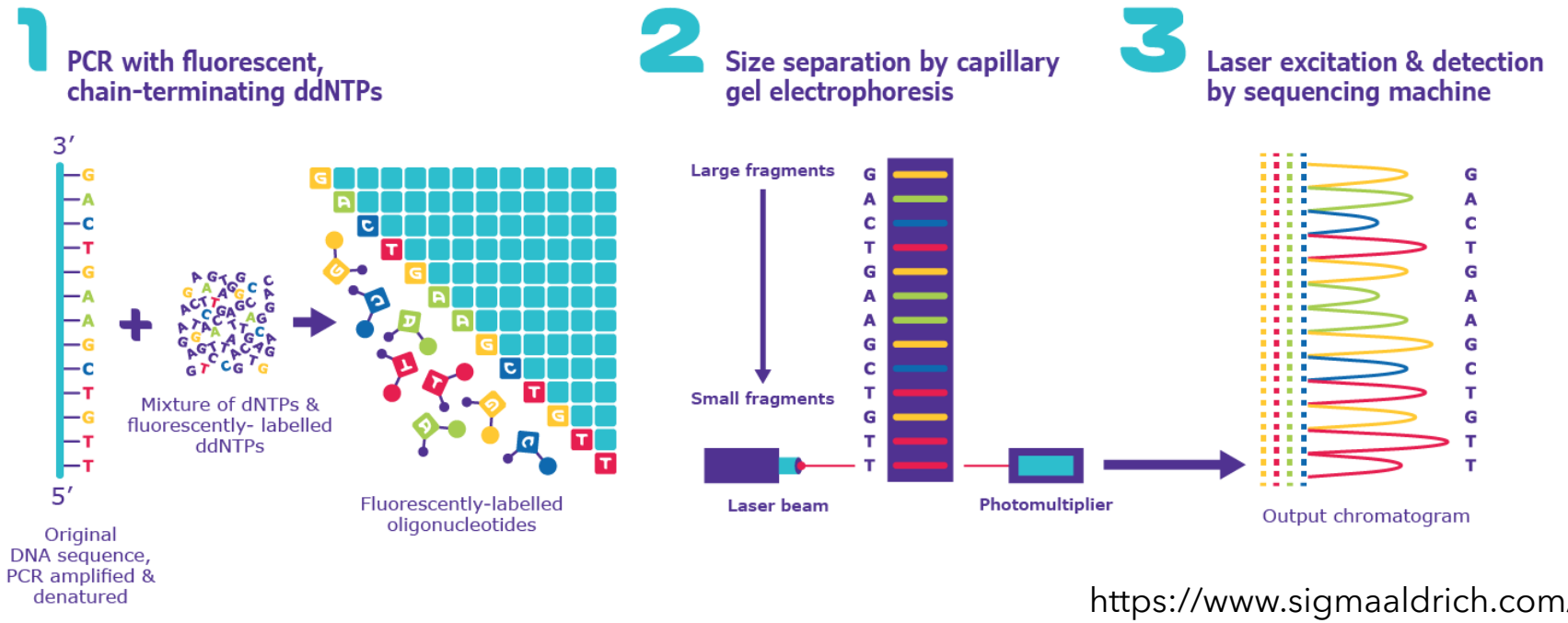
# Sanger sequencing

Modification of PCR

- single primer extension
- Incorporation of dNTPs and ddNTPs

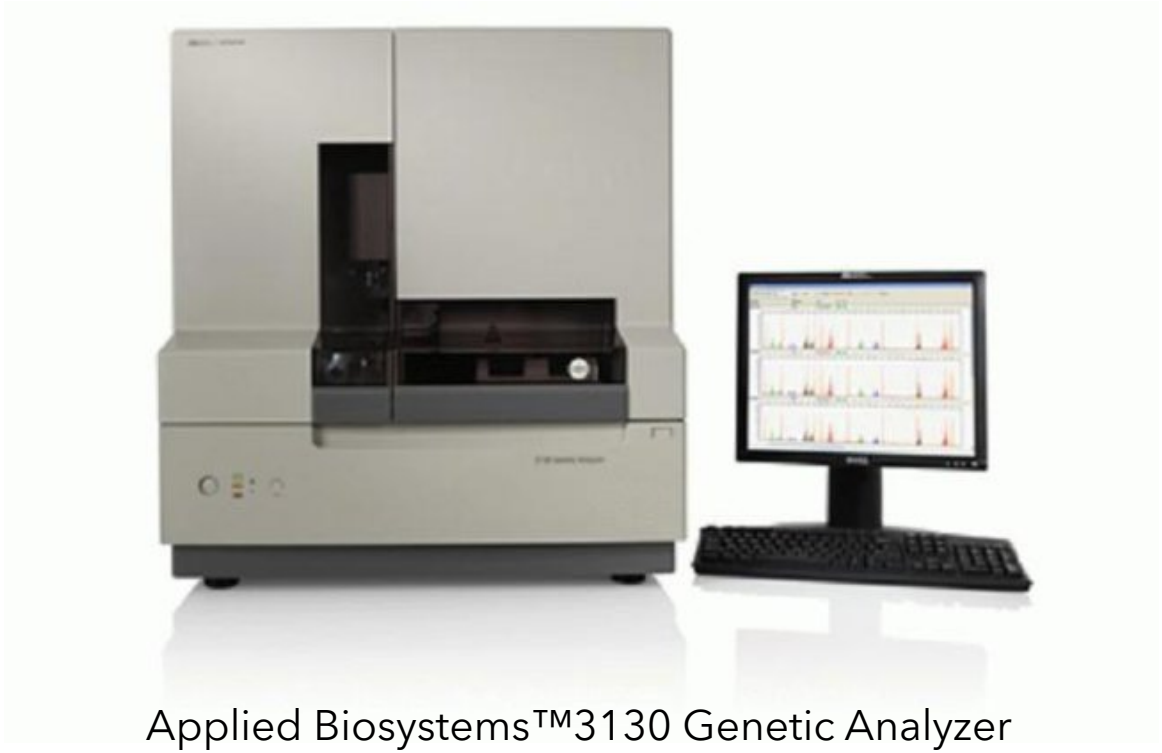
Applications

- Basic method for sequence variant detection (mutations, breakpoint localization)



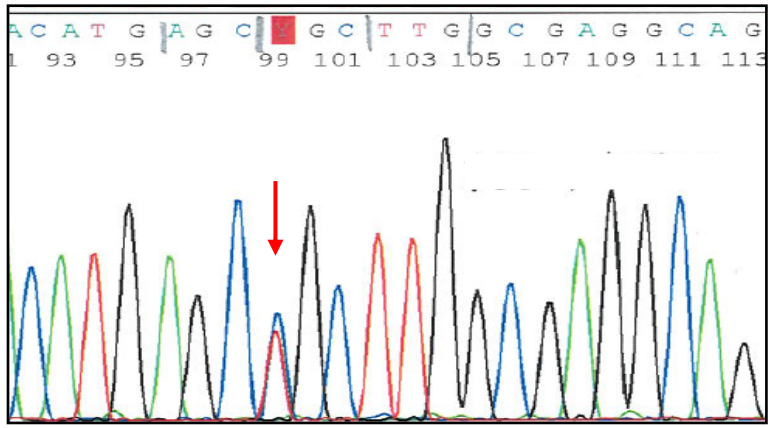

https://www.sigmaaldrich.com/technical-documents/articles/biology/sanger-sequencing.html

# Sanger sequencing

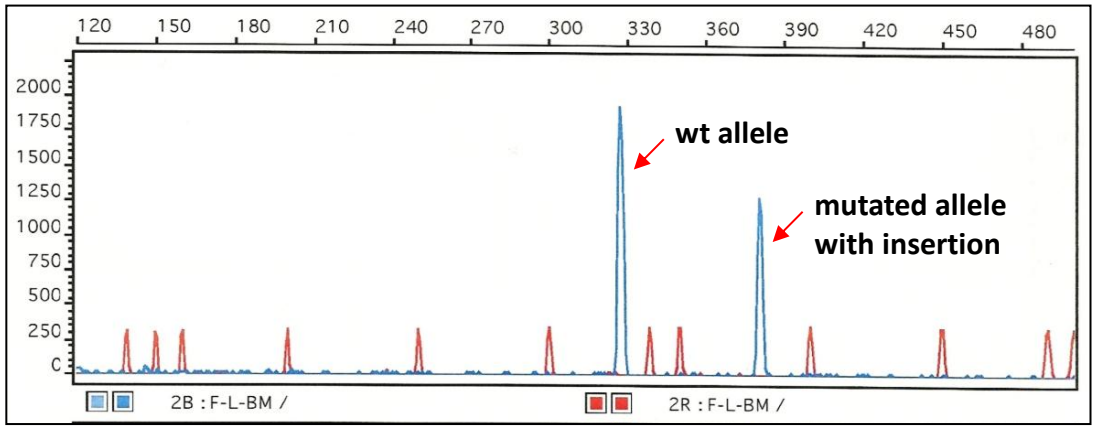


Applied Biosystems™3130 Genetic Analyzer

Sequencing analysis output



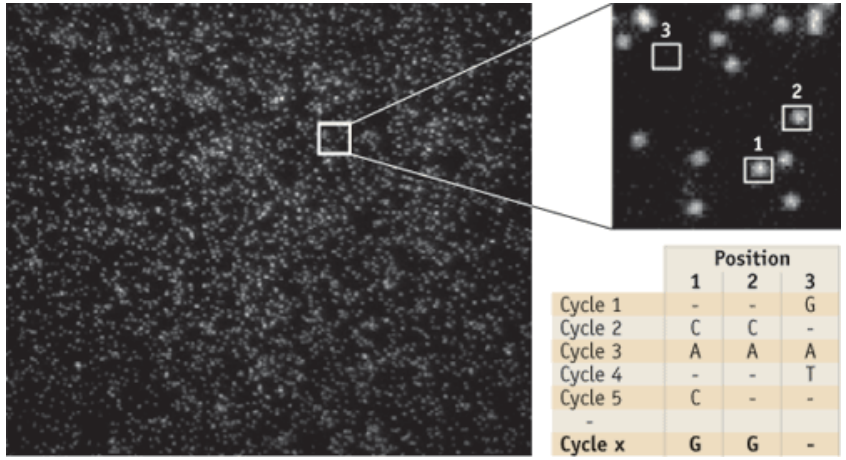

Fragment analysis – modification of the method



# 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)



# 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/rin,
1.2 GB/run

# NGS – regions of interest

genome

3 200 000 000 bp
30 x read depth

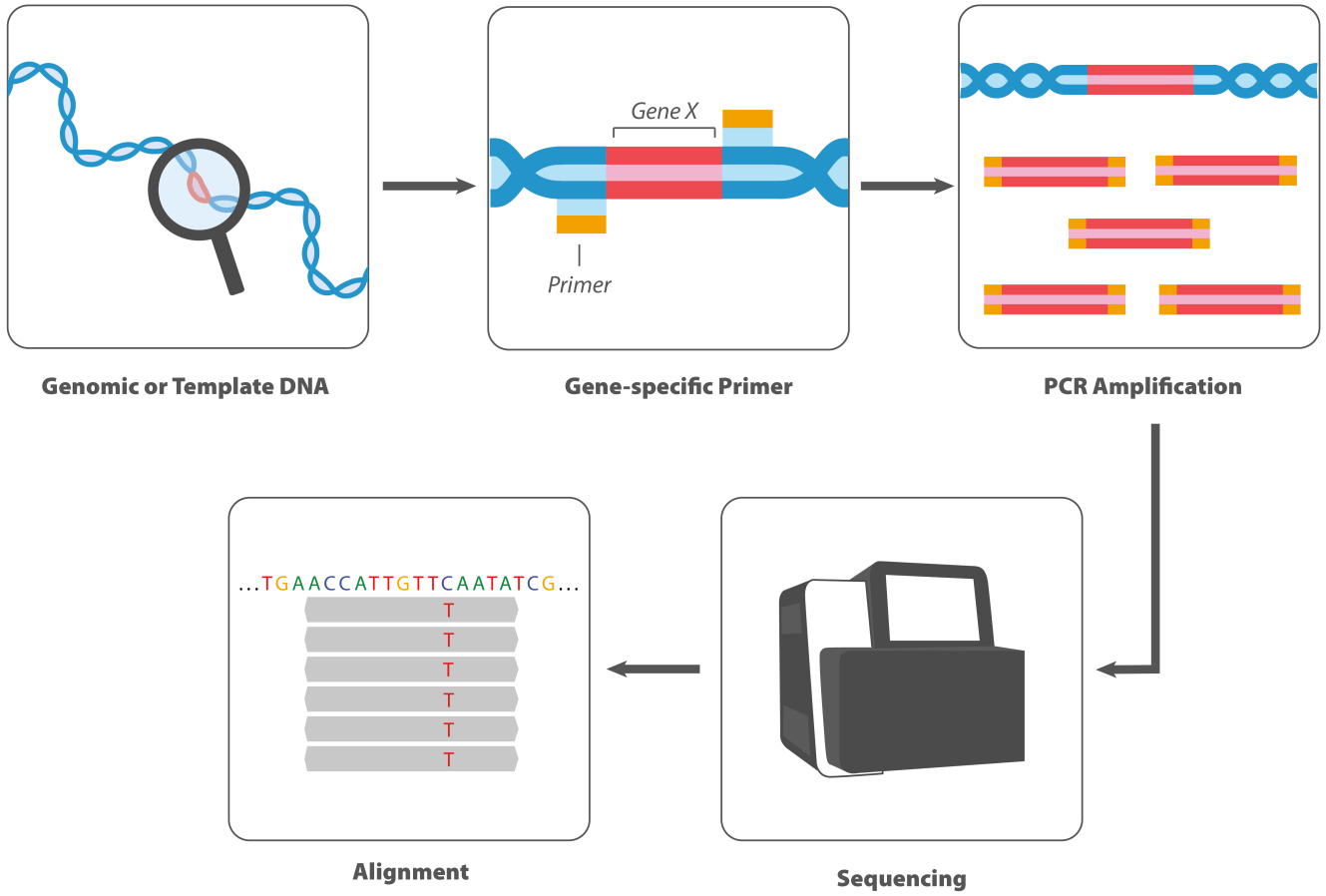
exome

20 000 genes
100 x read depth

selected genes or loci

< 100 genes
≥ 1000 x read depth

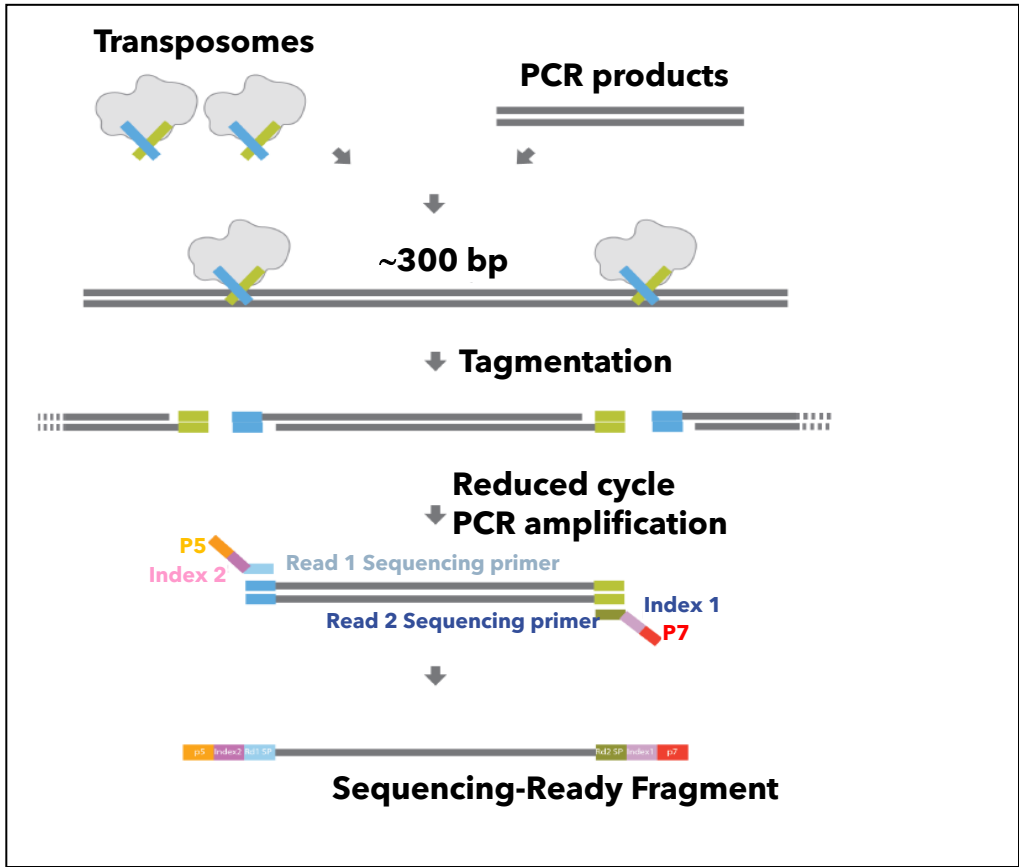
# Amplicon sequencing




https://www.abmgood.com/Amplicon-Sequencing-Service.html

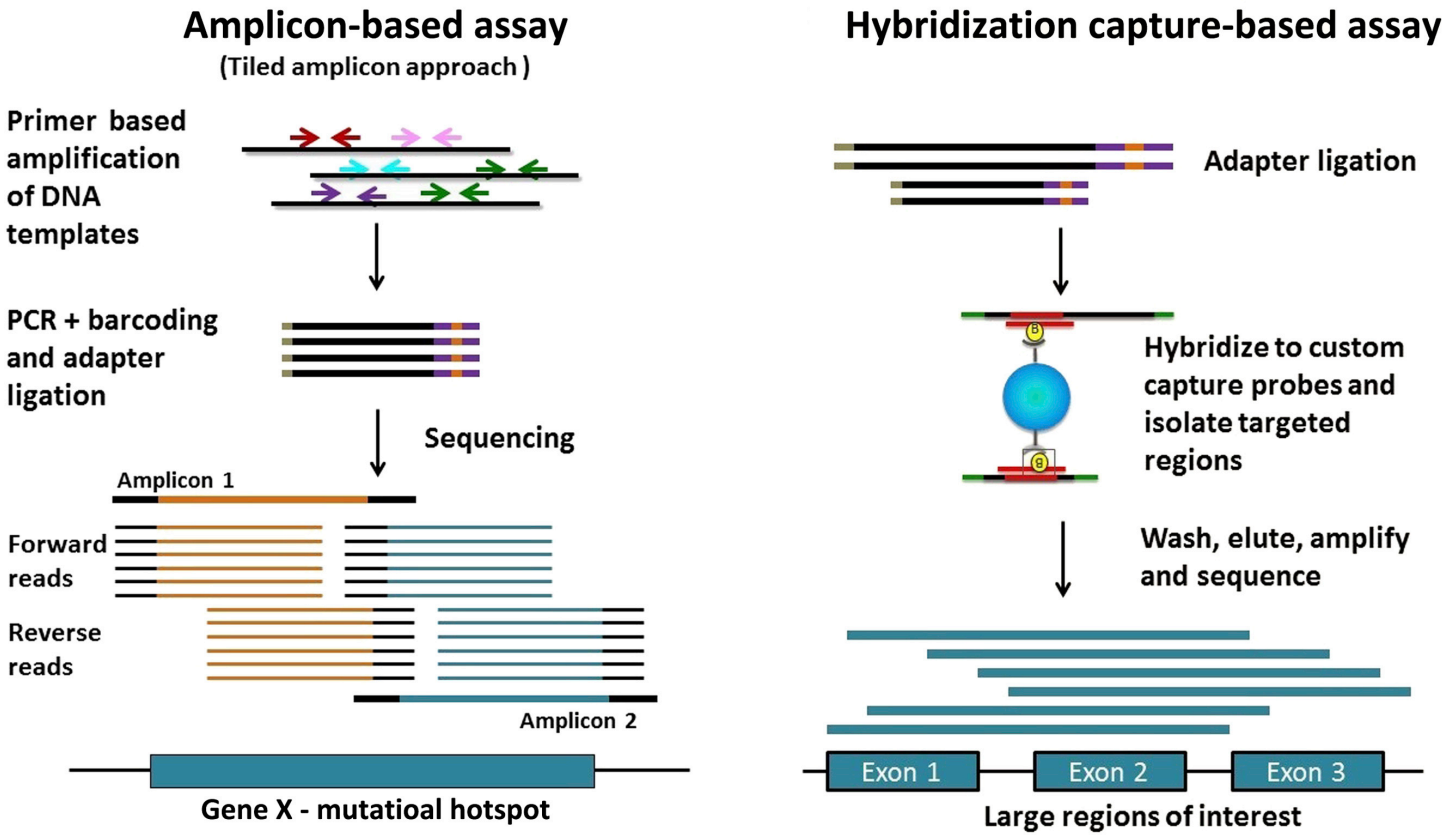
## Application: TP53 mutation analysis

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



# Panel sequencing

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

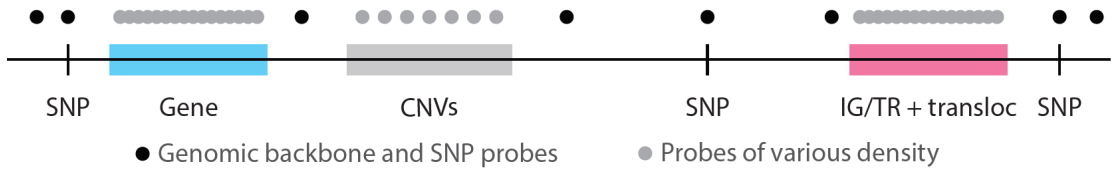



Jennings LJ et al. J Mol Diagn. 2017

# Panel sequencing

- LYNX panel – diagnostics of molecular markers in lymphoid malignancies

([1]CLL, [2]MCL, [3]FL, [4]DLBCL, [5]ALL, [6]Ph-like ALL)




## List of genes

| | | | | |
|---|---|---|---|---|
| ARID1A[1,3] | ASXL[1,5] | ATM[1,2] | BIRC3[1,2] | BRAF[1,3-5] |
| BTG1[6] | CARD11[1-4] | CCND1[2] | CD79A[1,4] | CD79B[1,2,4] |
| CDKN2A[1-5] | CDKN2B[3-5] | CHD2[1] | CREBBP[1,3-5] | CRLF2[5] |
| CSF2RA[6] | EBF1[6] | EGR2[1] | EP300[1,3,4] | EPOR[6] |
| ETV6[5] | EZH2[3-5] | FBXW7[1] | FIGNL1[6] | FLT3[5] |
| FOXO1[3] | HIST1H1E[1] | IKZF1[5] | IKZF2[6] | IKZF3[1,6] |
| IL2RB[6] | IL3RA[6] | IL7R[5] | JAK1[1,5] | JAK2[1,5] |
| JAK3[5] | KRAS[1,5] | MEF2B[2-4] | MGA[1] | KMT2A[1,5] |
| KMT2D[1-4] | MYC[3,5] | MYD88[1-4] | NF1[1,5] | NFKBIE[1] |
| NOTCH1[1-4] | NOTCH2[2,4] | NRAS[1,5] | P2RY8[6] | PAG1[5] |
| PAX5[1,5] | PIM1[1,4] | PTEN[3-5] | PTPN11[1,5] | POT1[1] |
| RB1[1,5] | RPS15[1] | RUNX1[5] | SAMHD1[1] | SETD2[1,5] |
| SF3B1[1,2] | SH2B3[6] | SHOX[6] | TNFRSF14[3,4] | TP53[1-5] |
| TYK2[6] | UBR5[2] | WHSC1[2] | XPO1[1] | ZMYM3[1] |

exon-proximal probes | 3´UTR region included | introns included

### Rearrangements[1-5]

| | |
|---|---|
| IGH@ | 79 subgenes |
| IGK@ | 45 subgenes |
| IGL@ | 42 subgenes |
| TRA@ | 96 subgenes |
| TRB@ | 64 subgenes |
| TRG@ | 13 subgenes |
| TRD@ | 11 subgenes |

### Translocations[2-4]

| | |
|---|---|
| CCND1/IGH | t(11;14) |
| BCL2/IGH | t(14;18) |
| BCL6/IGH | t(3;14) |

### CNVs[1-6]

> 6 MB across whole genome

### Reccurent deletions[1,2]

> 300 kb/1 Mb

Del17p
Del11q
Del13q

### Trisomy[1,2]

Tri12

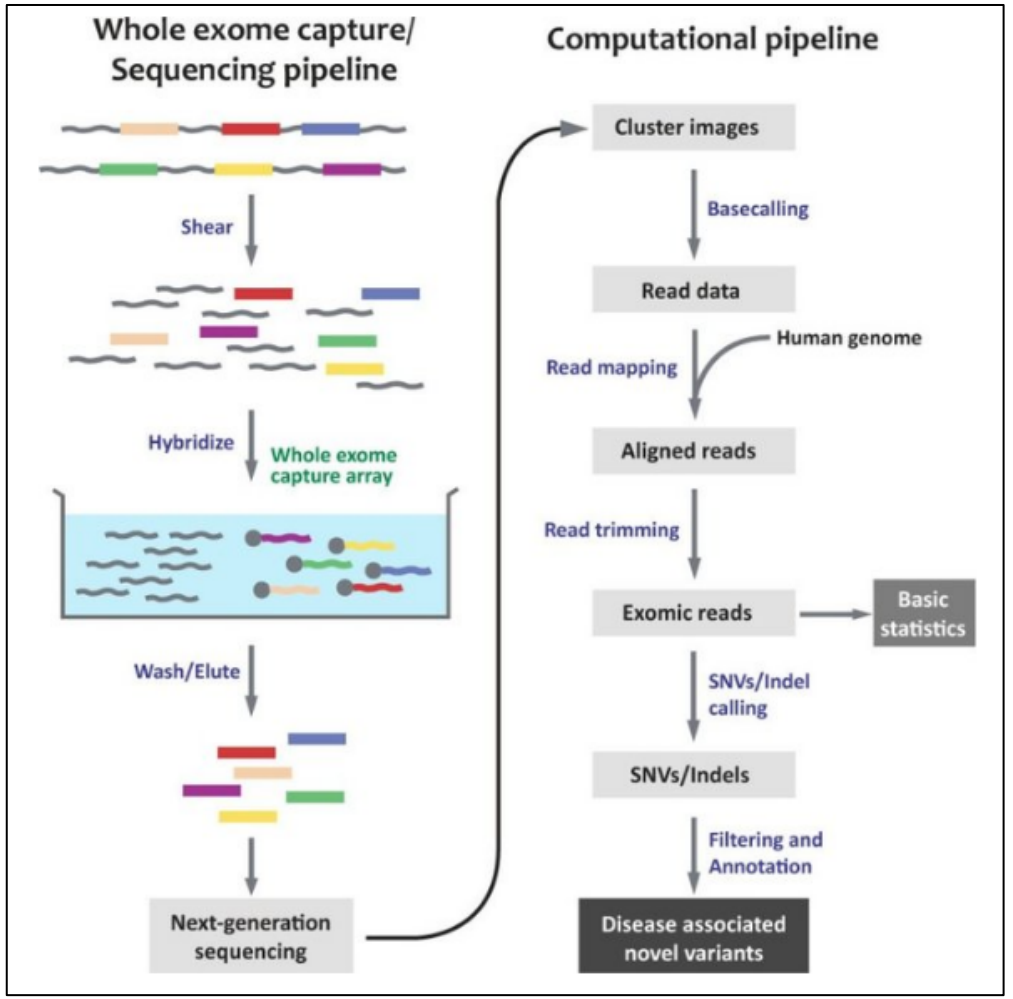
### cnLOH[1-6]

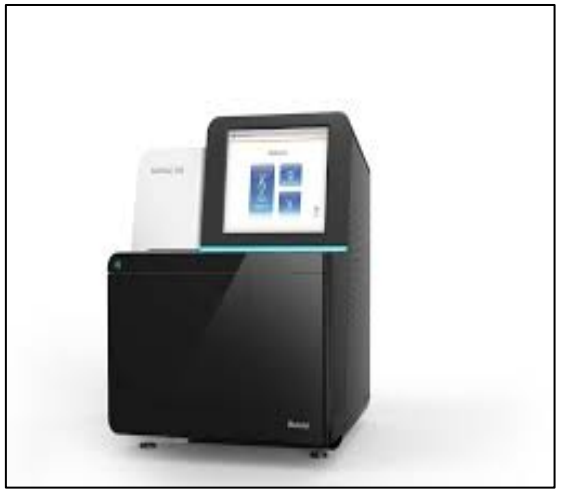
A A

according to SNP probe density

# Whole exome sequencing (WES)

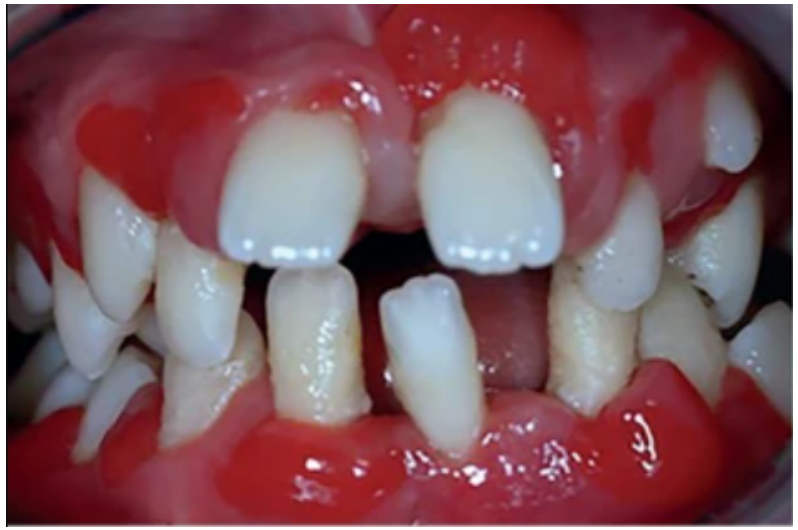
- Mainly experimental approach for exploring unknown variants
- Used in
  - genetic counseling for identification of causative variants
  - discovery of novel genetic markers
  - searching for treatment targets

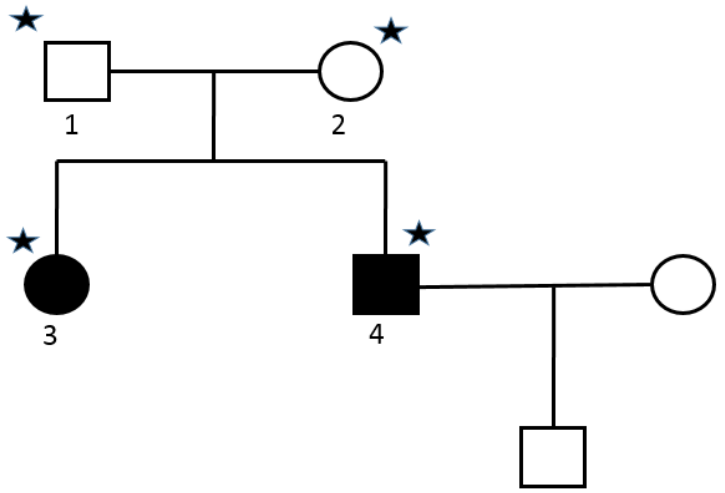


# WES – case report of Shwachman-Diamond syndrome

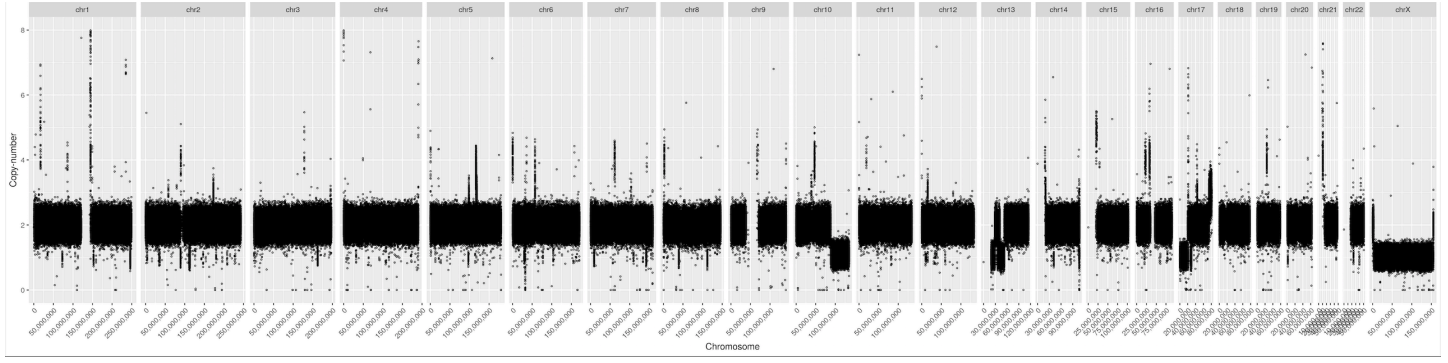
- a multisystem autosomal recessive disorder
- clinical features: pancreatic exocrine insufficiency, hematologic dysfunction, and skeletal abnormalities
- haematological malignancies (e.g. myelodysplastic syndrome and acute myeloid leukemia) occur in one third of patients
- homozygous or compound heterozygous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

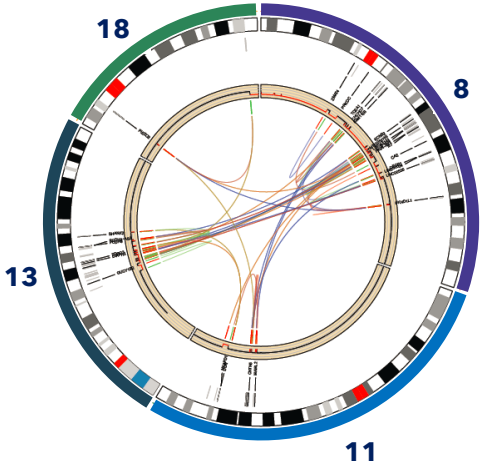




# Whole genome sequencing (WGS)

- 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…

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CEITEC MU A35
Internal Medicine – Hematology and Oncology, University Hospital Brno

Thank you for your attention!