MUNI
MED

# Molecular genetics and cytogenetics laboratory and methods

KARLA PLEVOVÁ

27.9.2022

# Statement

This presentation is intended exclusively for educational purposes.

Every form of misuse, including copying, distribution and sharing on public online platforms or social media is strictly prohibited and can be punished.

# Outline of the presentation

What to expect from a molecular genetics and cytogenetics laboratory (MGC lab)?

How does it look in the MGC lab?

What methods are available in the MGC lab?

# What to expect from a MGC lab?

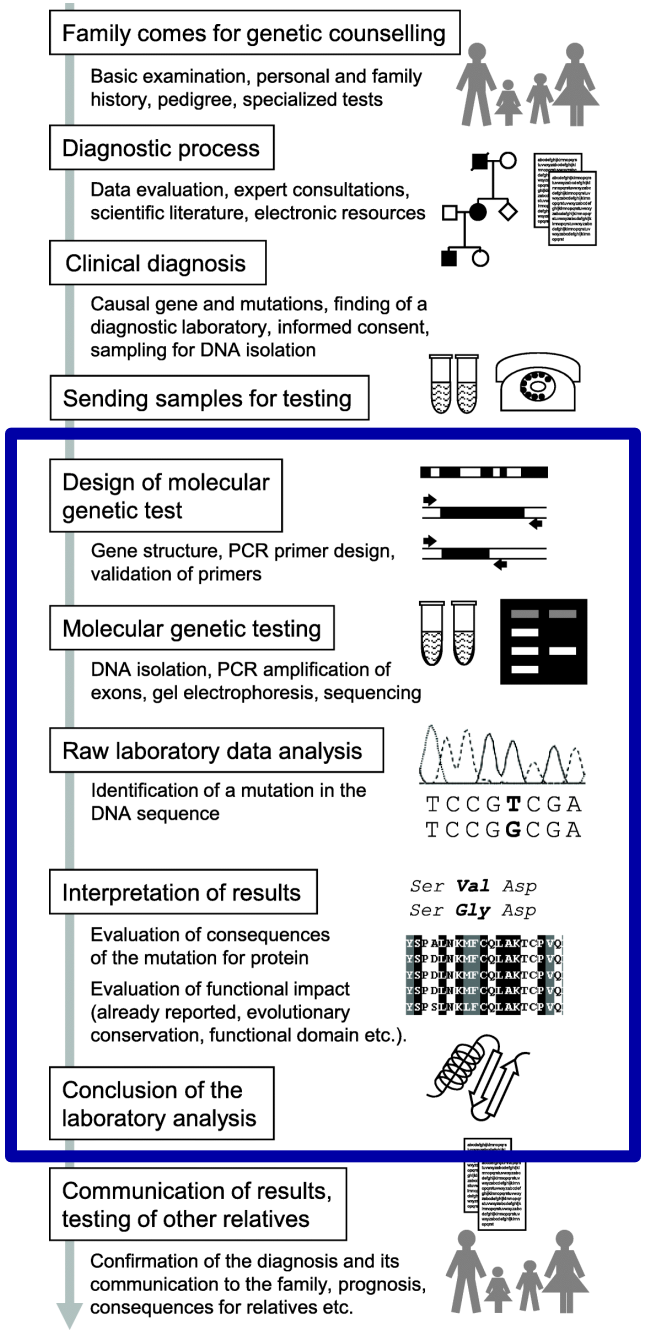
## MGC LAB = PARTNER

Specifics of a MG lab – a need for assays designed for individual families or individual patients
→ a high proportion of laboratory developed tests compared to other diagnostic labs in hospitals

Discuss with the staff, learn what methods they use, know what the methods can be good for

# Application of results

Establishing and refining diagnosis
Prenatal and preimplantation testing
Hereditary predisposition assessment
Disease prognostication
Treatment optimization
Disease activity monitoring
Disease complication diagnostics




Prochazkova et al, BMC Medical Education 2019

# Technical aspects of the laboratory methods

Target regions, analytes
Specificity and sensitivity, limit of detection, …
Tools for data analysis and their limitations
Time for processing – few hours or few days?
Standardization and validation
Regular quality assessment
Compliance with legislation regulations

→ a basis for laboratory test request

→ expectations and outcomes

Laboratory manual!

# Result reporting

The report is an essential part of any laboratory test

Content – concise but comprehensive:

- What exactly was tested
- What method was used
- What results were obtained
- Who reports the results
- Who reviewed the results
- What preanalytic, analytic, and postanalytic factors could influence the results
- What cannot be identified (e.g. failed or low covered regions)

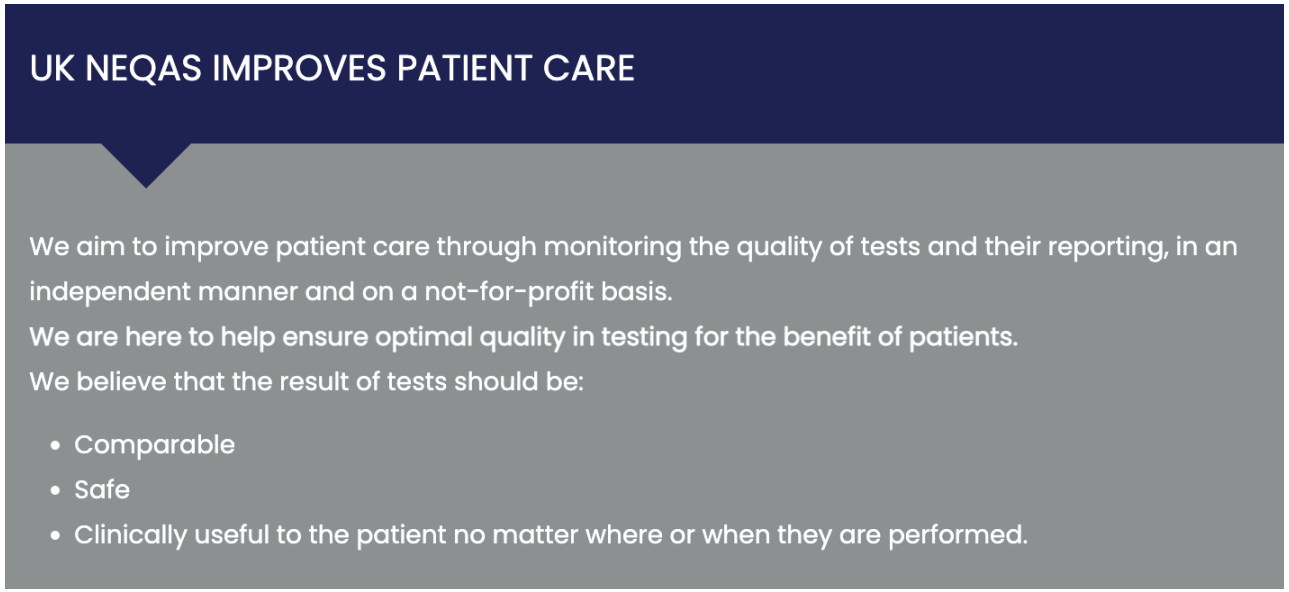

Comply with ISO 15189 technical requirements for medical laboratories

# Quality control assessment

Internal QC

External QC

- national
- international


https://ukneqas.org.uk/about-us/

Needed for method (and laboratory) accreditation

Compliance with international regulations for *in vitro* diagnostic methods

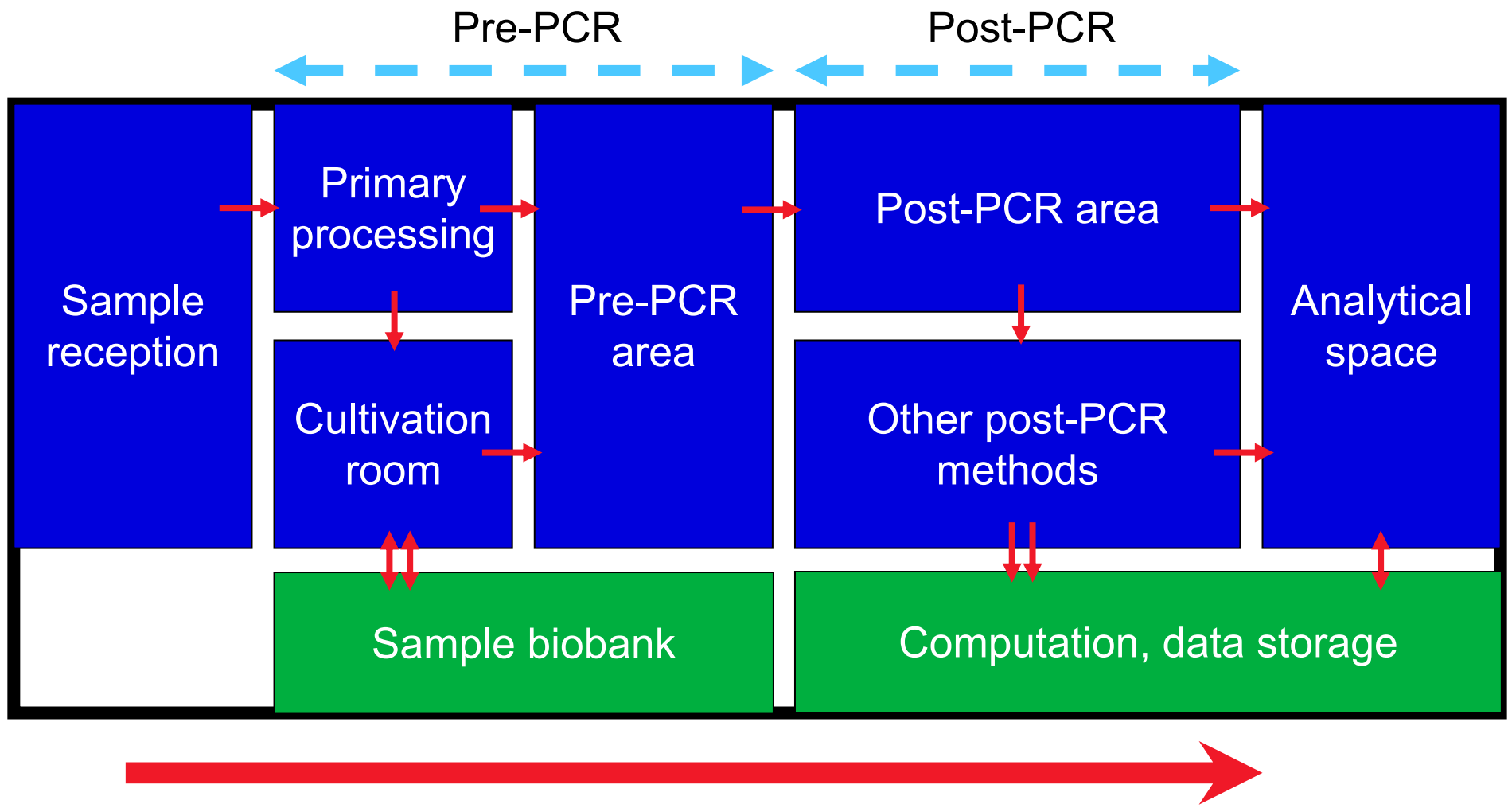
# Practical example

## BRONCO diagnostic panel for hereditary cancer syndromes

- What genetic syndrome am I looking for?
- Are suspect genes included in the panel?
- What defects could I expect?
- How quickly do I need the result?
- …
- Is the method suitable for answering my questions?

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ACD | BRCA1 | CHD2 | ELANE | FANCE | IKZF1 | MDM4 | NF2 | POT1 | RB1 | SLC37A4 | UBE2V2 |
| AIP | BRCA2 | CHEK1 | EPCAM | FANCF | IKZF2 | MED12 | NFKBIE | PPM1D | RBBP8 | SLX4 | UBE4B |
| ALK | BRCC3 | CHEK2 | EPHX1 | FANCG | IKZF3 | MEN1 | NFKBIZ | PREX2 | RECQL | SMAD4 | UIMC1 |
| ANKRD26 | BRCC3 | chr9:21512 | EPOR | FANCI | IL7R | MET | NHEJ1 | PRF1 | RECQL4 | SMARCA4 | USB1 |
| AP3B1 | BRE | CLSPN | ERCC1 | FANCL | IRF4 | MGA | NOTCH1 | PRKAR1A | RECQL5 | SMARCB1 | VHL |
| APC | BRIP1 | CRLF2 | ERCC2 | FANCM | ITPKB | MGA | NPM1 | PRKDC | RET | SMARCE1 | VPS13B |
| APEX1 | BTG1 | CSF3R | ERCC3 | FBXW7 | JAK1 | MGMT | NRAS | PTCH1 | RFC1 | SRP72 | VPS45 |
| ASXL1 | BTK | CSNK1D | ERCC4 | FH | JAK2 | MLH1 | NSD1 | PTEN | RFC2 | STK11 | WAS |
| ATG2B | BUB1B | CSNK1E | ERCC5 | FLT1 | JAK3 | MLH3 | OGG1 | PTTG2 | RFC4 | SUFU | WIPF1 |
| ATM | C11ORF30 | CWF19L2 | ERCC6 | FLT3 | KAT5 | MMP8 | P2RY8 | RAB27A | RHBDF2 | TAZ | WRN |
| ATMIN | C19ORF40 | CXCR4 | ESR1 | G6PC3 | KCNJ5 | MPL | PALB2 | RAC2 | RIPK1 | TCL1A | WT1 |
| ATR | C3 | CYLD | ESR2 | GADD45A | KIT | MRE11A | PARP1 | RAD1 | RNF146 | TELO2 | XPA |
| ATRIP | CALR | DCLRE1C | ETV6 | GATA1 | KLHL6 | MSH2 | PAX5 | RAD17 | RNF168 | TERF2 | XPC |
| AURKA | CASP8 | DDB2 | EXO1 | GATA2 | KRAS | MSH3 | PCNA | RAD18 | RNF8 | TERT | XPO1 |
| AXIN1 | CCND1 | DDX3X | EXT1 | GFI1 | LAMTOR2 | MSH5 | PHB | RAD23B | RPA1 | TLR2 | XRCC1 |
| BABAM1 | CDC73 | DDX41 | EXT2 | GPC3 | LIG1 | MSH6 | PHOX2B | RAD50 | RPS15 | TLR4 | XRCC2 |
| BAP1 | CDH1 | DHFR | EYA2 | GRB7 | LIG3 | MSR1 | PIK3CG | RAD51 | RUNX1 | TMEM127 | XRCC3 |
| BAP1 | CDK12 | DICER1 | EZH2 | GSKIP | LIG4 | MUS81 | PLA2G2A | RAD51AP | SAMHD1 | TOPBP1 | XRCC4 |
| BARD1 | CDK4 | DMC1 | FAM175A | HAX1 | LMO1 | MUTYH | PLCG2 | RAD51B | SBDS | TP53 | XRCC5 |
| BCOR | CDKN1B | DNAJC21 | FAM175B | HELQ | LRP1B | MYD88 | PMS1 | RAD51C | SDHAF2 | TSC1 | XRCC6 |
| BIRC3 | CDKN1C | DPYD | FAN1 | HIST1H1E | LYST | NAT1 | PMS2 | RAD51D | SDHB | TSC2 | ZMYM3 |
| BLM | CDKN2A | EBF1 | FANCA | HNF1A | MAX | NBN | POLB | RAD52 | SETBP1 | TSHR | ZNF350 |
| BMPR1A | CDKN2B | EGFR | FANCB | HOXB13 | MCPH1 | NCAM1 | POLB | RAD54B | SETX | UBE2A | ZNF365 |
| BRAF | CEBPA | EGR1 | FANCC | HRAS | MDC1 | NELFB | POLD1 | RAD54L | SF3B1 | UBE2B | |
| BRAP | CEP57 | EGR2 | FANCD2 | HUS1 | MDM2 | NF1 | POLE | RAD9A | SHPRH | UBE2I | |

# How does it look the MG lab?




# Sample processing and separation

Diverse input material (peripheral blood, tissue specimens etc.)

Sterile hoods (esp. in connection with cell cultivation and biobanking of samples)

Cell separation needed in specific contexts (e.g. analysis of somatic changes

# Materials used

Peripheral blood
Bone marrow
Liquid biopsies
Aspirates
Fine-needle biopsies
Fresh tissue
Formalin-fixed paraffin-embedded (FFPE) tissue
Swabs (e.g. buccal)

Postnatal genetics
X
Prenatal testing
X
Oncology

# Peripheral blood processing

Different cell population used according to the application:

Leukocytes

Mononuclear cells

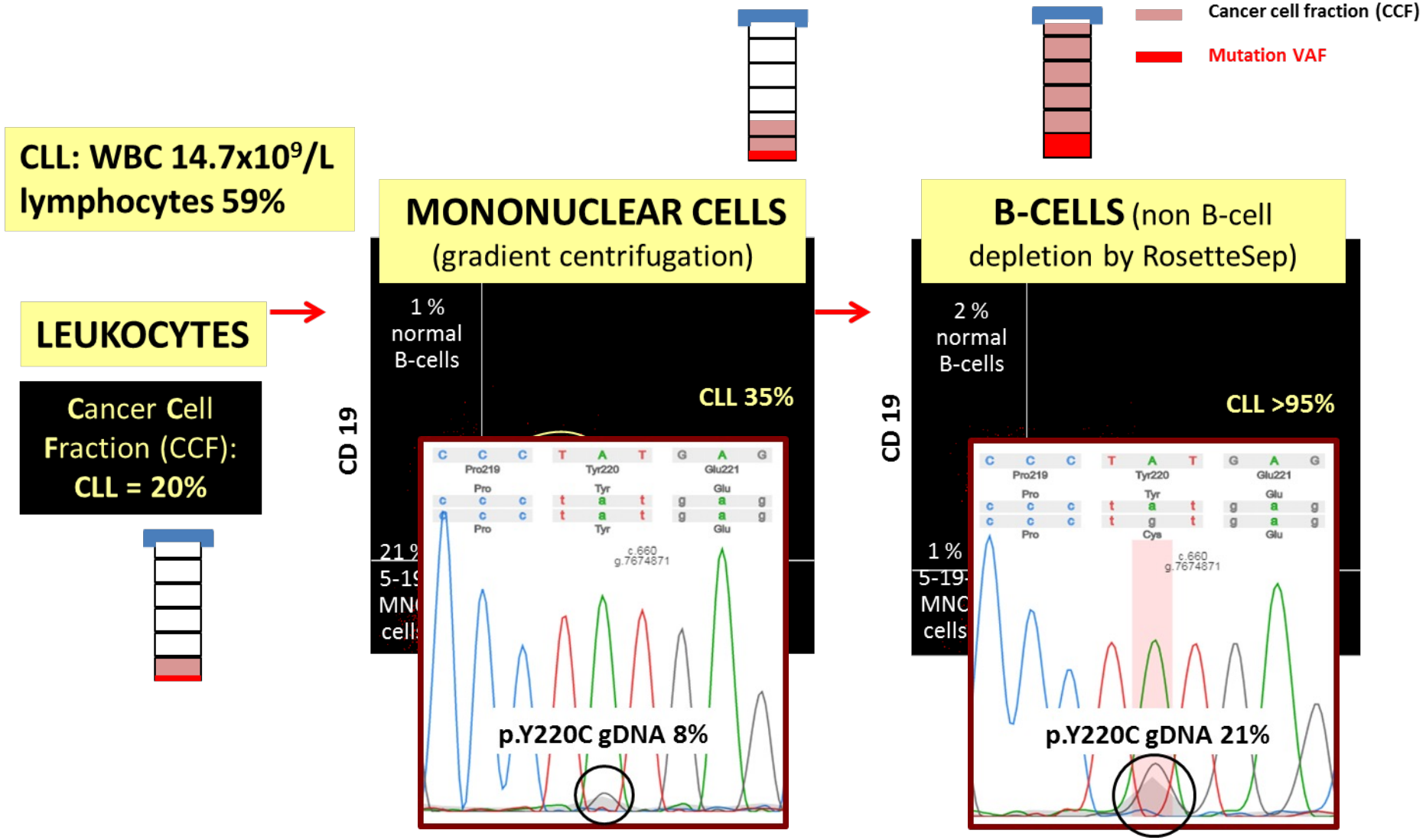
Granulocytes

Lymphocytes

Specific cell subpopulations

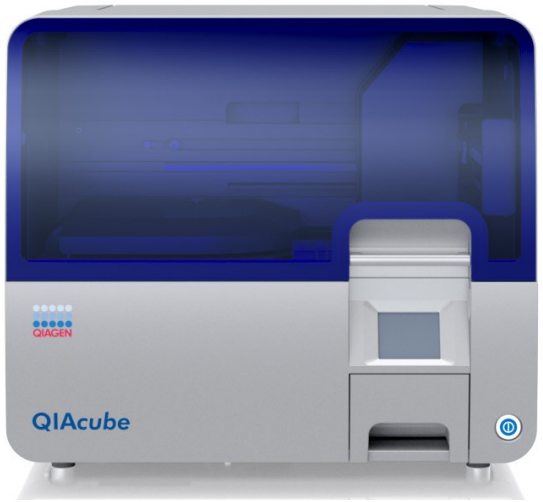
# Why to perform cell separation?

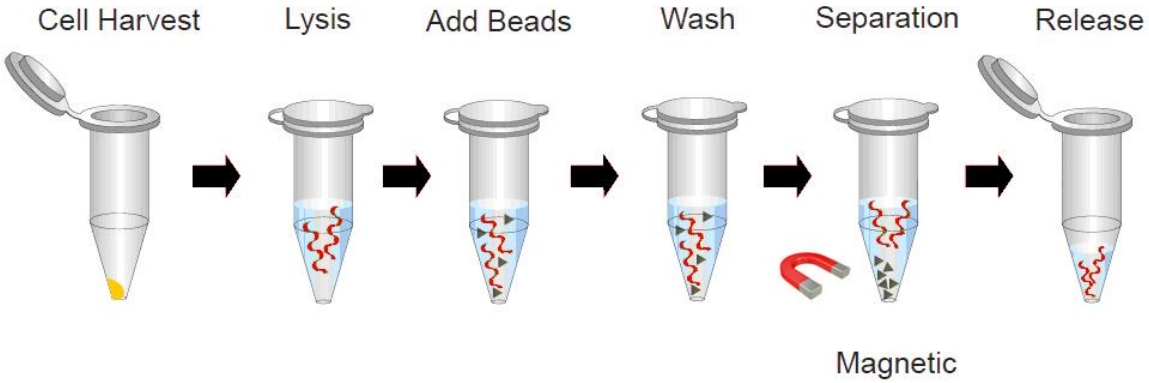
## Example of *TP53* gene testing in chronic lymphocytic leukemia

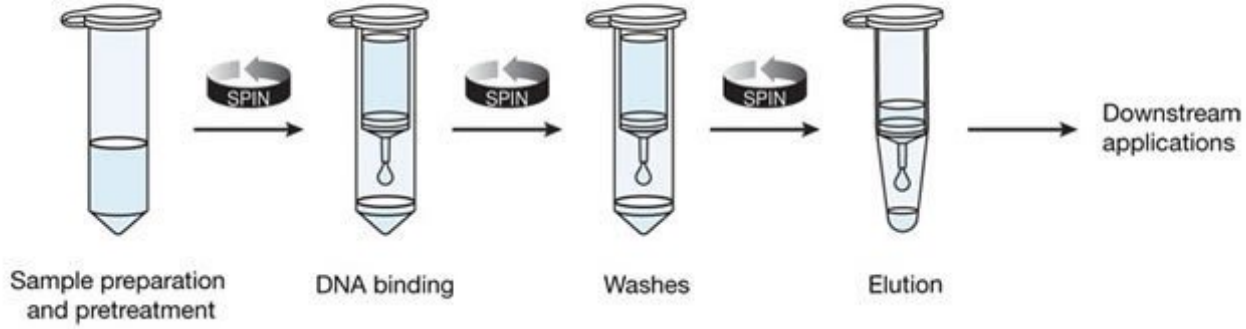


# Nucleic acid (RNA, DNA) isolation

pre-PCR area
Manual and automated sample processing
Adjusted to the purpose of the test

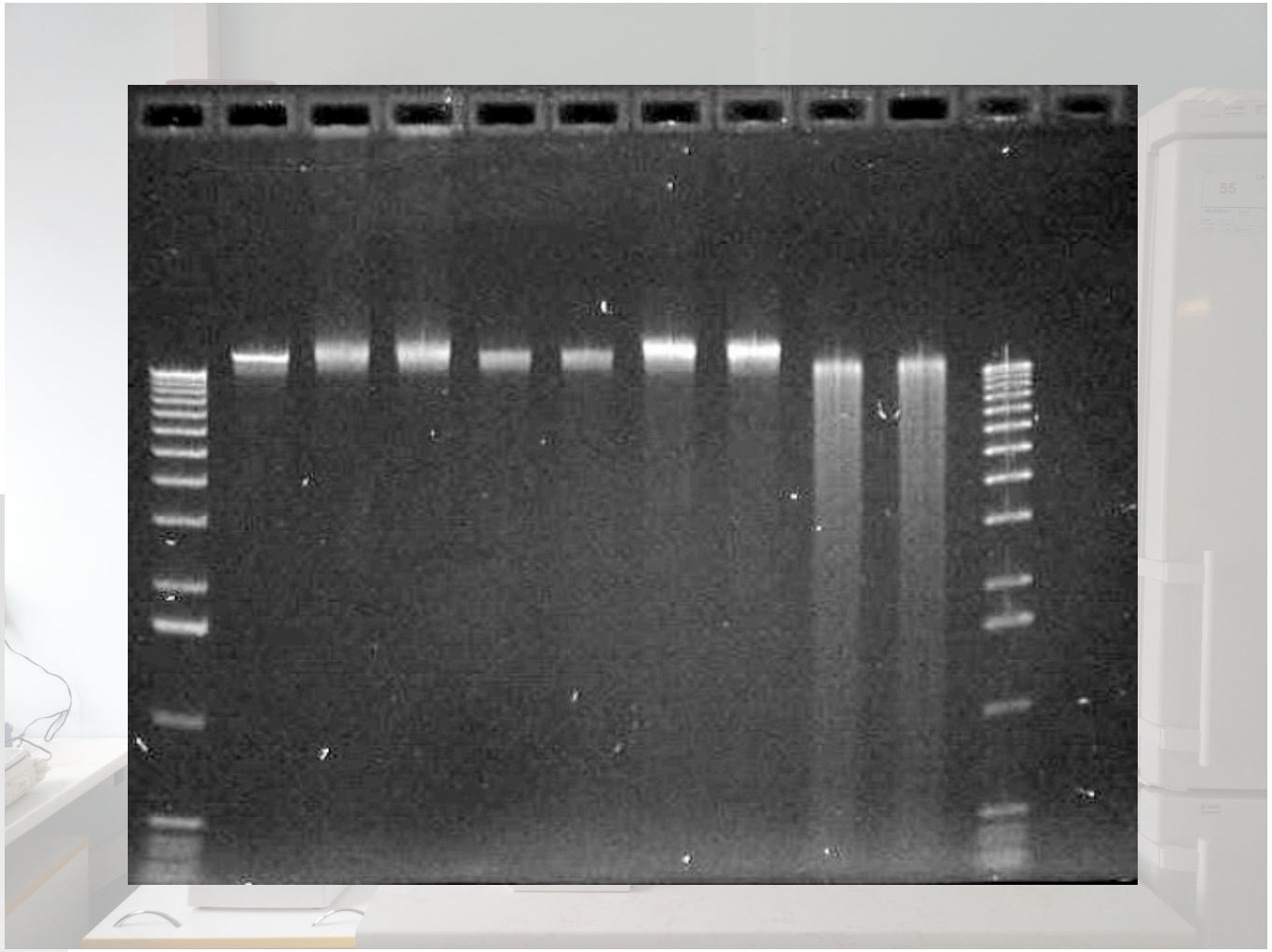



# Nucleic acid quantification and QC

Spectroscopic and fluorimetric methods
Electrophoretic methods





# Nucleic acid quality control

Alternative methods to gel electrophoresis
Lower material input



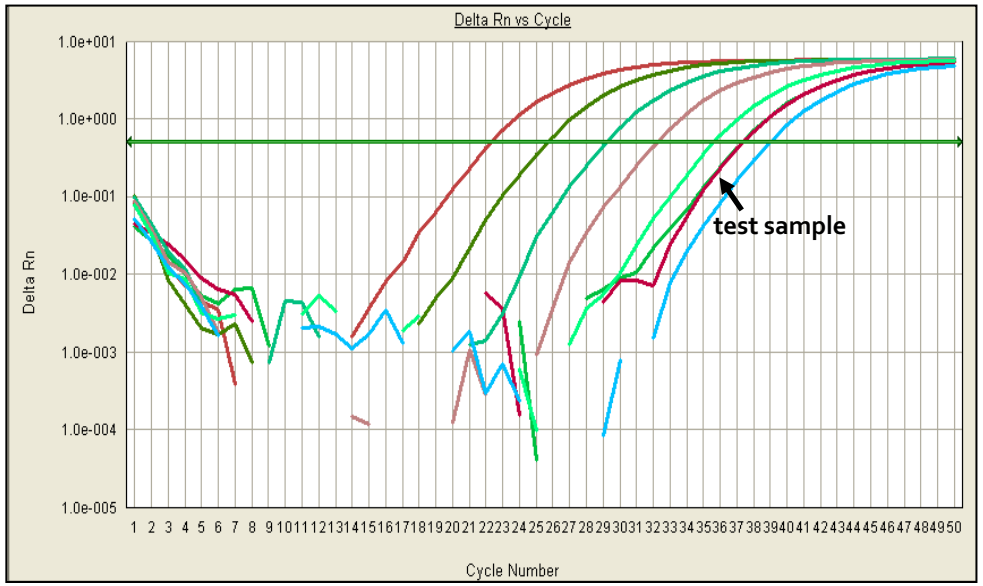


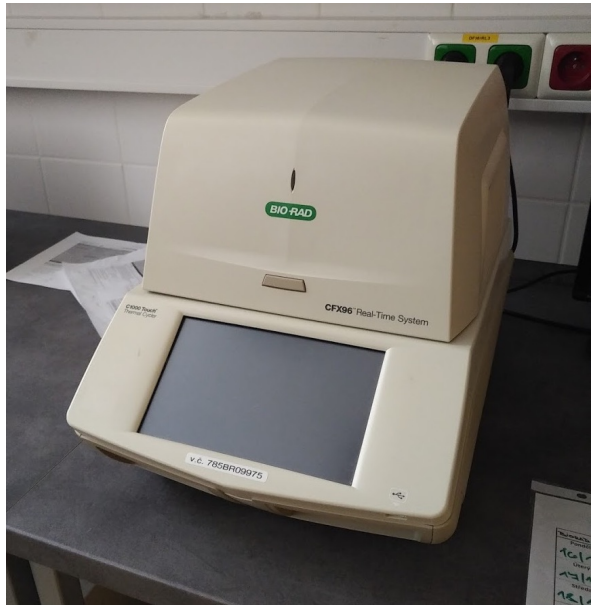

# Polymerase chain reaction (PCR)

Fundamental reaction of molecular biology and genetics
Amplification of regions of interests
PCR assembling in pre-PCR area
Carried out in thermocycler
Various modifications


# real-time PCR

Quantitative method – fluorescent detection of generated products
Need for specific primers and probes
Relative and absolute quantification



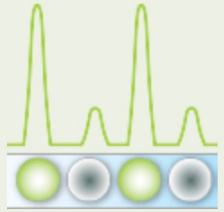




# Droplet digital PCR (ddPCR)

DNA sample with target sequence is partitioned into droplets

Target and background DNA are randomly distributed among 20,000 droplets

Target sequence is amplified by end-point PCR in each droplet

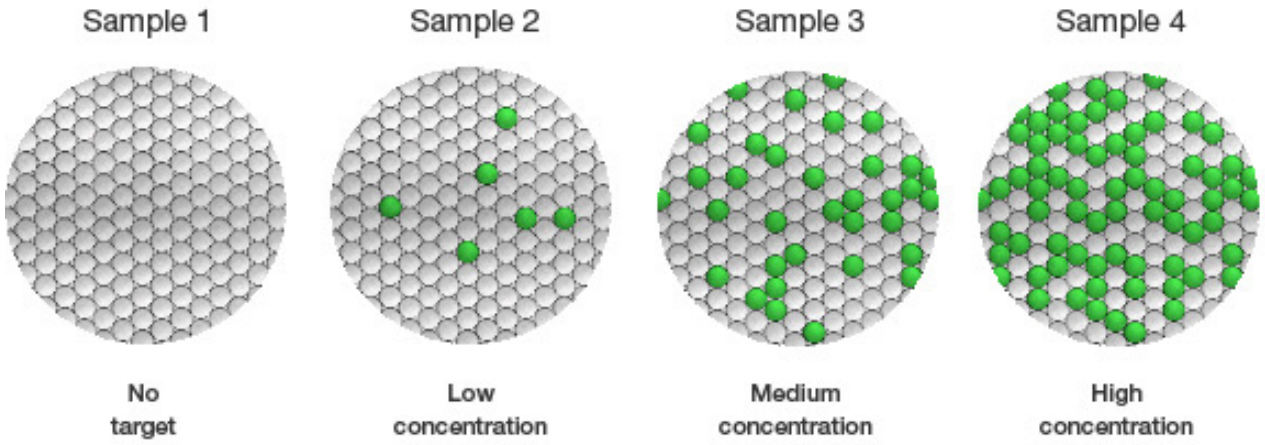


Positive droplets are counted to give precise quantification of target sequences in sample

Alternative method for marker absolute quantification

Highly precise

Need for specific instrumentation



# Next-generation sequencing (NGS)

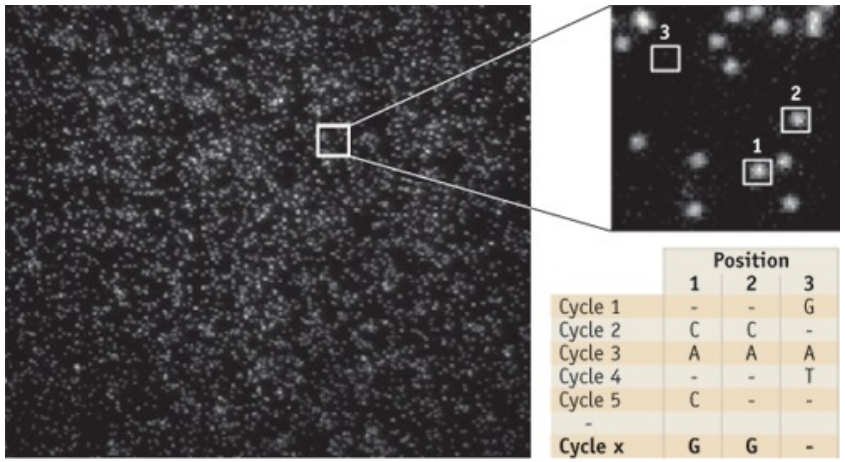
~ massively parallel sequencing (MPS)

PCR amplification of DNA fragments or direct sequencing of individual fragments (single molecule sequencing)

The most common approach – sequencing by synthesis (Illumina sequencers)

Millions of fragments are amplified simultaneously (vs capillary sequencer max 96 reactions)

Short reads (tens to hundreds base pairs)



# NGS - 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

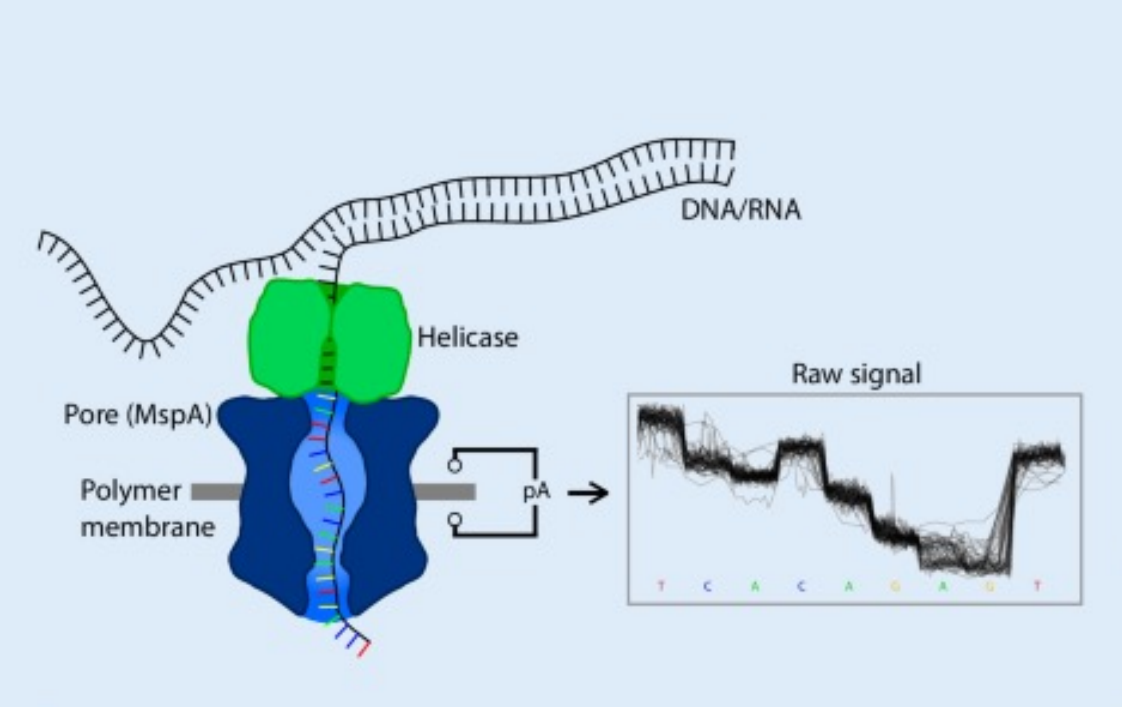
MiniSeq

0.07 genome/run,
7.5 GB/run

iSeq

0.01 genome/rin,
1.2 GB/run

# Short-read vs. long-read sequencing




Kraft & Kurth, Medizinische Genetik 2019

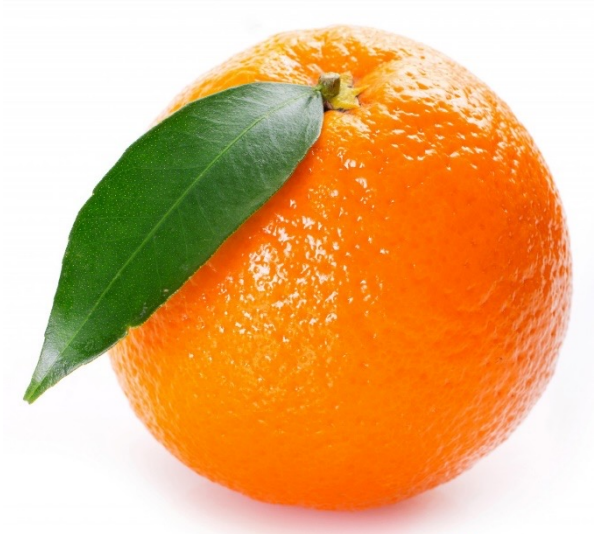
**B** Whole-genome short read data

**C** Whole-genome long read data

Schwarz et al, Medizinische Genetik 2021

# 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

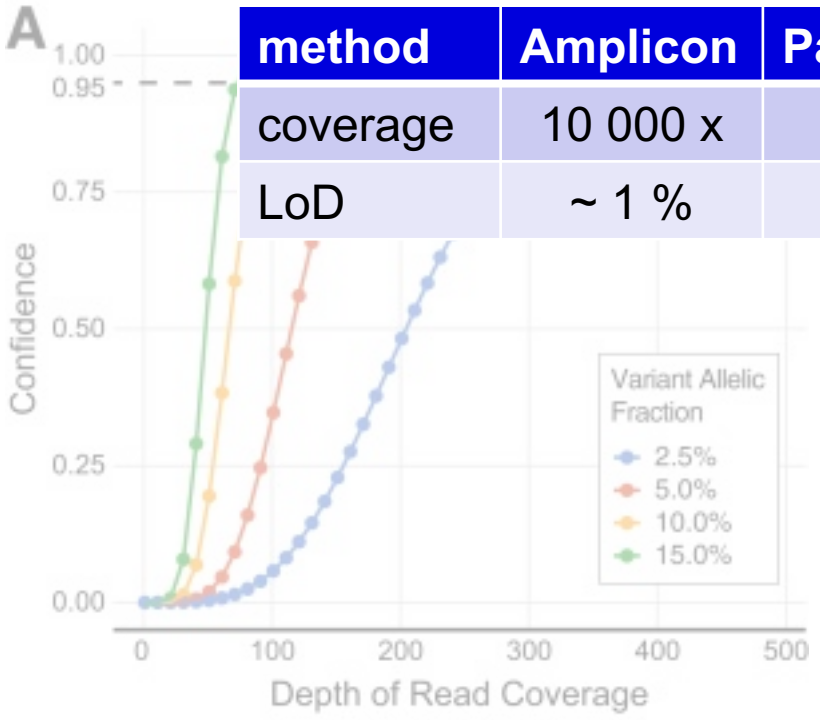
< 100 genes
≥ 1000 x read depth

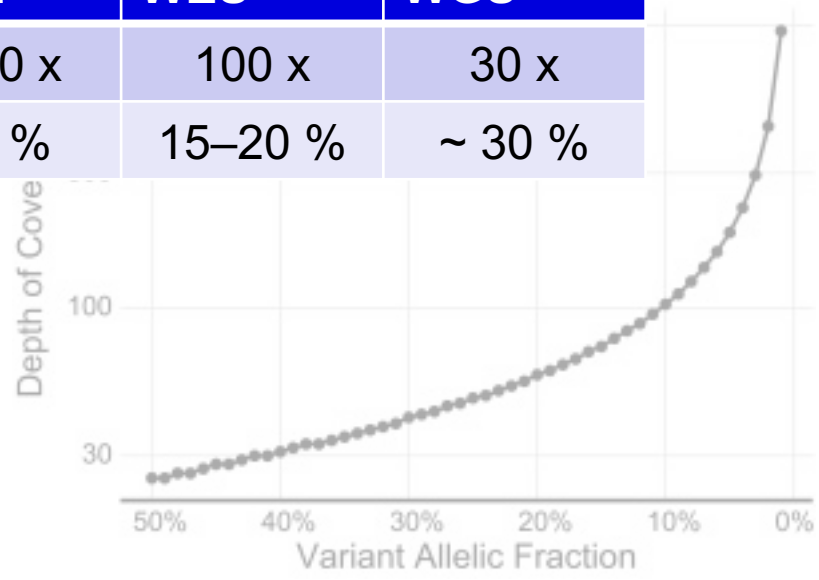
# Practical example

## Limit of detection (LoD) of various NGS methods

In theory… (binomial sampling statistics)

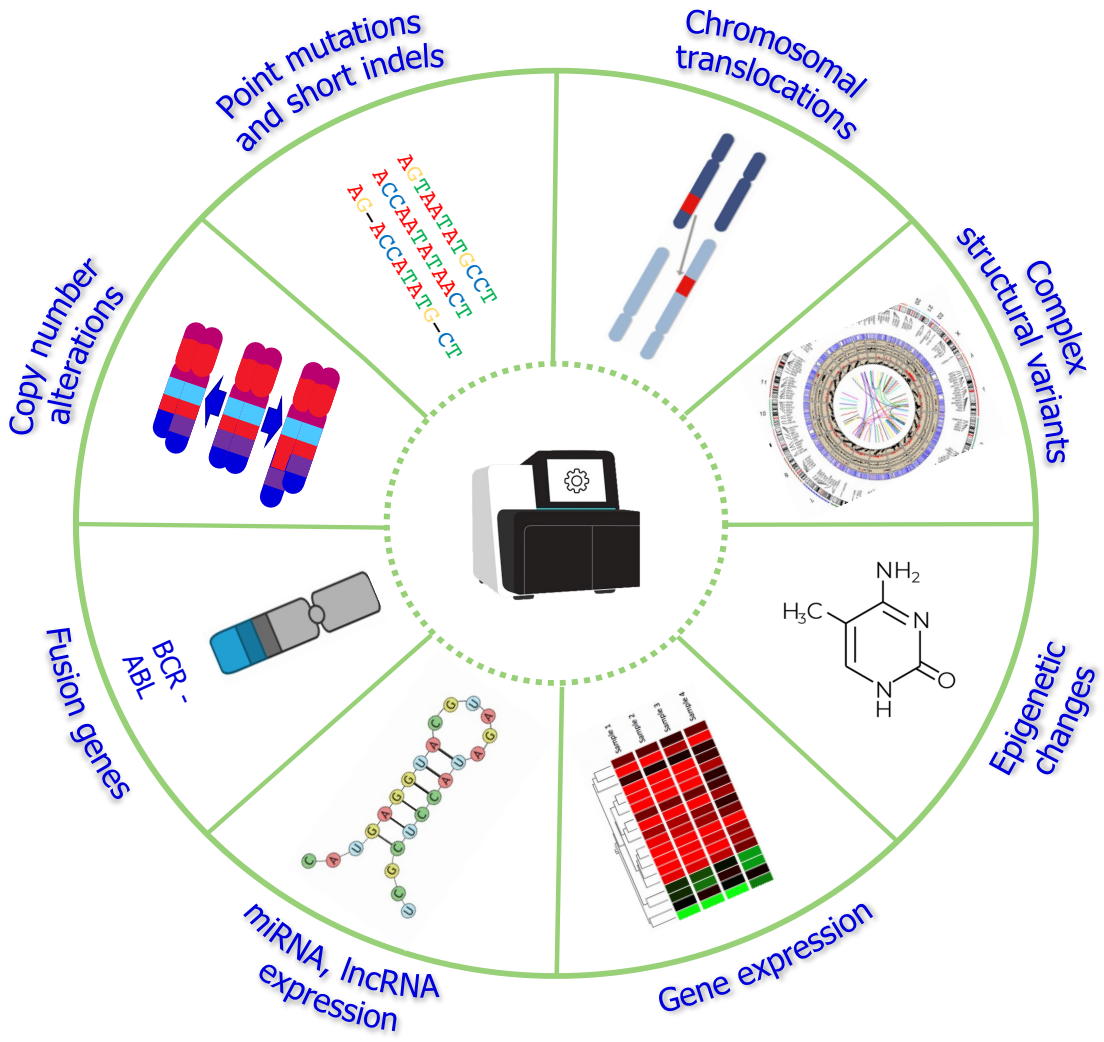
| method | Amplicon | Panel | WES | WGS |
|---|---|---|---|---|
| coverage | 10 000 x | 1000 x | 100 x | 30 x |
| LoD | ~ 1 % | ~ 5 % | 15–20 % | ~ 30 % |




McNulty et al, J Mol Diagn 2020

# NGS – diverse targeted markers



# Panel NGS

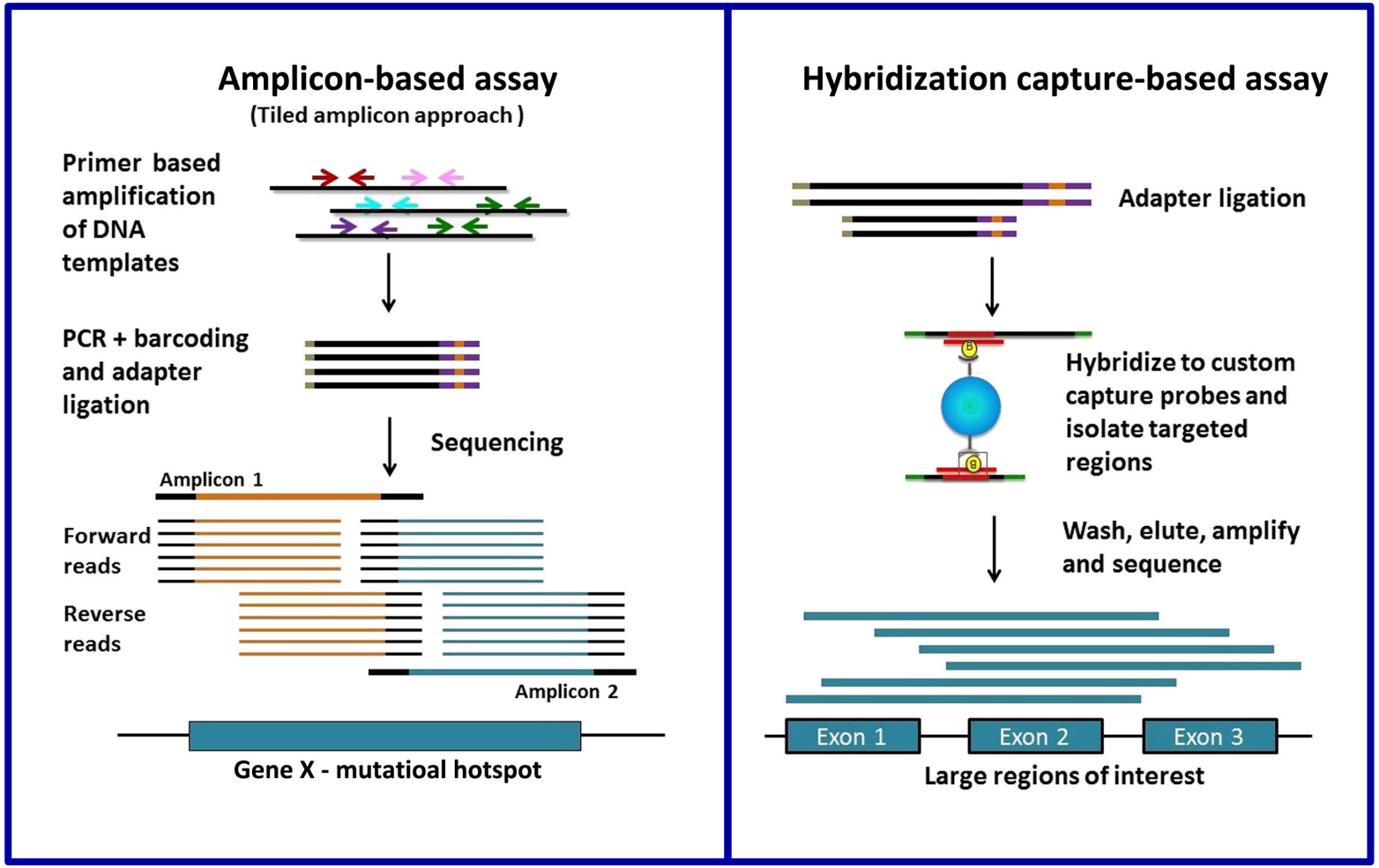
Sets of selected regions of interest

Target enrichment by amplification or hybridization

Why to use gene panels:

One disease can be associated with variants in different genes

Certain gene is diagnostically relevant for several diseases



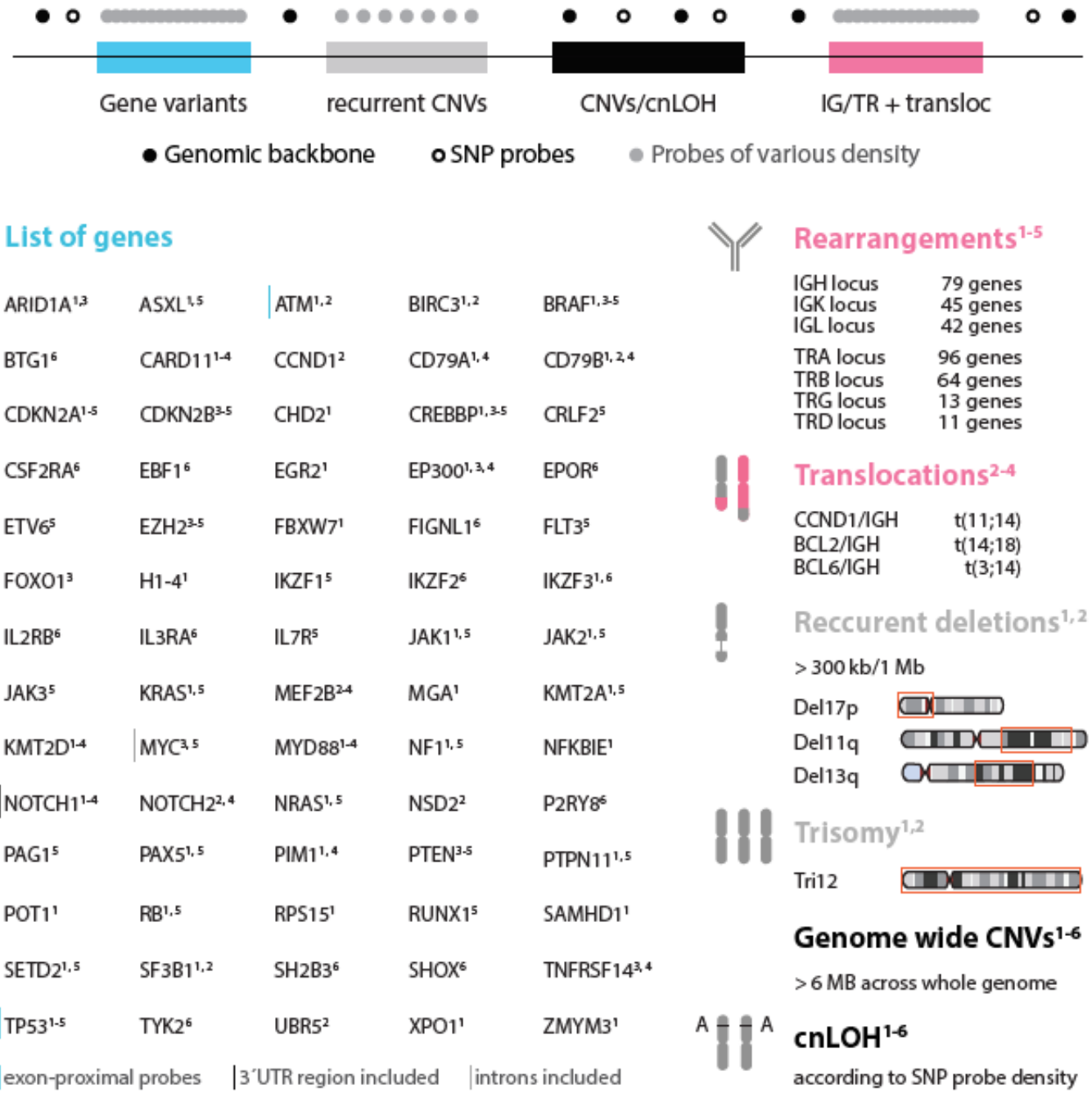

Jennings et al, J Mol Diagn 2017

# Practical example

## LYNX panel

– diagnostics of molecular markers in lymphoid malignancies

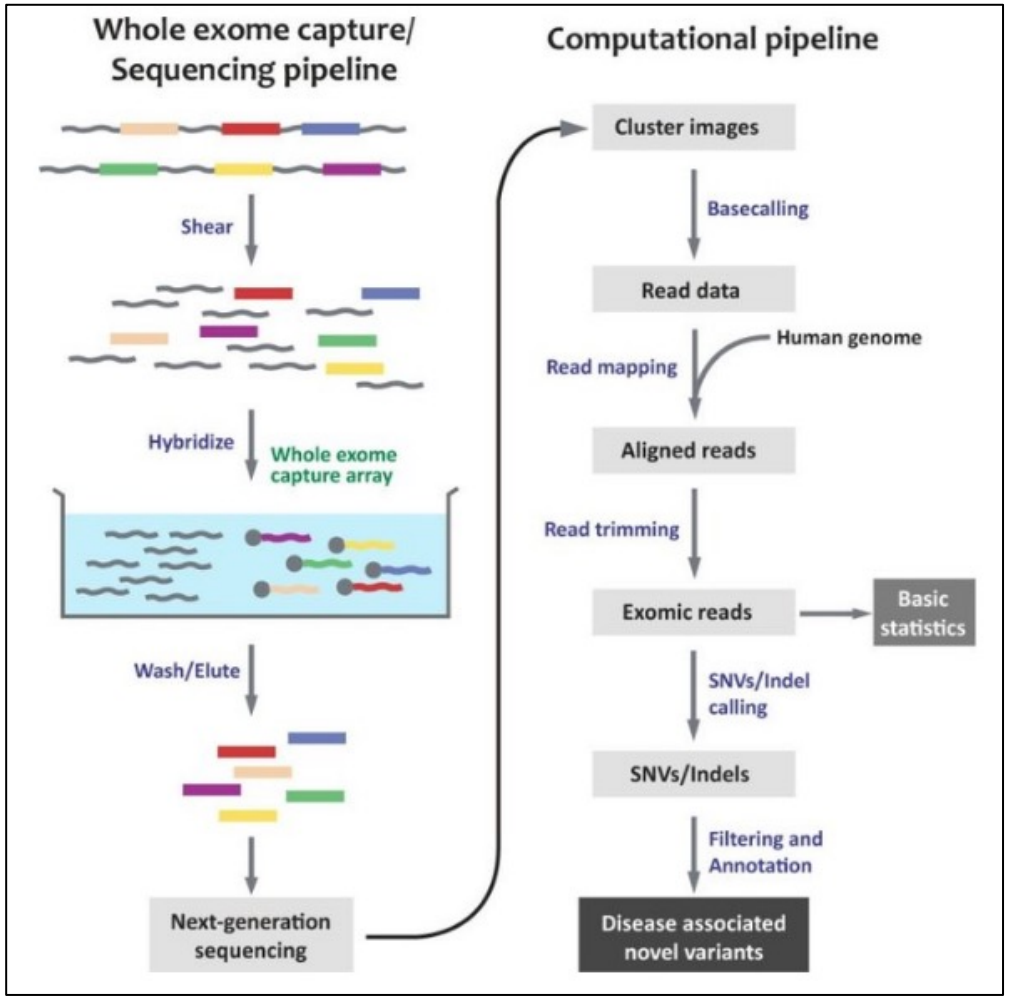
[1]CLL, [2]MCL, [3]FL, [4]DLBCL, [5]ALL, [6]Ph-like ALL




Navrkalova et al, J Mol Diagn 2021

# Whole exome sequencing (WES)

Identification of causative variants
Discovery of novel genetic markers
Searching for treatment targets



# Whole genome sequencing (WGS)

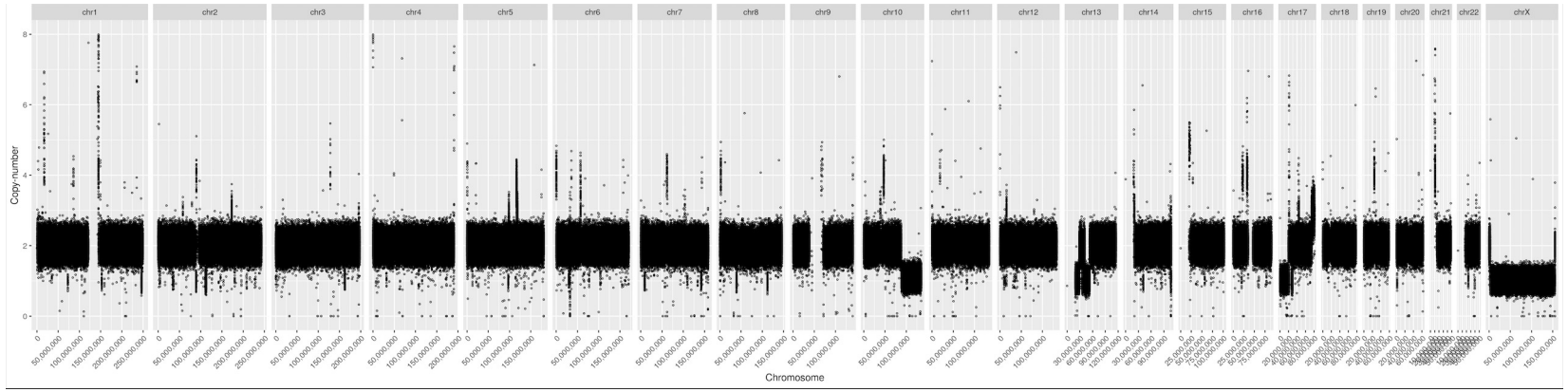
Mainly experimental method for exploring unknown variants

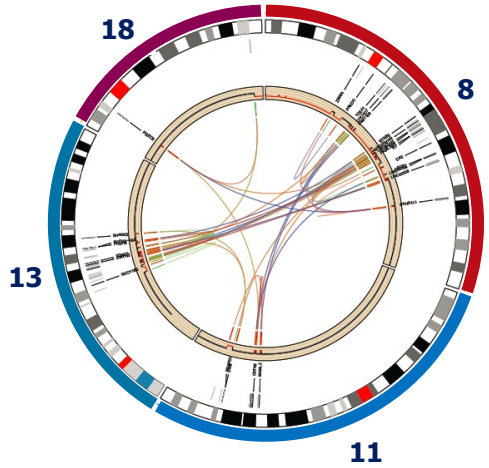
Applications similar to WES, additional information about non-coding regions and chromosomal abnormalities

Typical sequencing coverage ~ 30–100x – detection of somatic clonal or germline mutations

Shallow sequencing (~ 0.5-10x coverage) – genome-wide detection of chromosomal abnormalities, low yield of mutation detection

In clinical practice a potential benefit of combination of shallow and panel sequencing

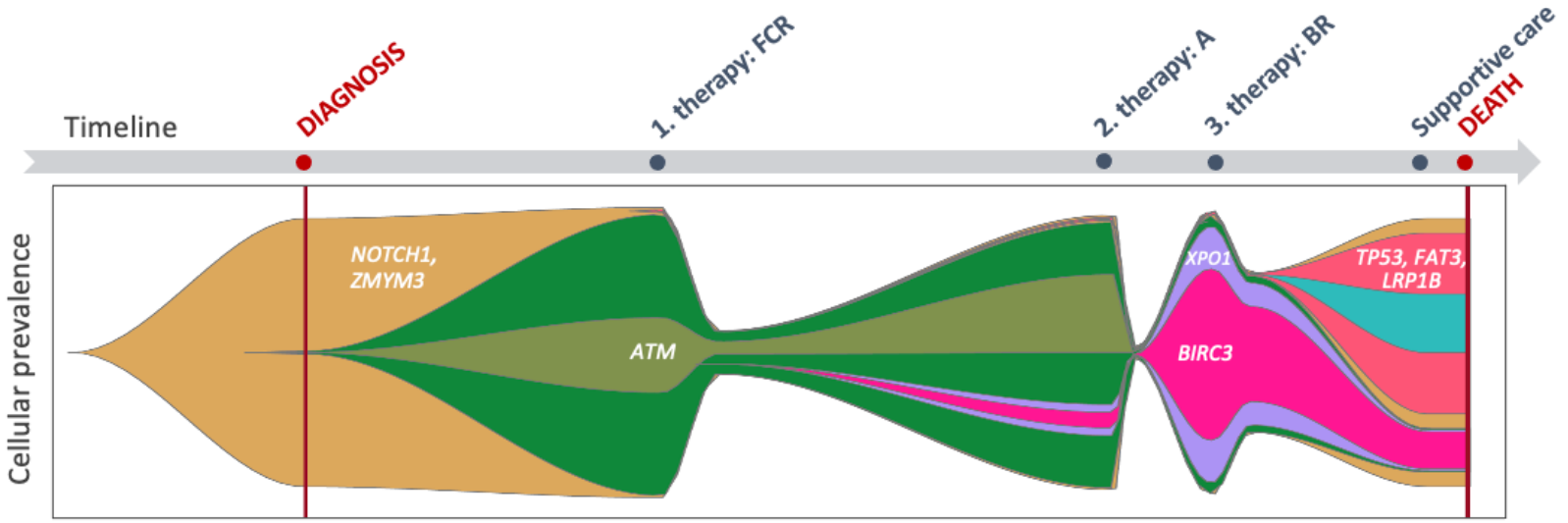


# Practical example

## Benefits of NGS in cancer diagnostics and monitoring

Reconstruction of clonal architecture and cancer evolution




Need for multidisciplinary team

– biologists, geneticists, computational scientists, bioinformaticians, statisticians

# Cytogenomics methods

## Comparison of sensitivity of the techniques

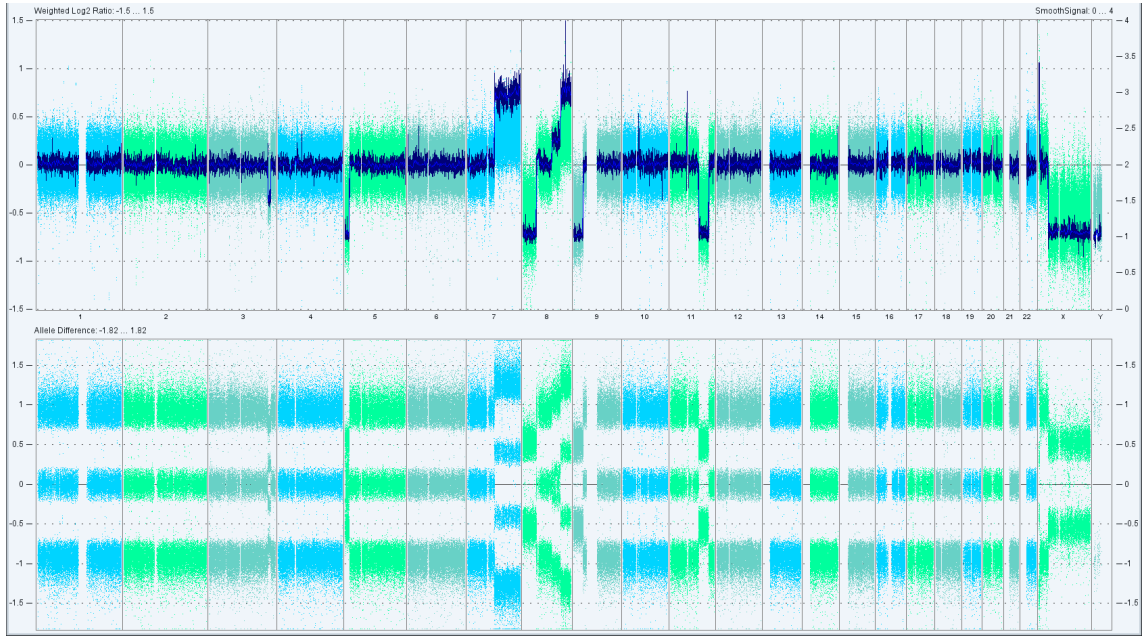
| | Aneu-ploidy | CNA | Poly-ploidy | Clonal heterogeneity | Focal amplification | Balanced rearrangements | Unbalanced rearrangements | cn-LOH |
|---|---|---|---|---|---|---|---|---|
| Classical cytogenetics | +++ | + | +++ | +++ | ++ | +++ | +++ | - |
| Interphase FISH | +++ | ++ | + | +++ | +++ |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

# Genomic arrays

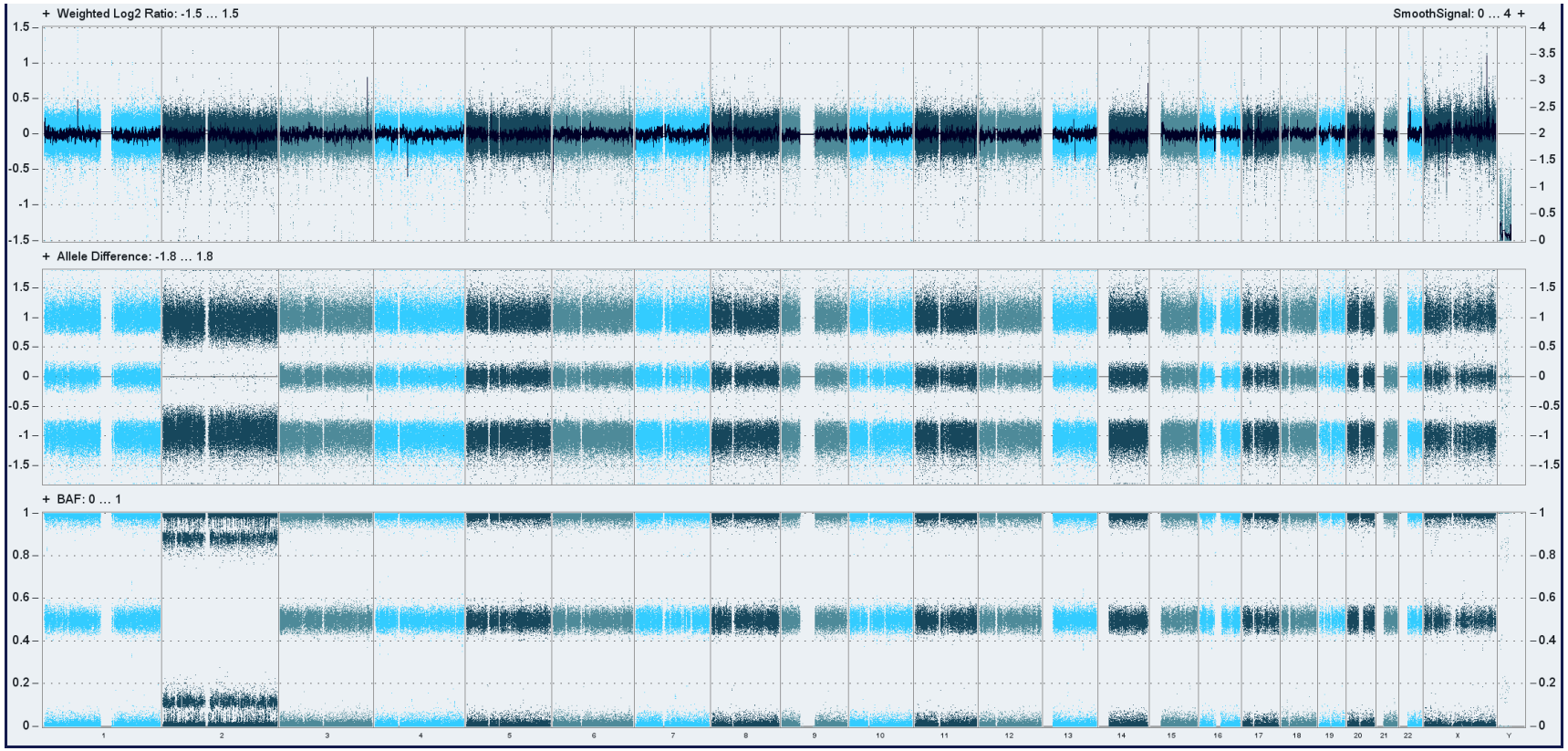
Molecular cytogenetic technique for detection of genomic gains and losses
Detection of copy-neutral loss of heterozygosity
Not possible to detect balanced rearrangements
Precise breakpoint localization, identification of affected genes
High resolution, genome-wide
Working with DNA, no need for viable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



# Practical example

## Muscular dystrophy

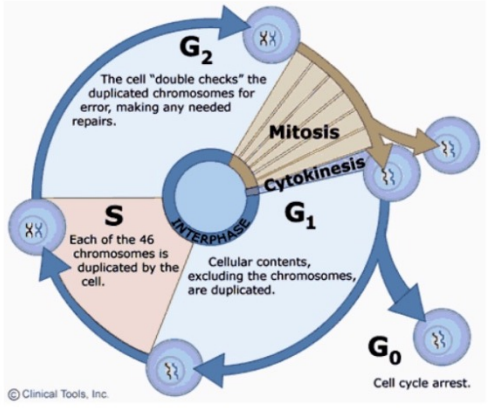
Panel NGS – imbalance of SNPs on chr2
Genomic array – UPD chr2 in 80% of cells – germline mosaicism

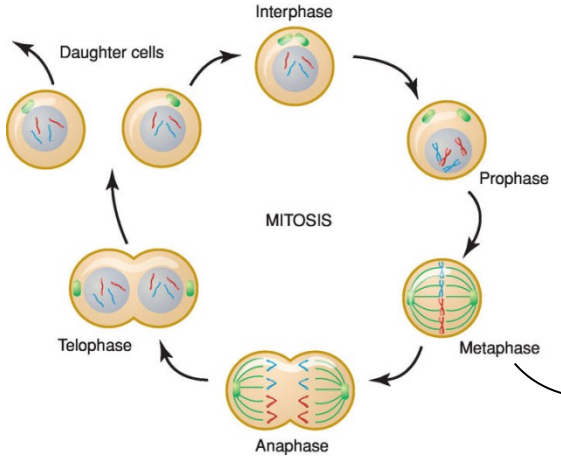


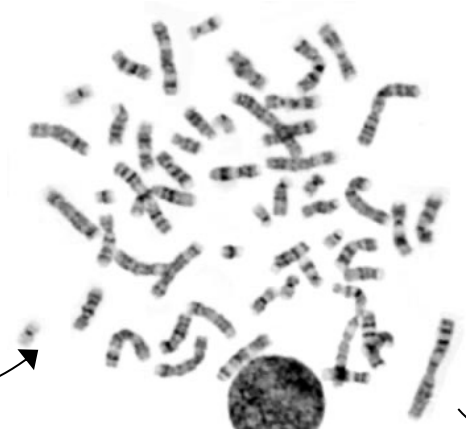
# Equipment for genomic arrays

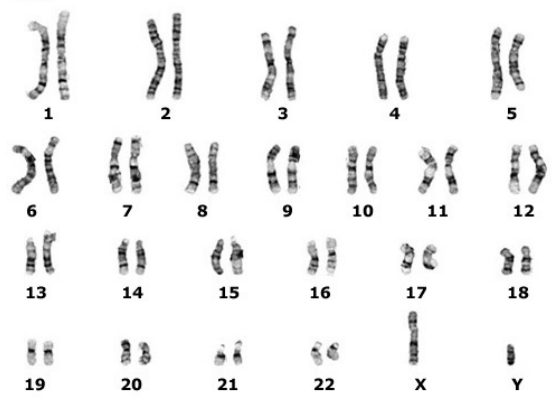

Affymetrix GeneChip System

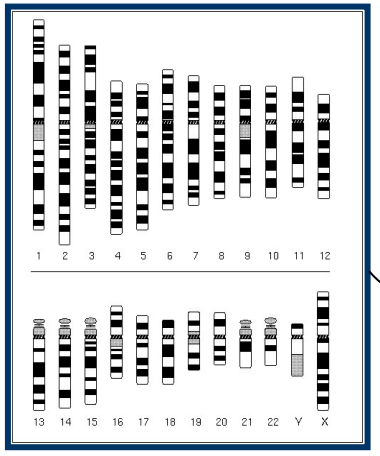
# Classical cytogenetics
## chromosome banding techniques

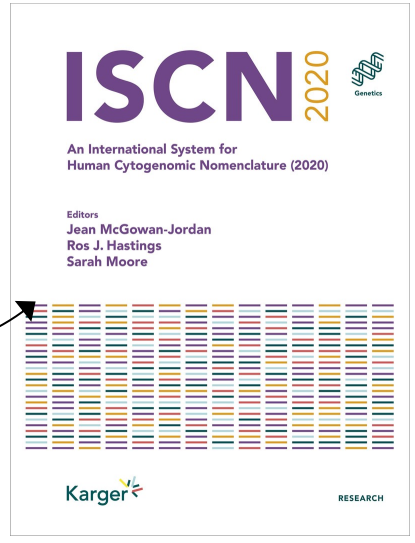





Methods not requiring PCR
Imaging methods
Cheap
Clonal composition assessment

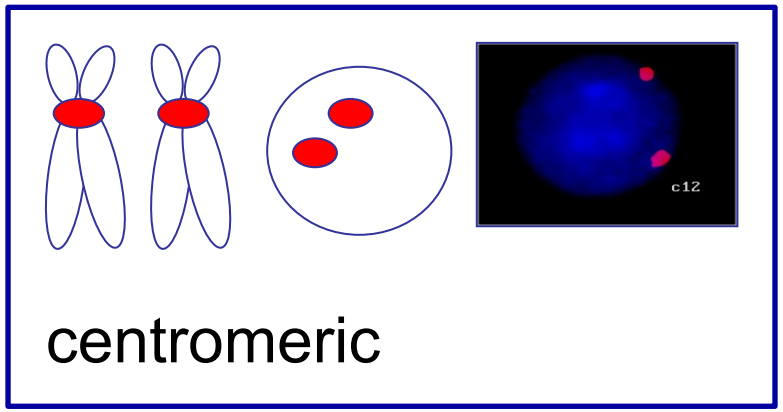
# Molecular cytogenetics

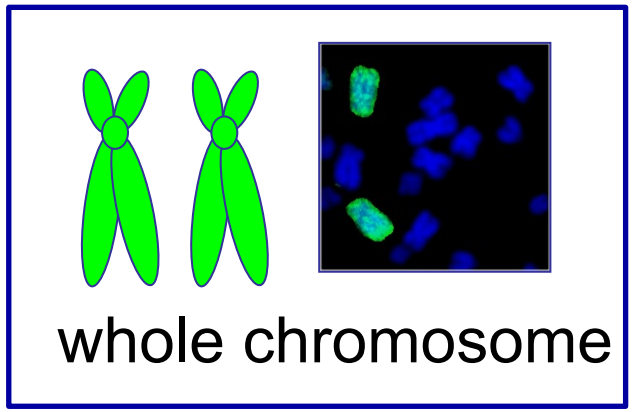
Fluorescent in situ hybridization (FISH)

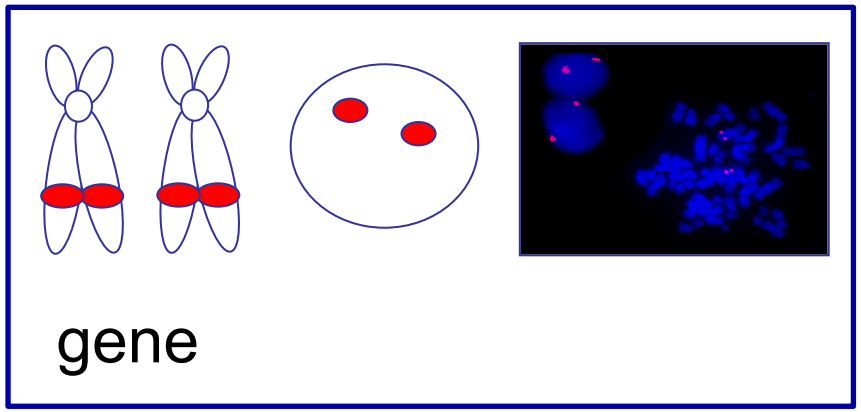
Targets specific regions based on DNA sequence

Detection of chromosomal abnormalities with diagnostic, prognostic and predictive value

Probe types:

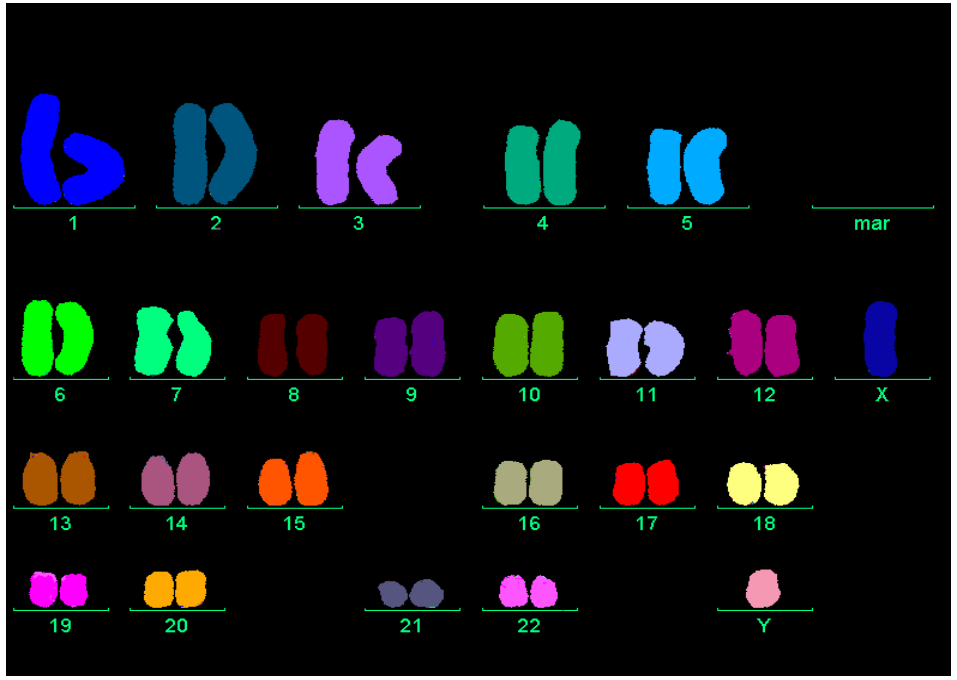


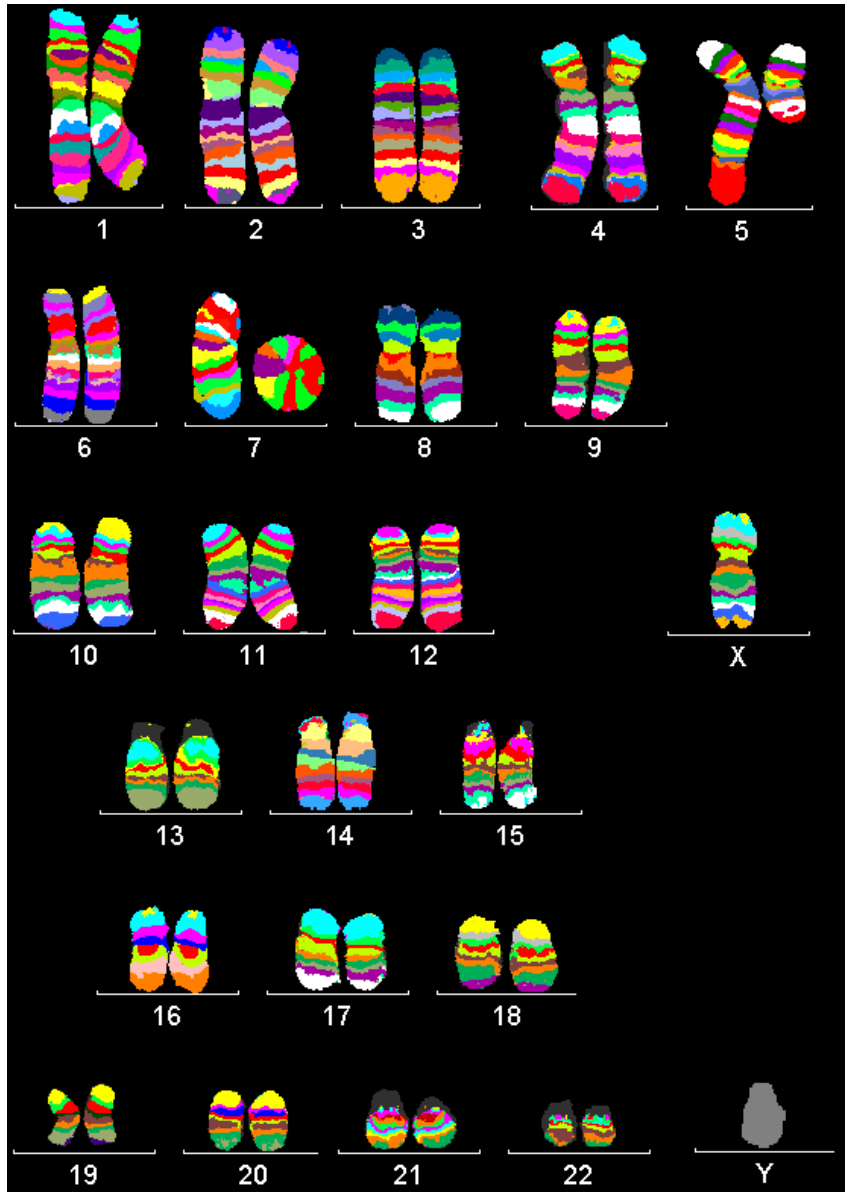



# Molecular cytogenetics

FISH methods for genome-wide analysis



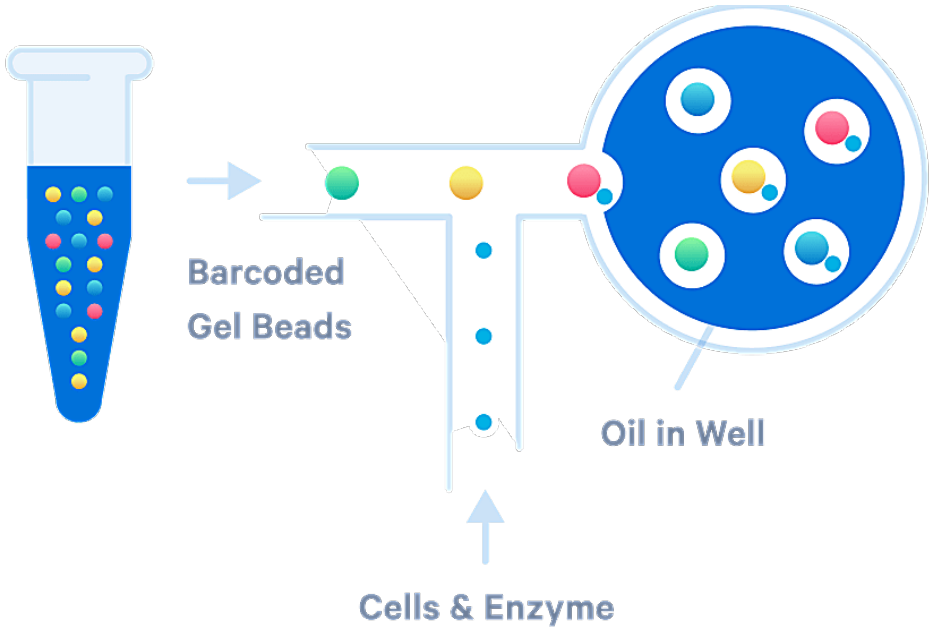

mFISH

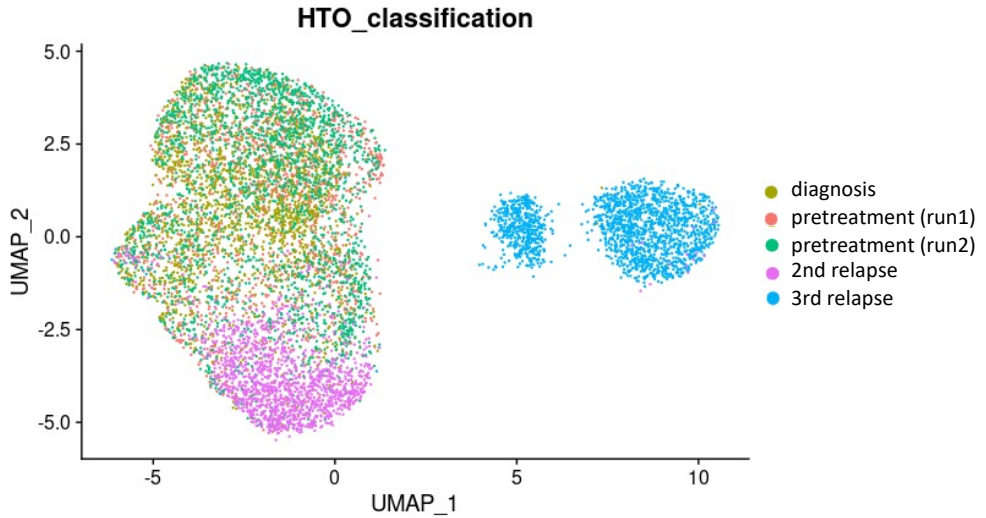



mBAND

# Single-cell technologies

Experimental methods
Applications in cancer research, immunology, developmental biology, …
Analysis of DNA variants, chromatin activity, RNA expression profiles, protein expression, …
Coexistence of cellular features in single cells




https://www.10xgenomics.com/

# Take-home messages

- Discuss
- Standardize
- Integrate

## The end…

Contact:
karla.plevova@mail.muni.cz or plevova.karla@fnbrno.cz

Thank you for your attention!