MOLECULAR GENETICS AND CYTOGENETICS LABORATORY AND METHODS

KARLA PLEVOVÁ

20.11.2020

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 the MGC lab?
- What methods are available in the MGC lab?

WHAT TO EXPECT FROM A MGC LAB?

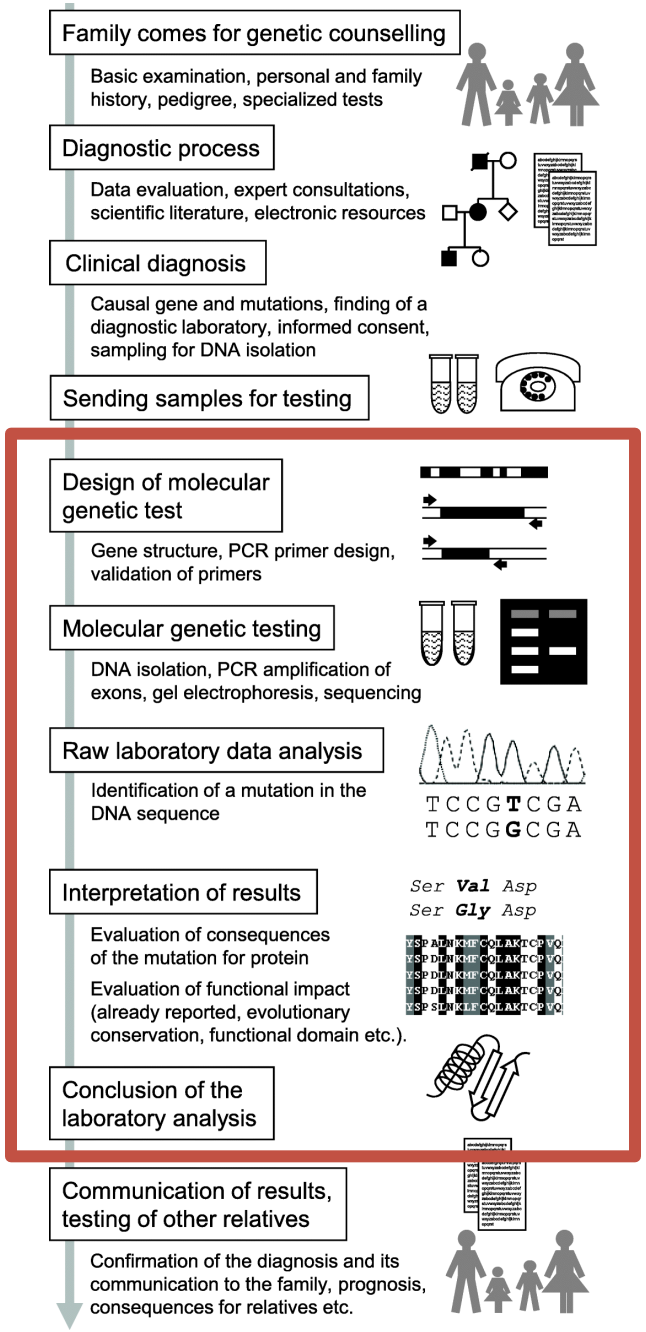
MGC LAB = PARTNER

- 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
- Hereditary predisposition assessment
- Disease prognostication
- Treatment optimization
- Disease activity monitoring
- Disease complication diagnostics



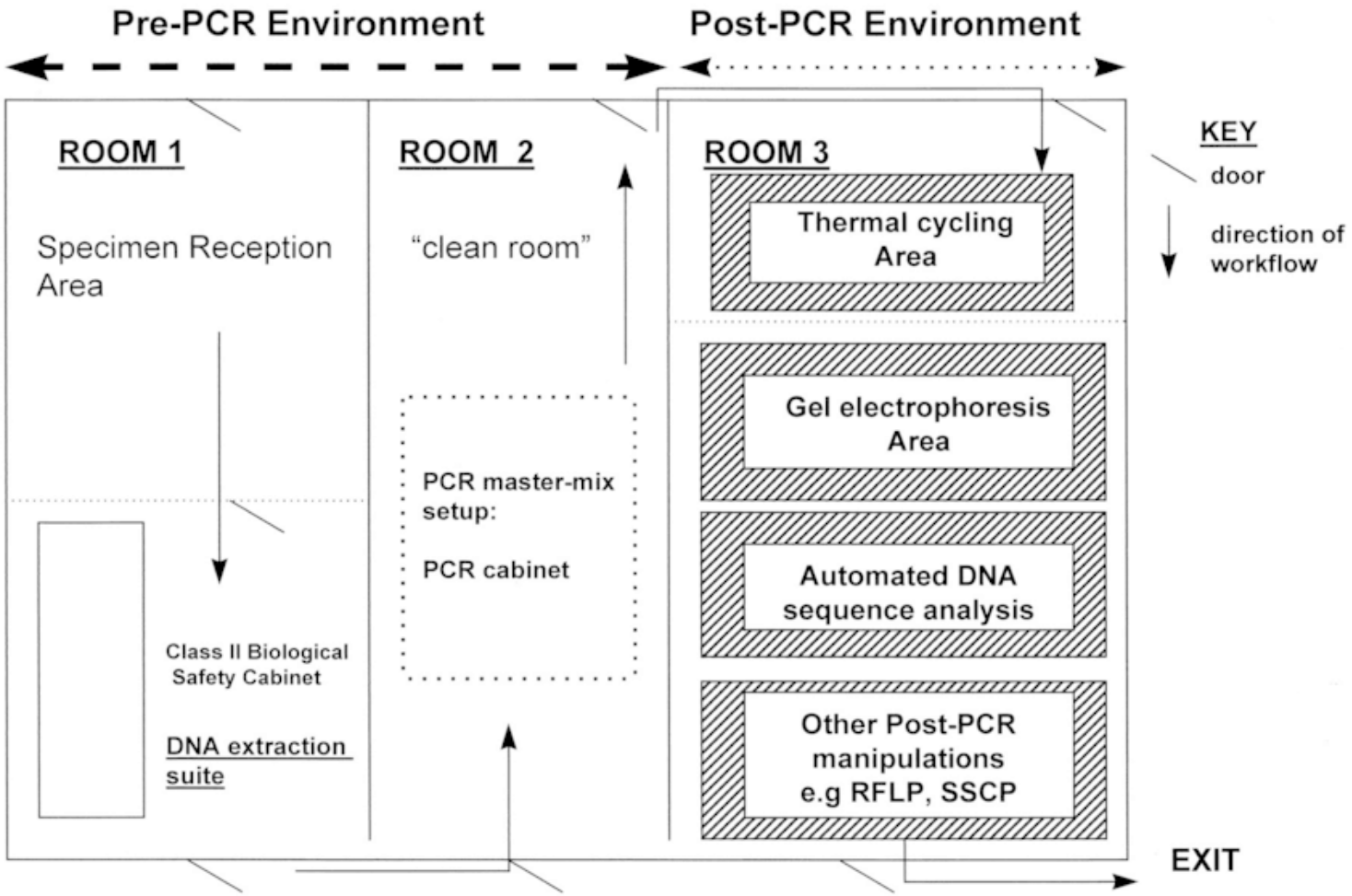
Prochazkova et al, BMC Medical Education 2019

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

laboratory report – interpretation ← a basis for laboratory test request

HOW DOES IT LOOK THE MG LAB?



Millar et al, J Clin Microbiol 2002

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

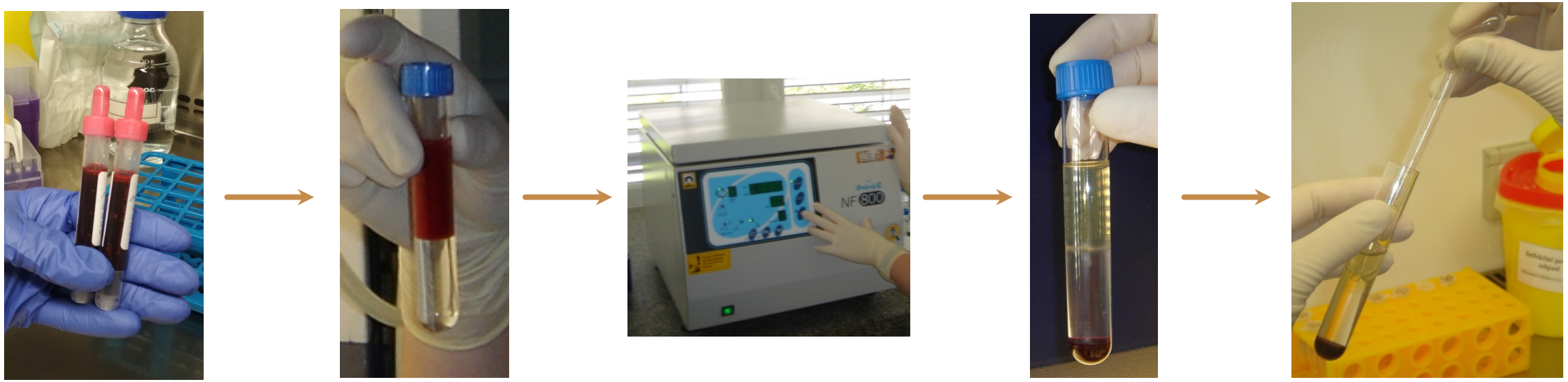
MATERIALS USED

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

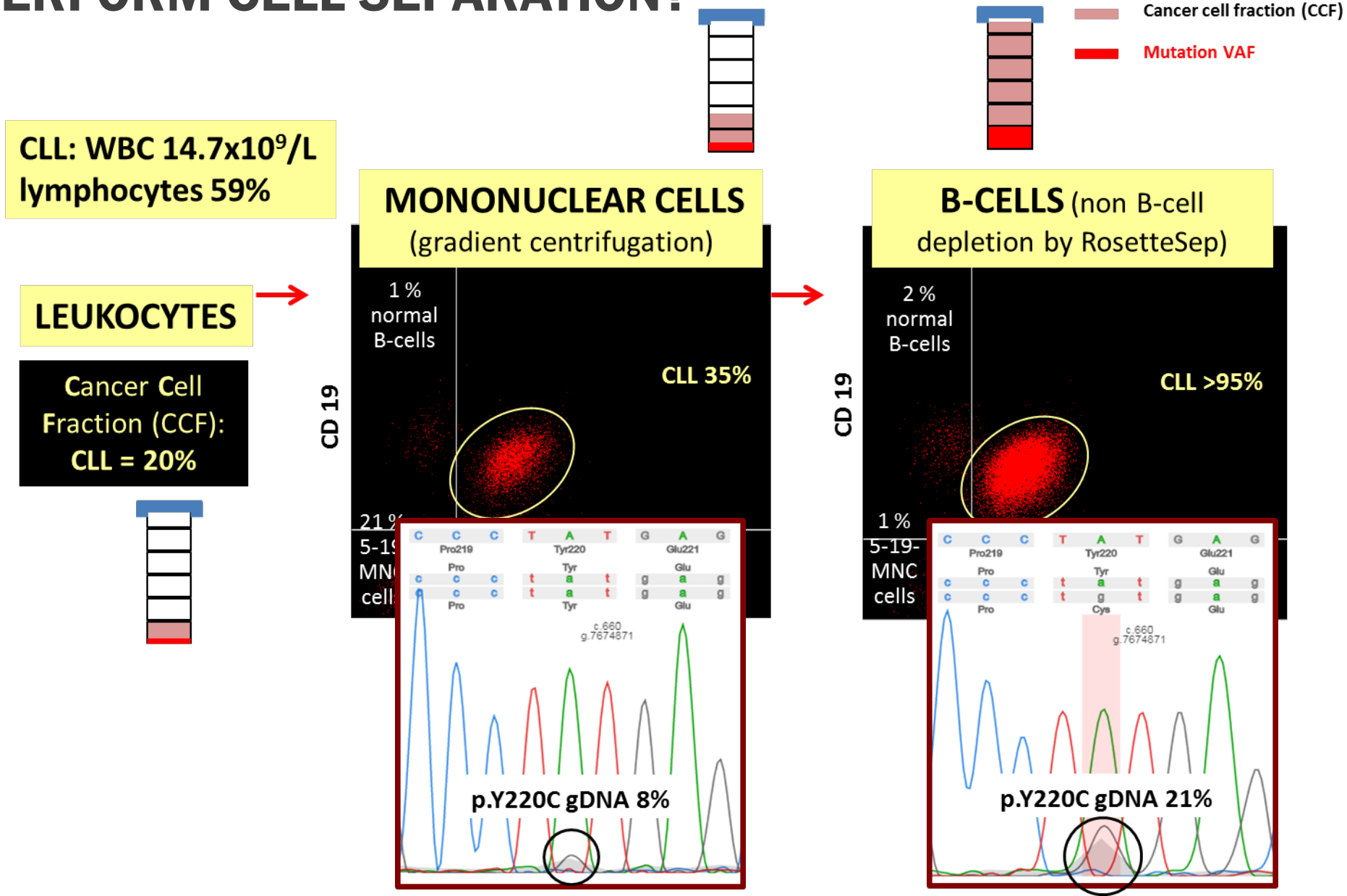
Postnatal genetics
X
Prenatal testing
X
Oncology

PERIPHERAL BLOOD PROCESSING

- Different cell population used according to the application:
  - **Leukocytes**
  - Mononuclear cells
  - Granulocytes
  - Lymphocytes
  - Specific cell subpopulations



WHY TO PERFORM CELL SEPARATION?

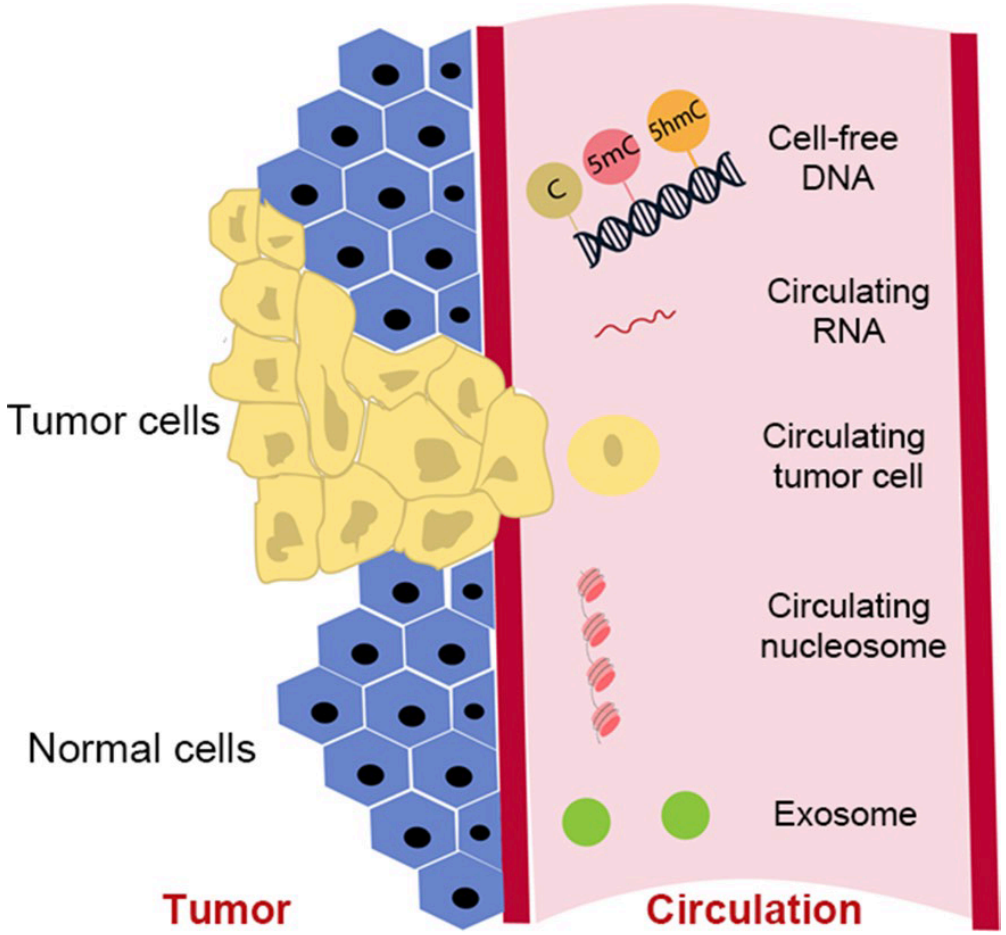


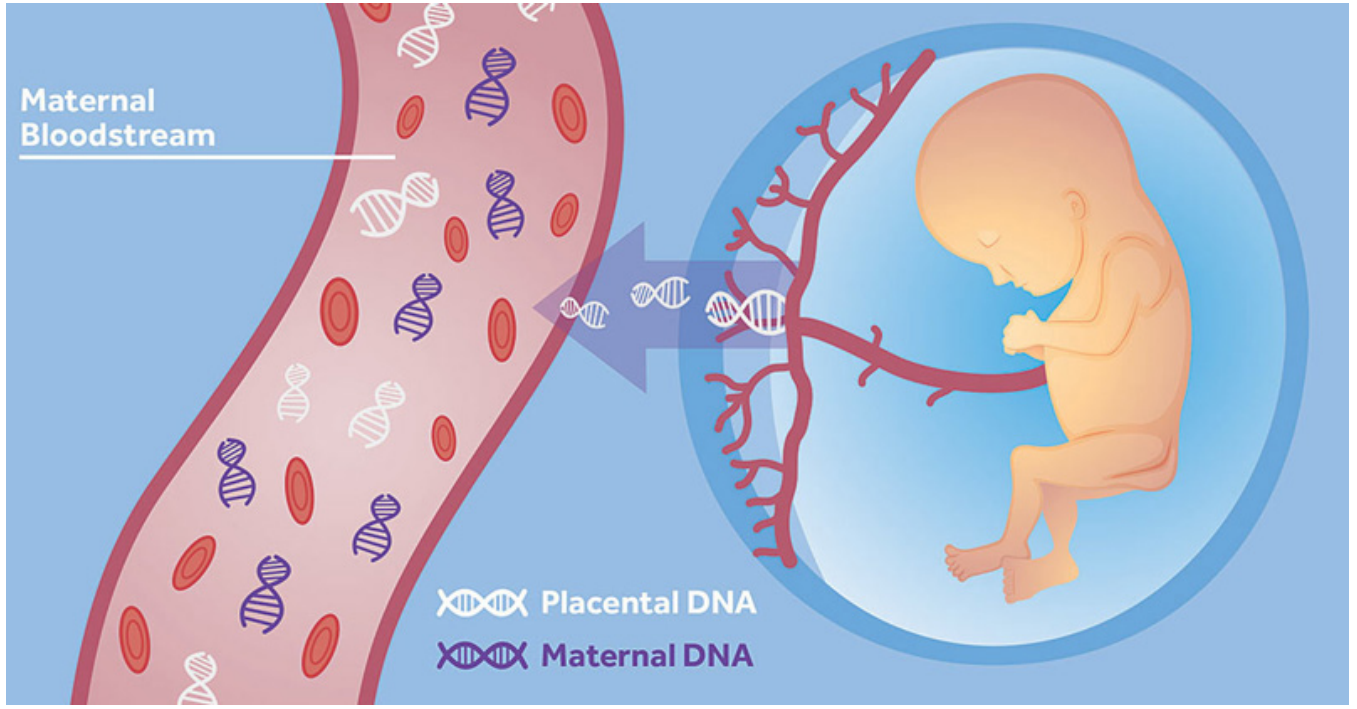
CELL CULTURE

- Culturing of peripheral blood, bone marrow, tissue sections, …

LIQUID BIOPSIES

When invasive biopsies are not an option



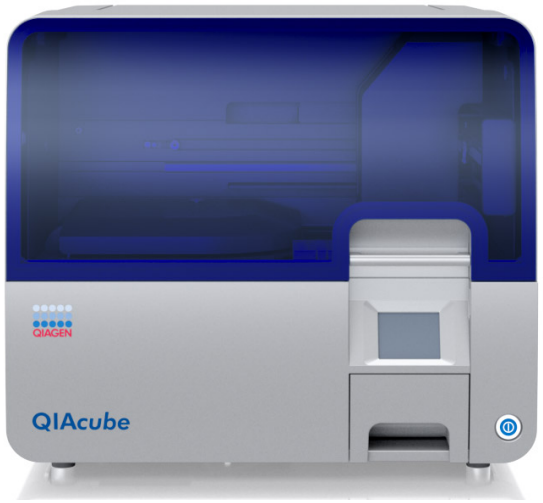


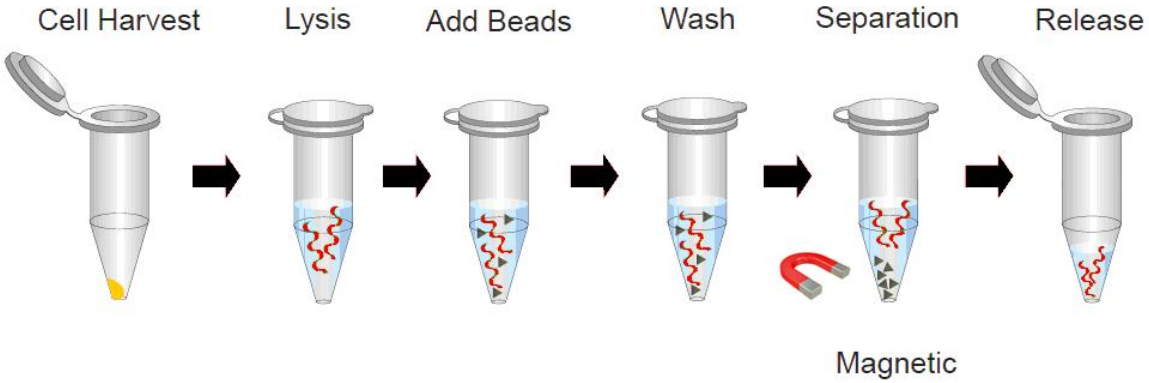
Very low amount of material

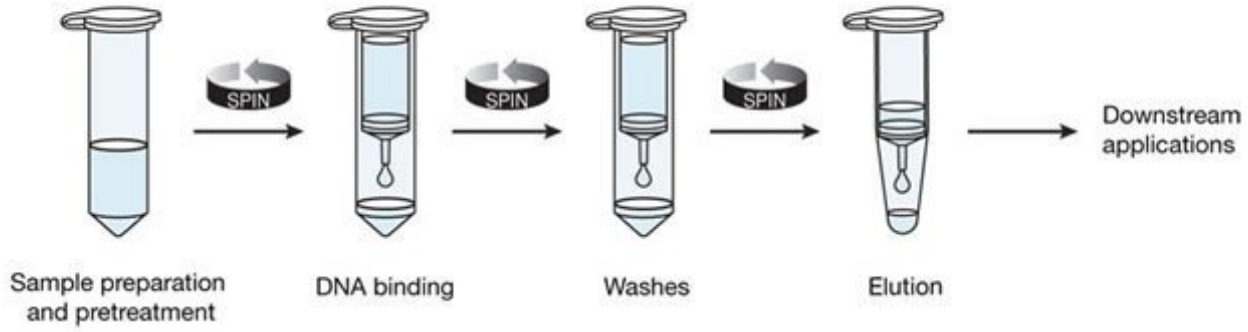
- Plasma / serum
- Urine
- Joint fluid
- Cerebrospinal fluid

NUCLEIC ACID (RNA, DNA) ISOLATION

- pre-PCR area
- Manual and automated sample processing

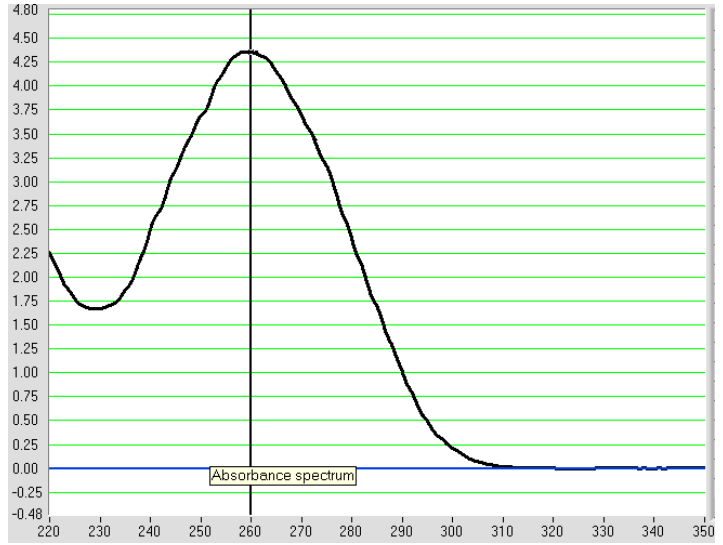






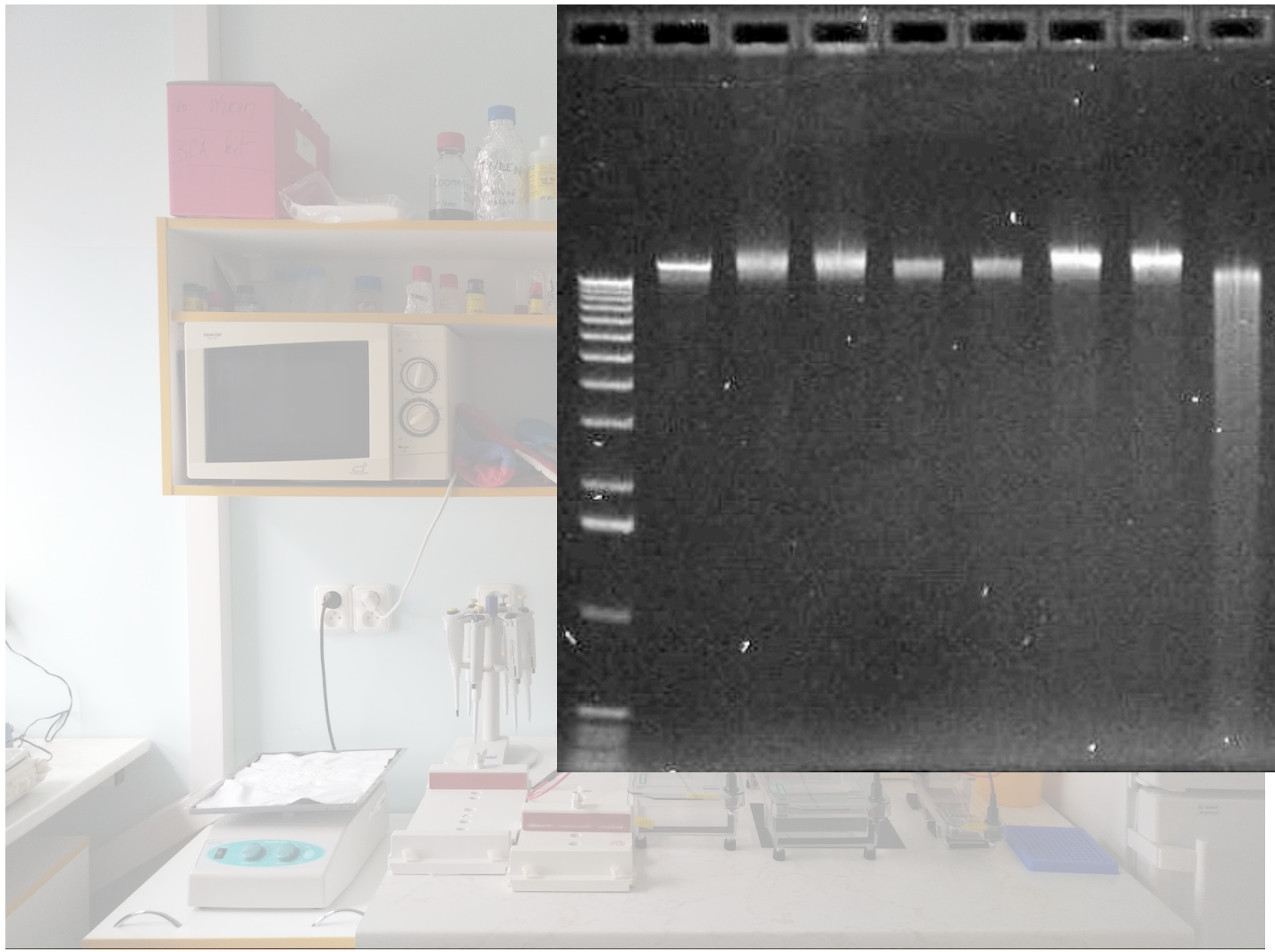
NUCLEIC ACID QUANTIFICATION

- Spectroscopic and fluorimetric methods



NUCLEIC ACID QUALITY CONTROL

- Electrophoretic methods



NUCLEIC ACID QUALITY CONTROL

- Alternative methods to gel electrophoresis
- Lower material input

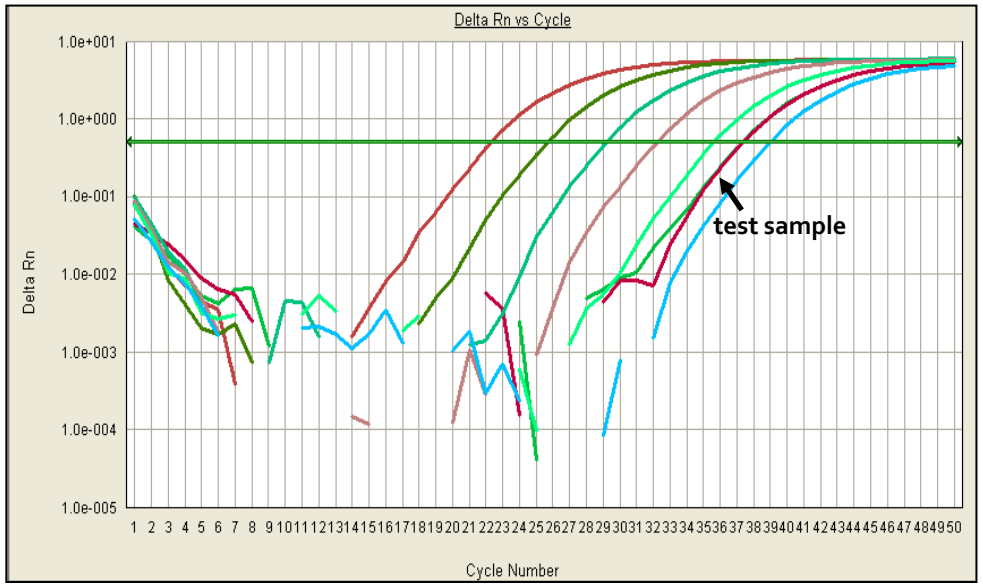
POLYMERASE CHAIN REACTION (PCR)

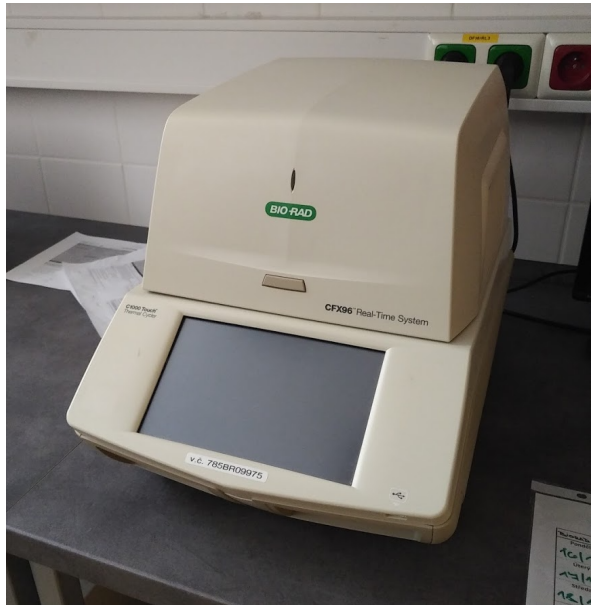
- Fundamental reaction of molecular biology and genetics
- Amplification of regions of interests
- PCR assembling in pre-PCR area
- Carried out in thermocycler
- Various modifications
- PCR components
  - Template DNA
  - Primers
  - Nucleotides
  - Polymerase

PCR THERMOCYCLER ROOM

REAL-TIME PCR

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





DROPLET DIGITAL PCR (DDPCR)

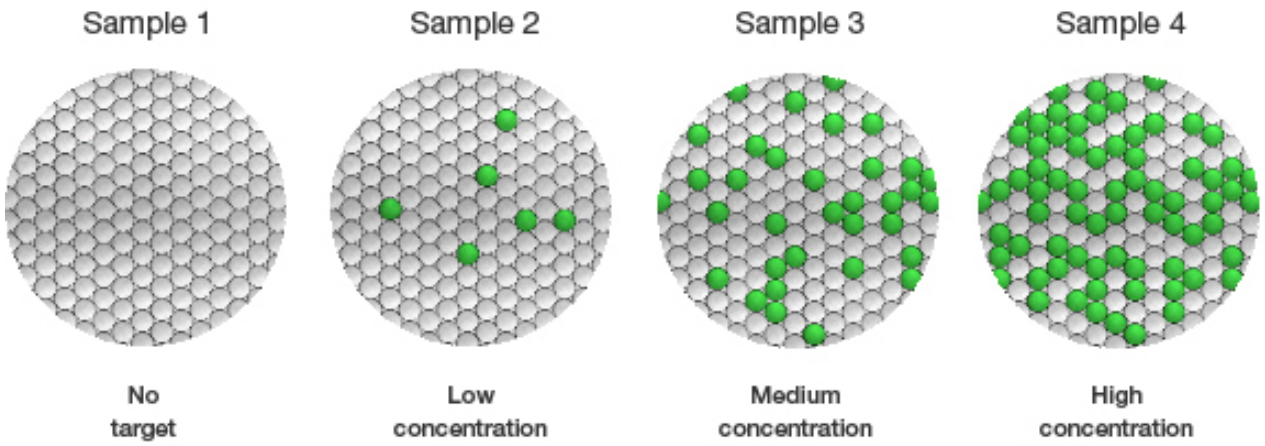
1 DNA sample with target sequence is partitioned into droplets

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

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

4 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



POST-PCR AREA

- Performing QC and downstream analyses
  - DNA sequencing – Sanger, NGS
  - Genomic arrays
  - …

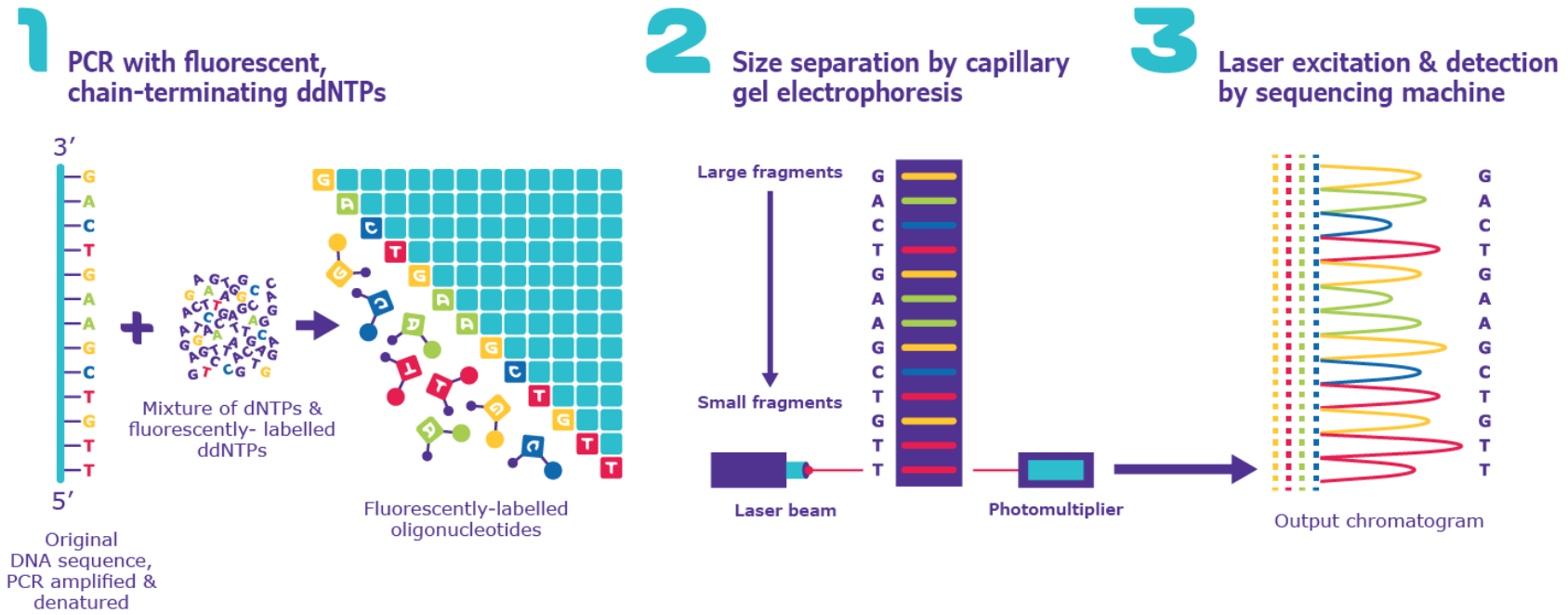
SANGER SEQUENCING

Modification of PCR

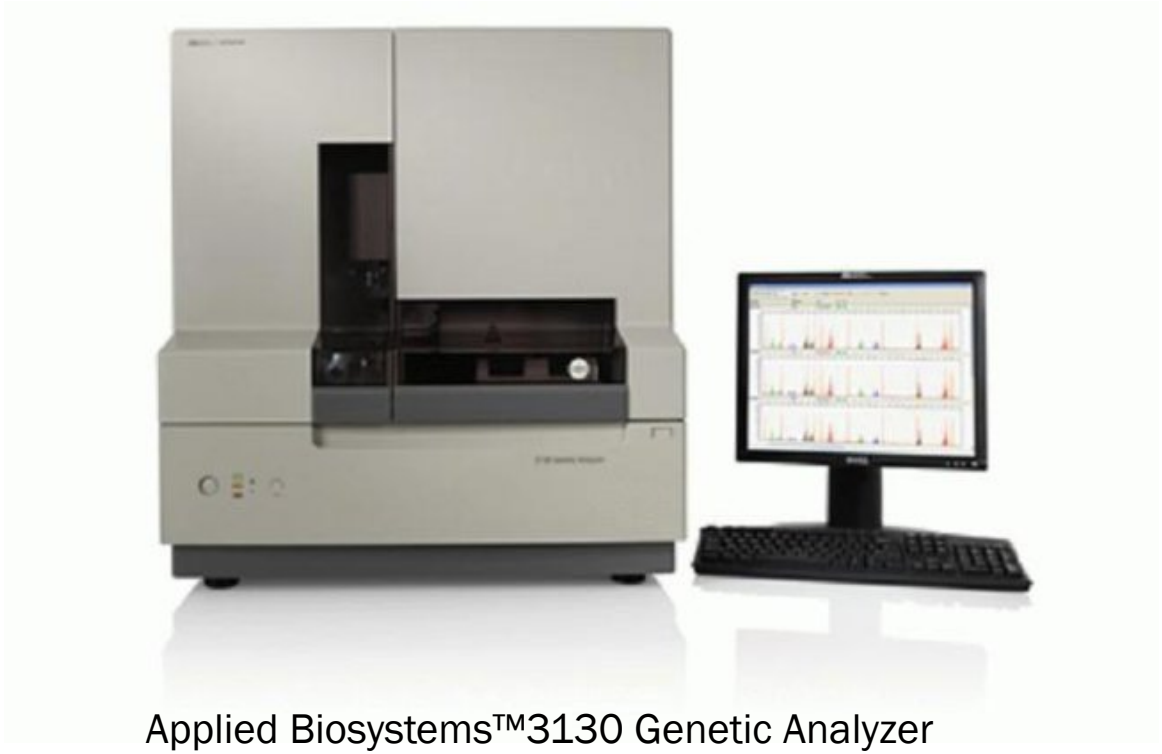
- single primer extension
- Incorporation of dNTPs and ddNTPs

Applications

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

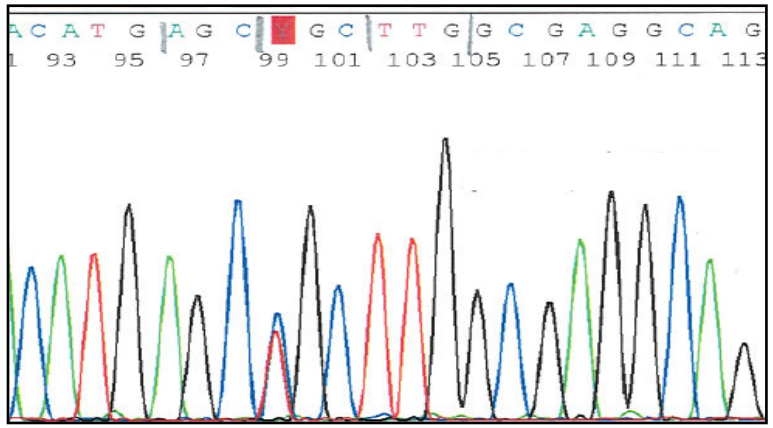


SANGER SEQUENCING

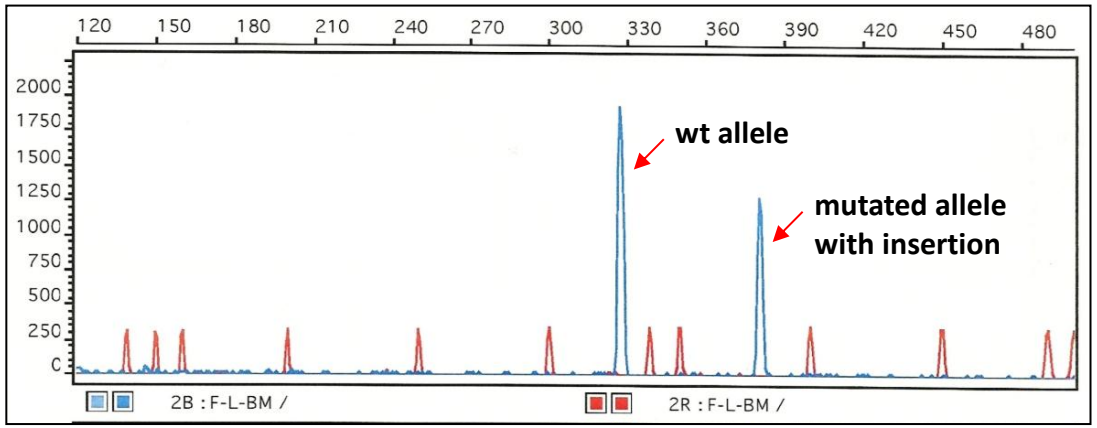


Applied Biosystems™3130 Genetic Analyzer

Sequencing analysis output



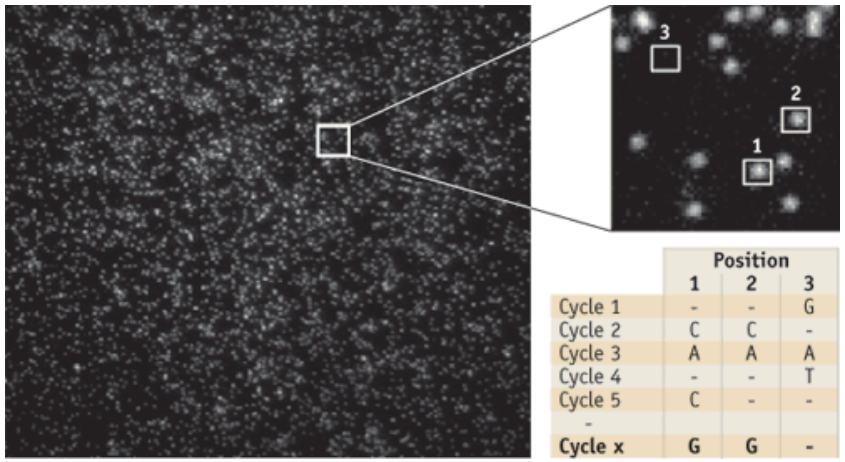
Fragment analysis – modification of the method



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

NGS – REGIONS OF INTEREST

genome

3 200 000 000 bp
30 x read depth

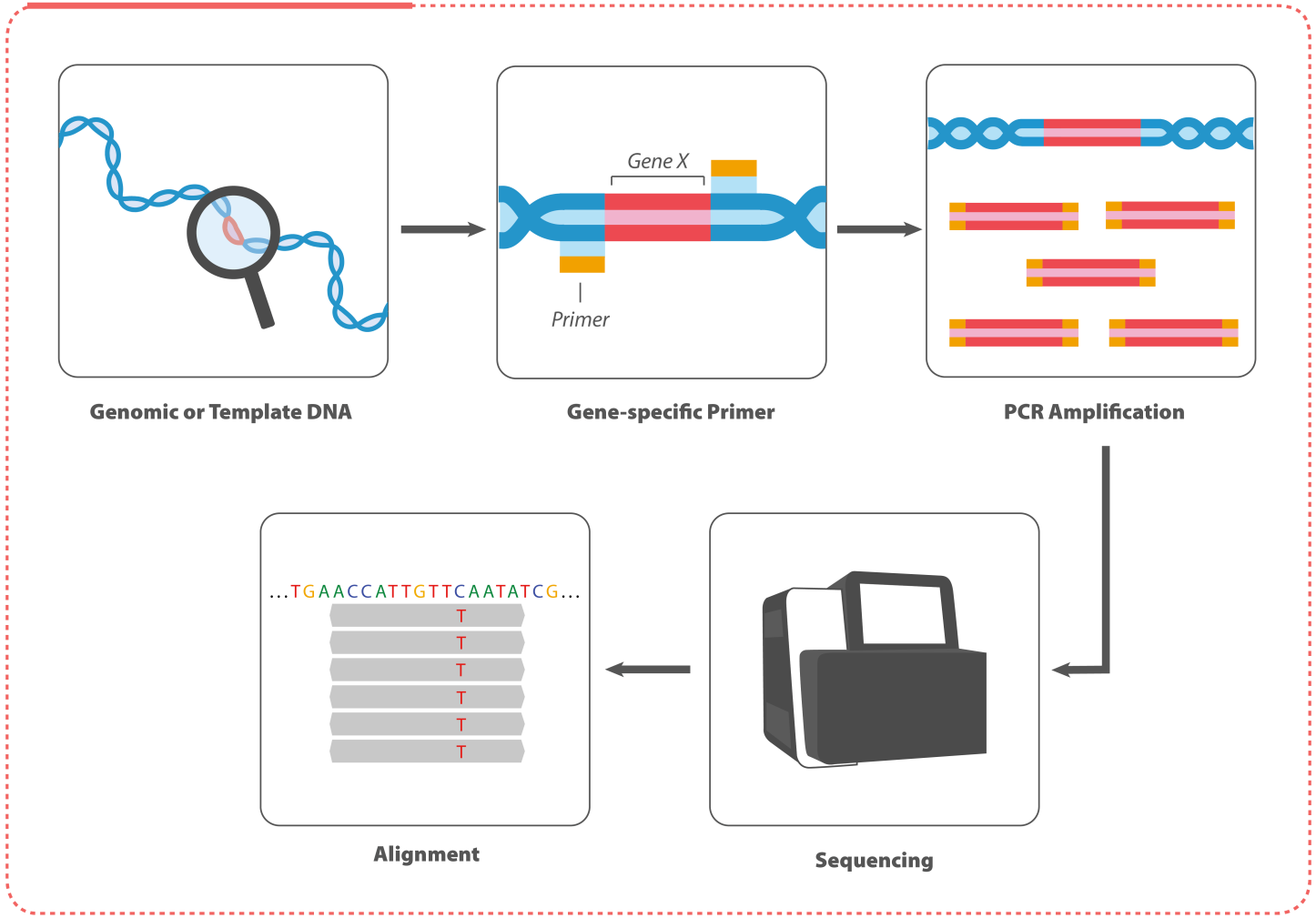
exome

20 000 genes
100 x read depth

selected genes or loci

< 100 genes
≥ 1000 x read depth

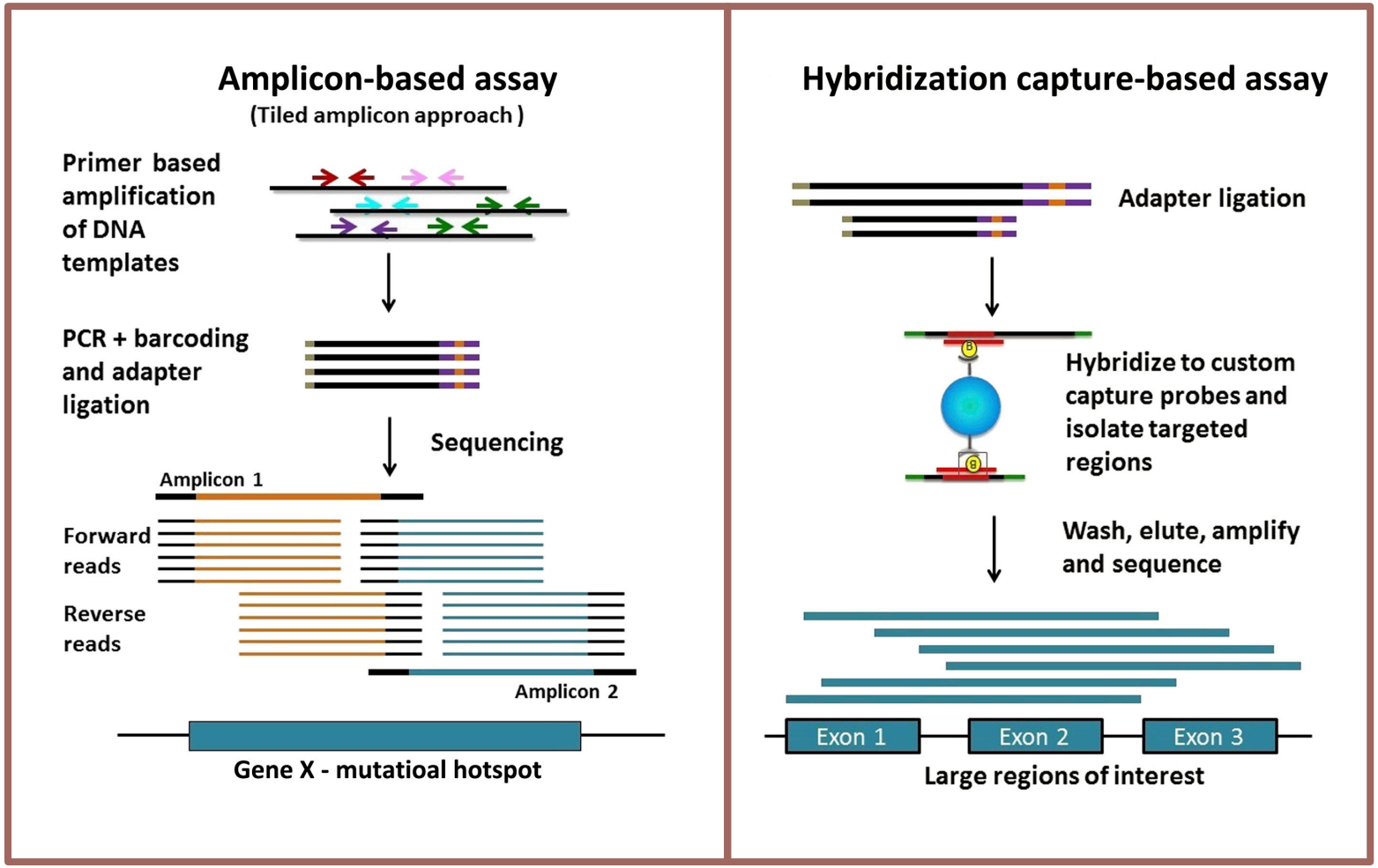
AMPLICON SEQUENCING



PANEL SEQUENCING

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

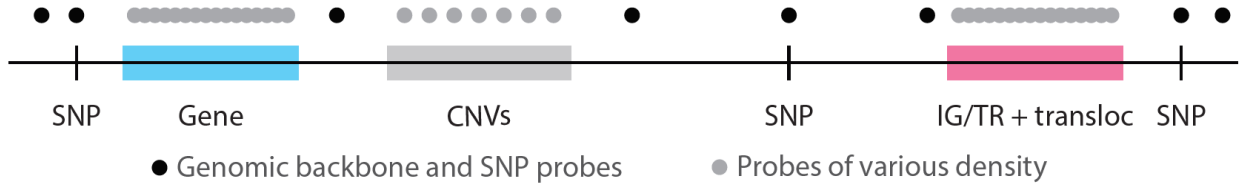
- Why to use gene panels:
  - One disease can be caused by mutations in different genes
  - Certain genes are diagnostically relevant for several diseases



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

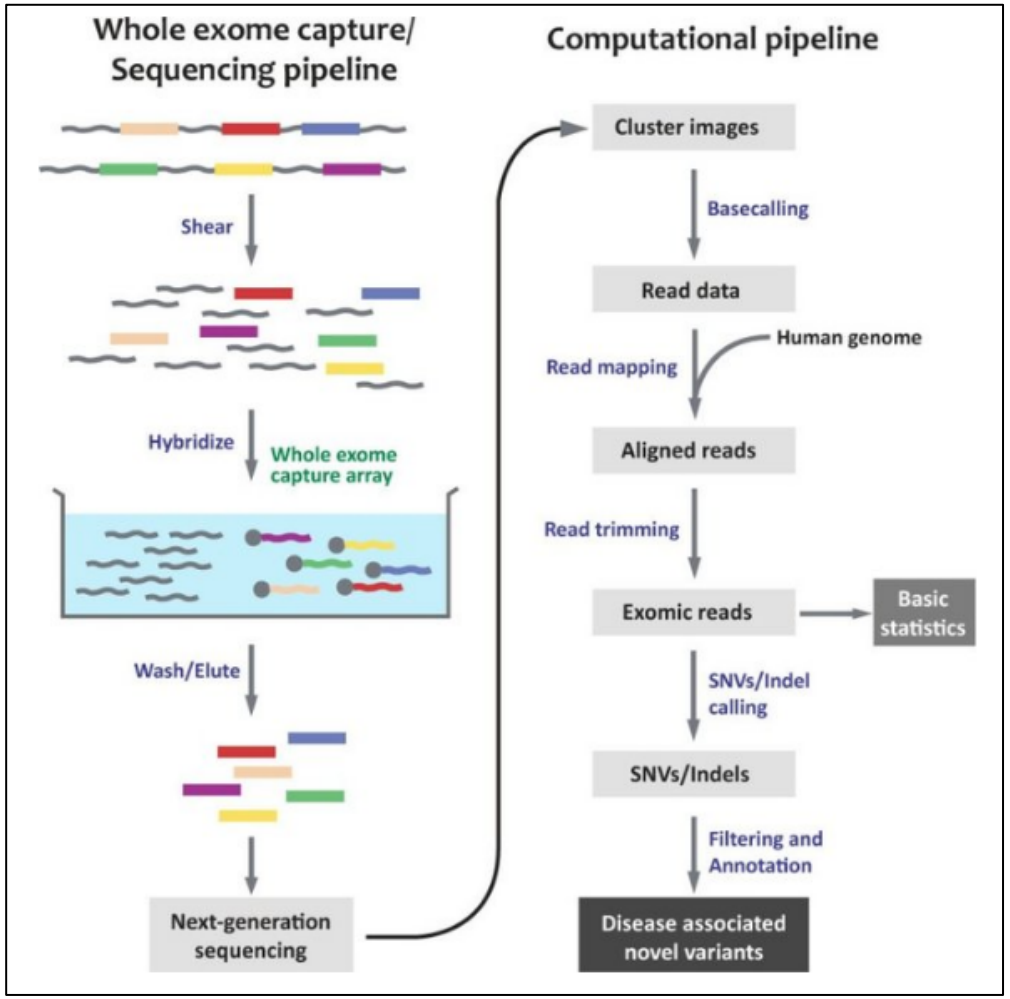
A — A

cnLOH[1-6]

according to SNP probe density

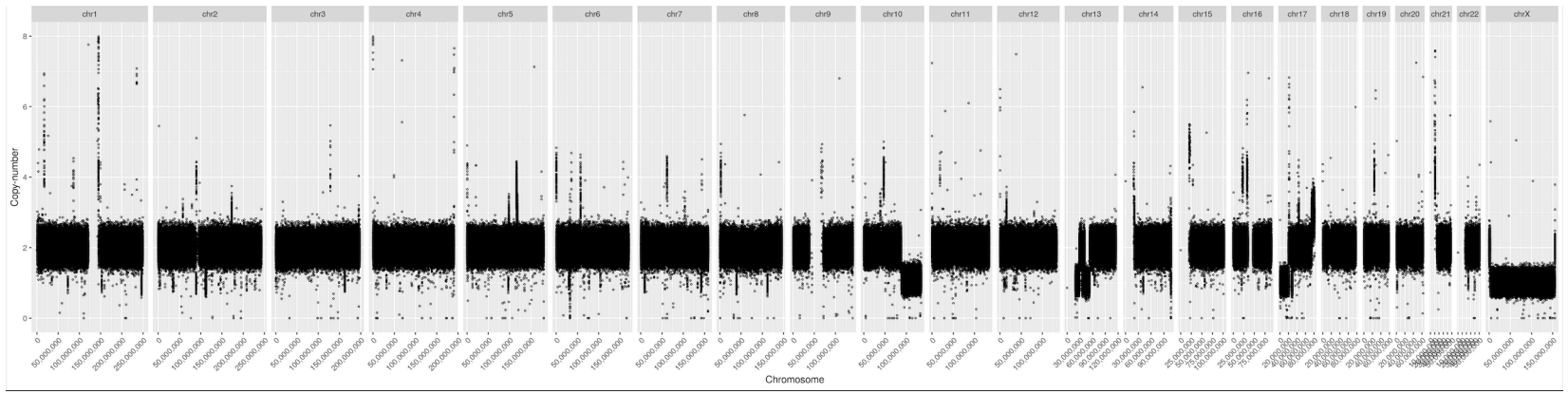
WHOLE EXOME SEQUENCING (WES)

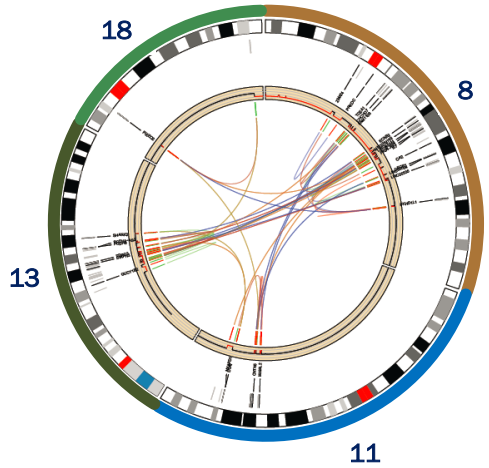
- identification of causative variants
- discovery of novel genetic markers
- searching for treatment targets



WHOLE GENOME SEQUENCING (WGS)

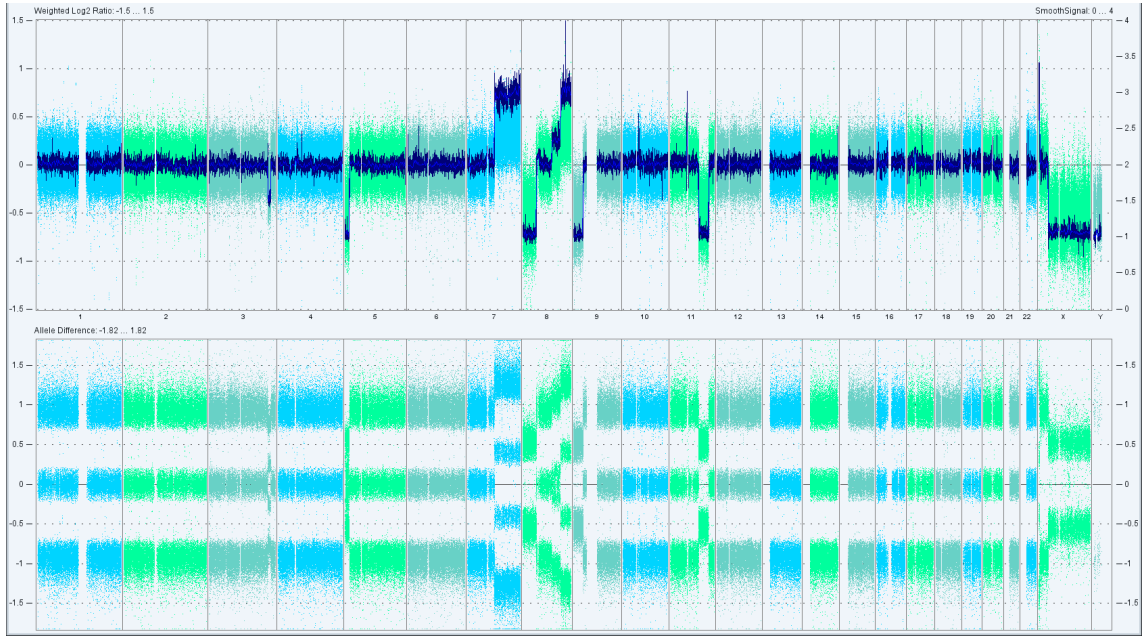
- Mainly experimental method for exploring unknown variants
- Applications similar to WES, additional information about non-coding regions and chromosomal abnormalities
- Typical sequencing coverage ~ 30–100x – detection of somatic clonal or germline mutations
- Shallow sequencing (~ 0.5-10x coverage)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





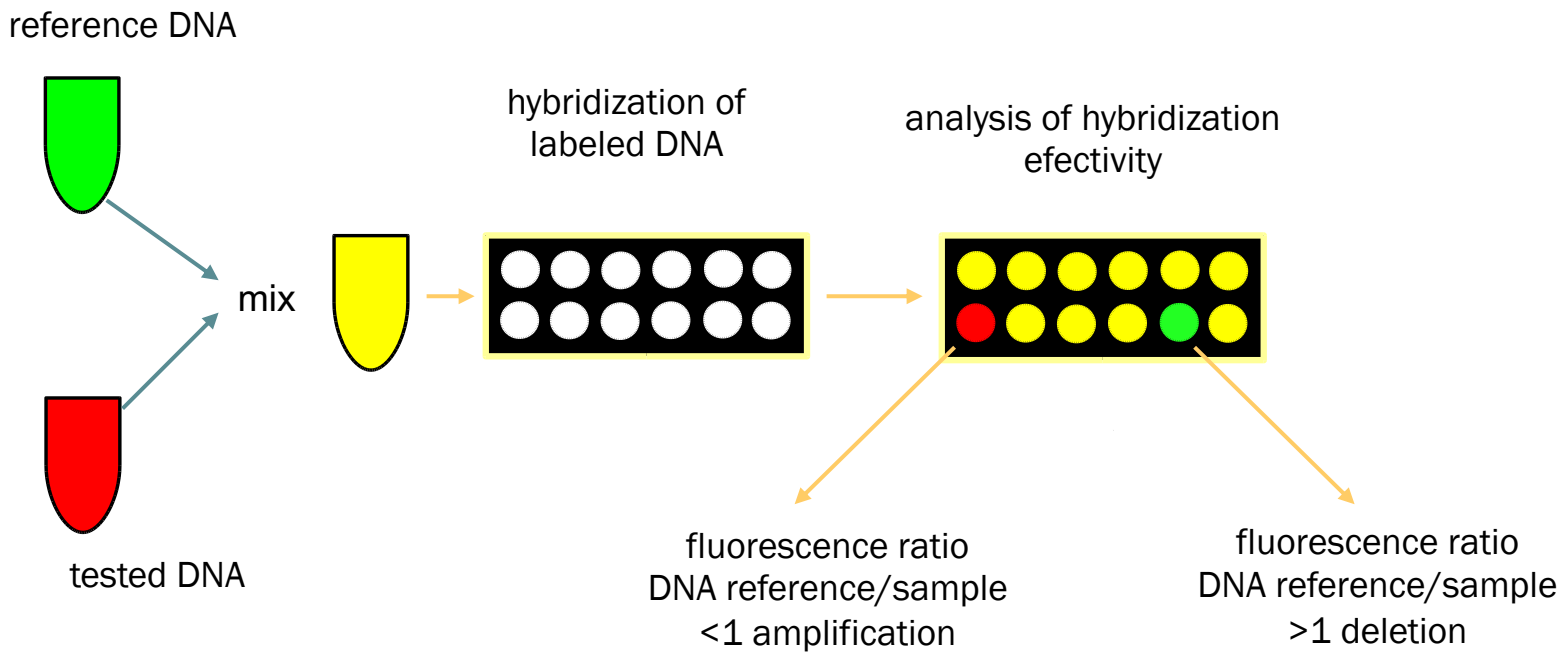
GENOMIC ARRAYS

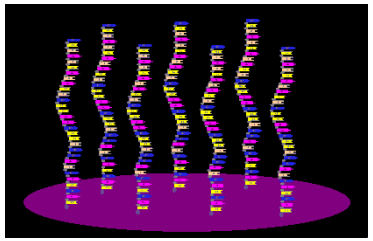
- Molecular cytogenetic technique for detection of genomic gains and losses
- Detection of copy-neutral loss of heterozygosity
- Not possible to detect balanced rearrangements
- Precise breakpoint localization, identification of affected genes
- High resolution, genome-wide
- Working with DNA, no need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

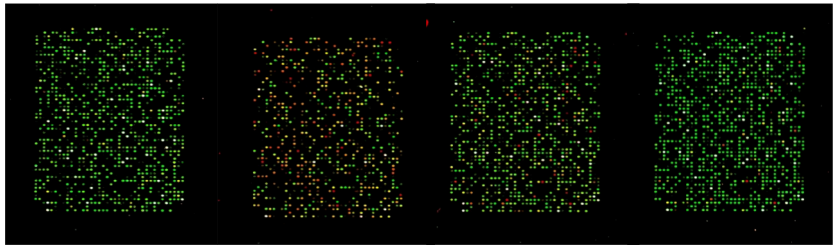


GENOMIC ARRAYS

ARRAY-BASED COMPARATIVE GENOMIC HYBRIDIZATION (ACGH)

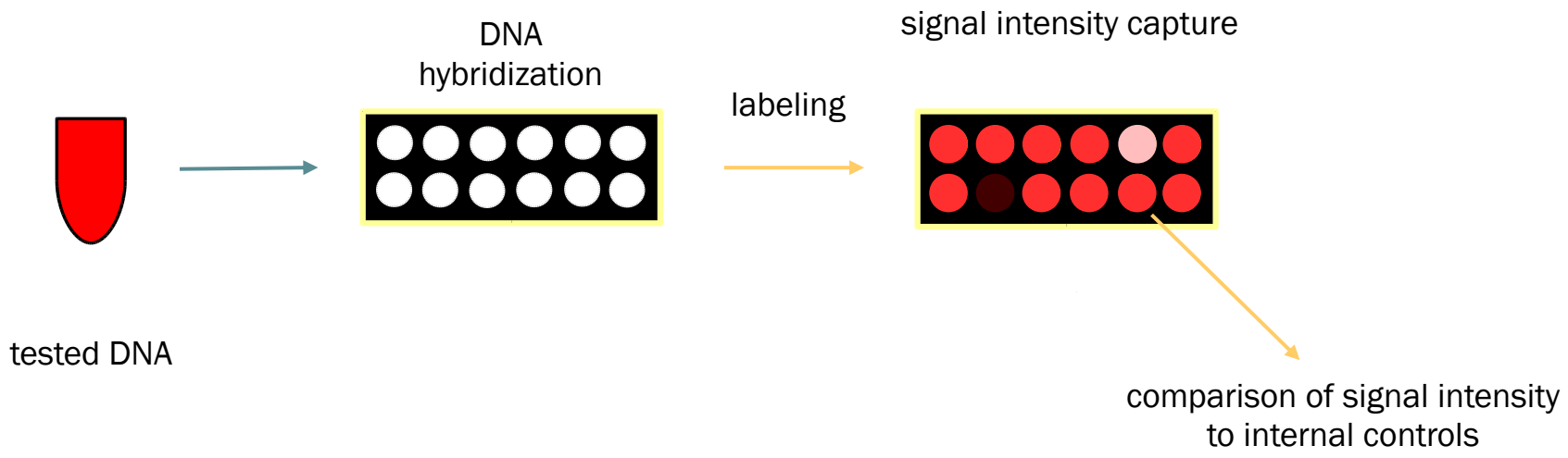


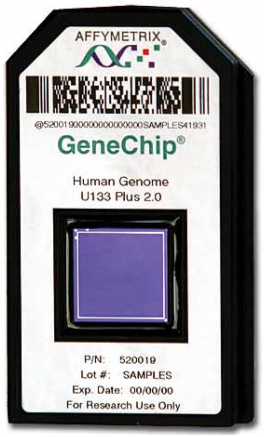


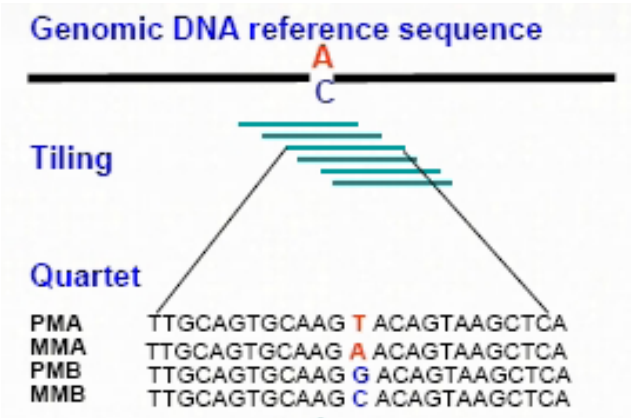


GENOMIC ARRAYS

SINGLE NUCLEOTIDE POLYMORPHISM (SNP) ARRAY





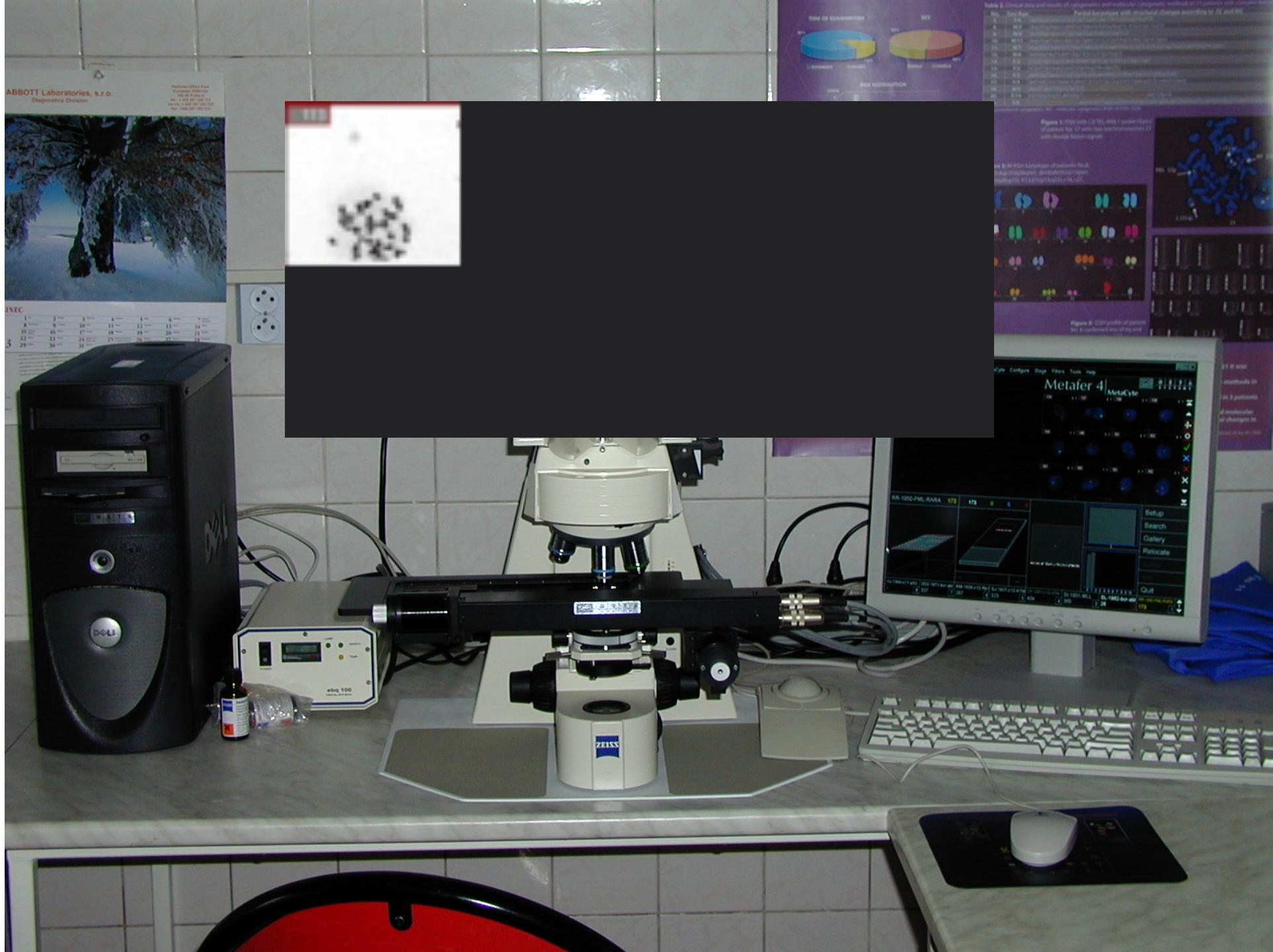


EQUIPMENT FOR GENOMIC ARRAYS

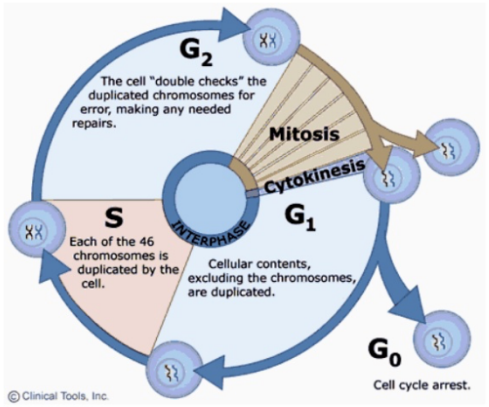
Affymetrix GeneChip System

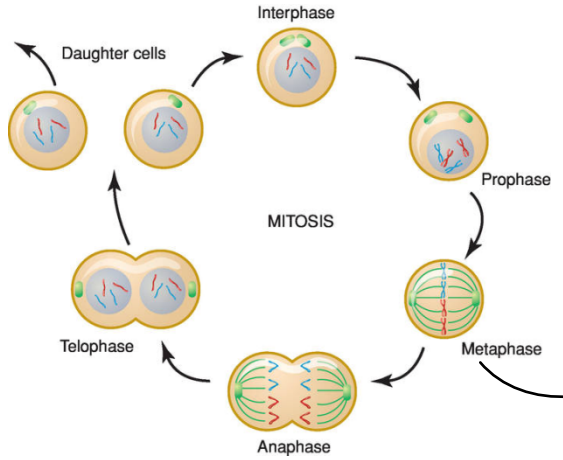
CYTOGENETICS LAB

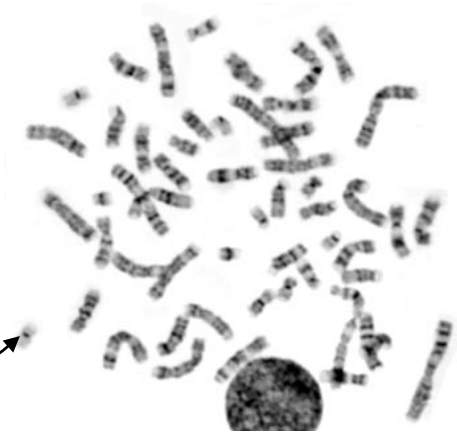
- Primary sample processing
- Cell culturing
- Methods not requiring PCR
- Imaging methods

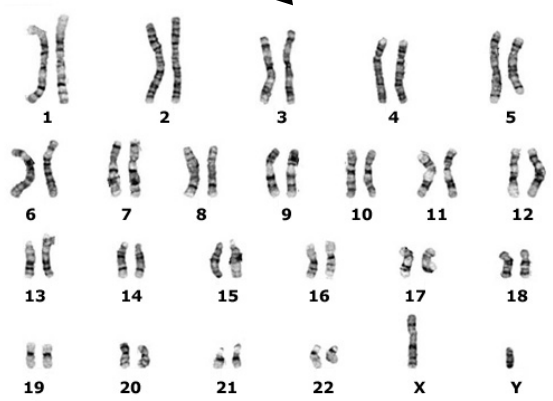


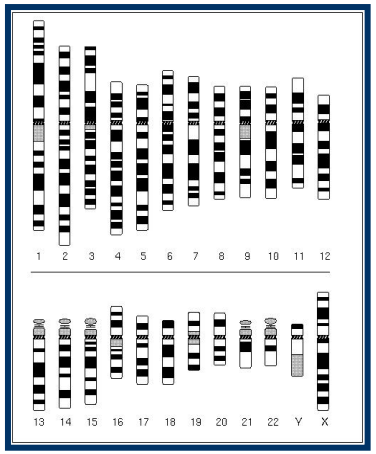
CLASSICAL CYTOGENETICS – CHROMOSOME BANDING TECHNIQUES

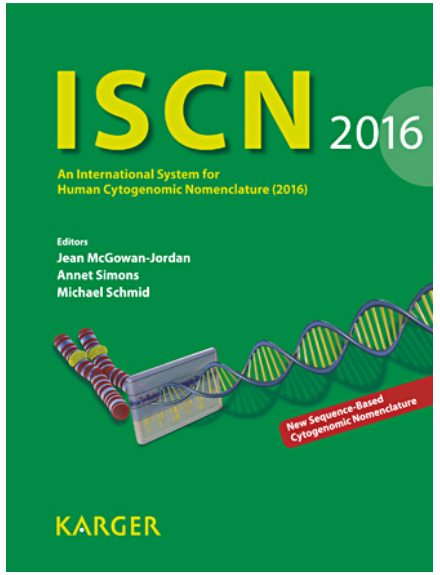








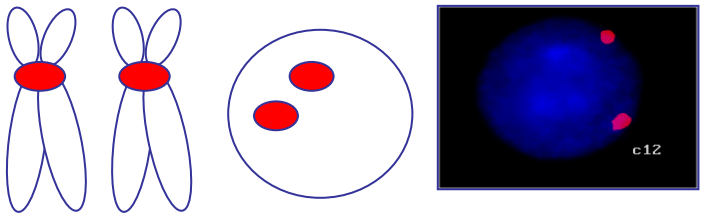




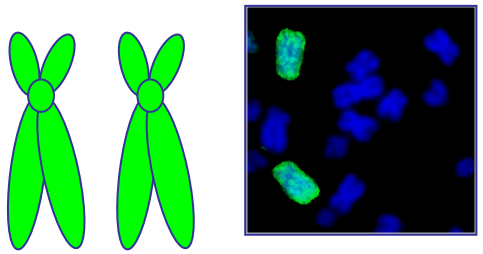
MOLECULAR CYTOGENETICS

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

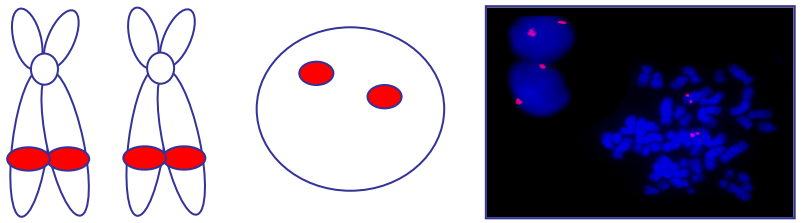
Probe types:



centromeric



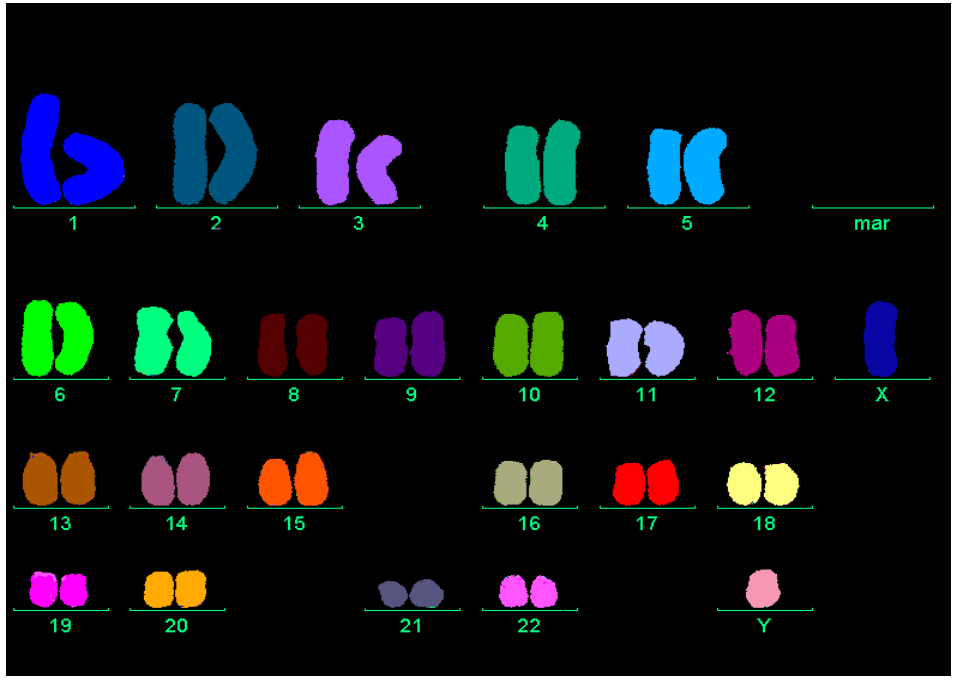
whole chromosome



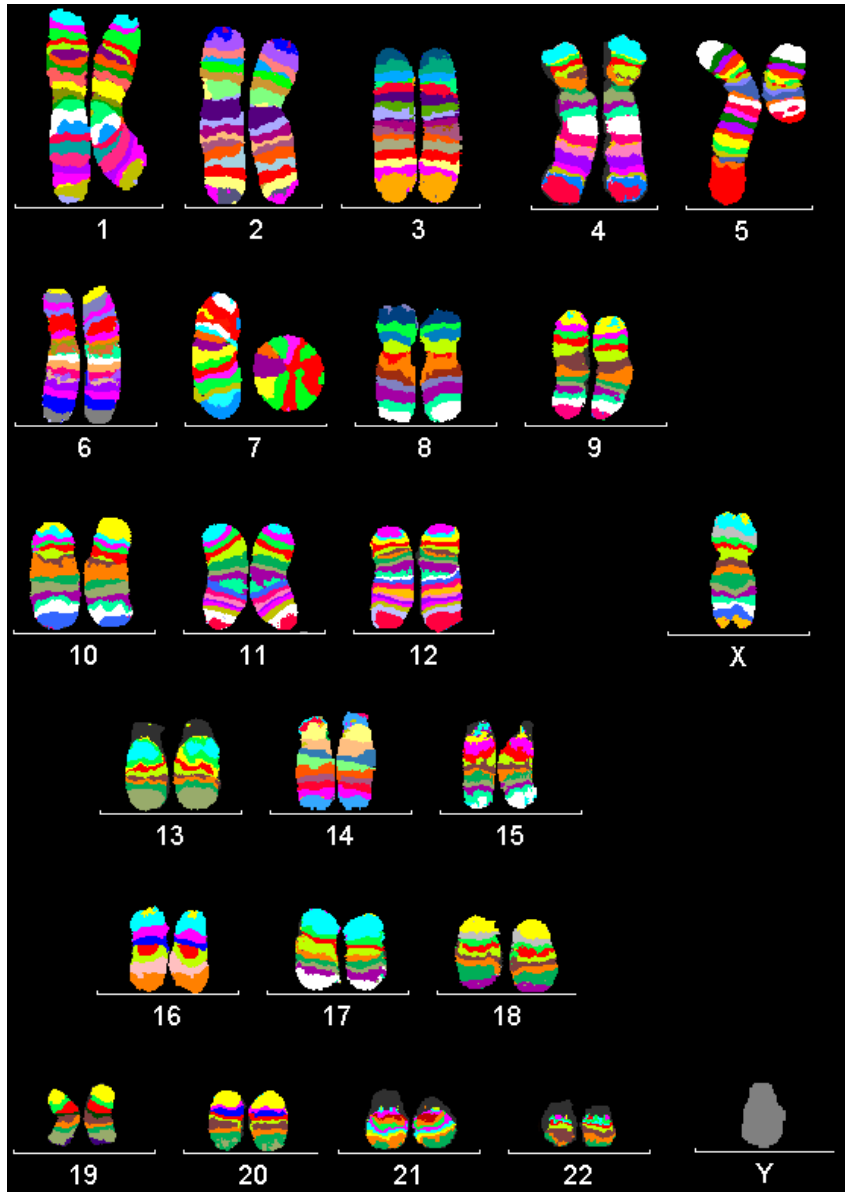
gene

MOLECULAR CYTOGENETICS

- FISH methods for genome-wide analysis



mFISH



mBAND

THE END...

Contact:
karla.plevova@mail.muni.cz or plevova.karla@fnbrno.cz
Internal Medicine – Hematology and Oncology, University Hospital Brno
CEITEC MU

Thank you for your attention!