MUNI
MED

Molecular genetics and cytogenetics laboratory and methods

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Statement

This presentation is intended exclusively for educational purposes.

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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?

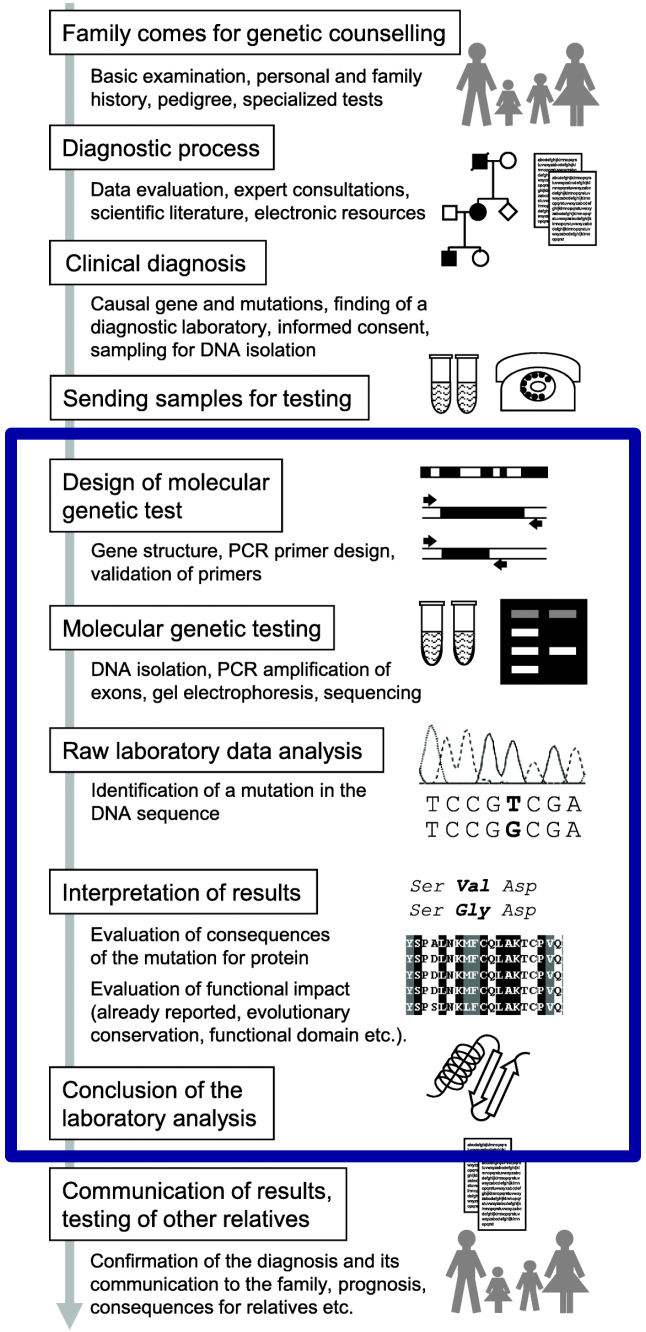
MGC LAB = PARTNER

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!

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	

Result of a laboratory test

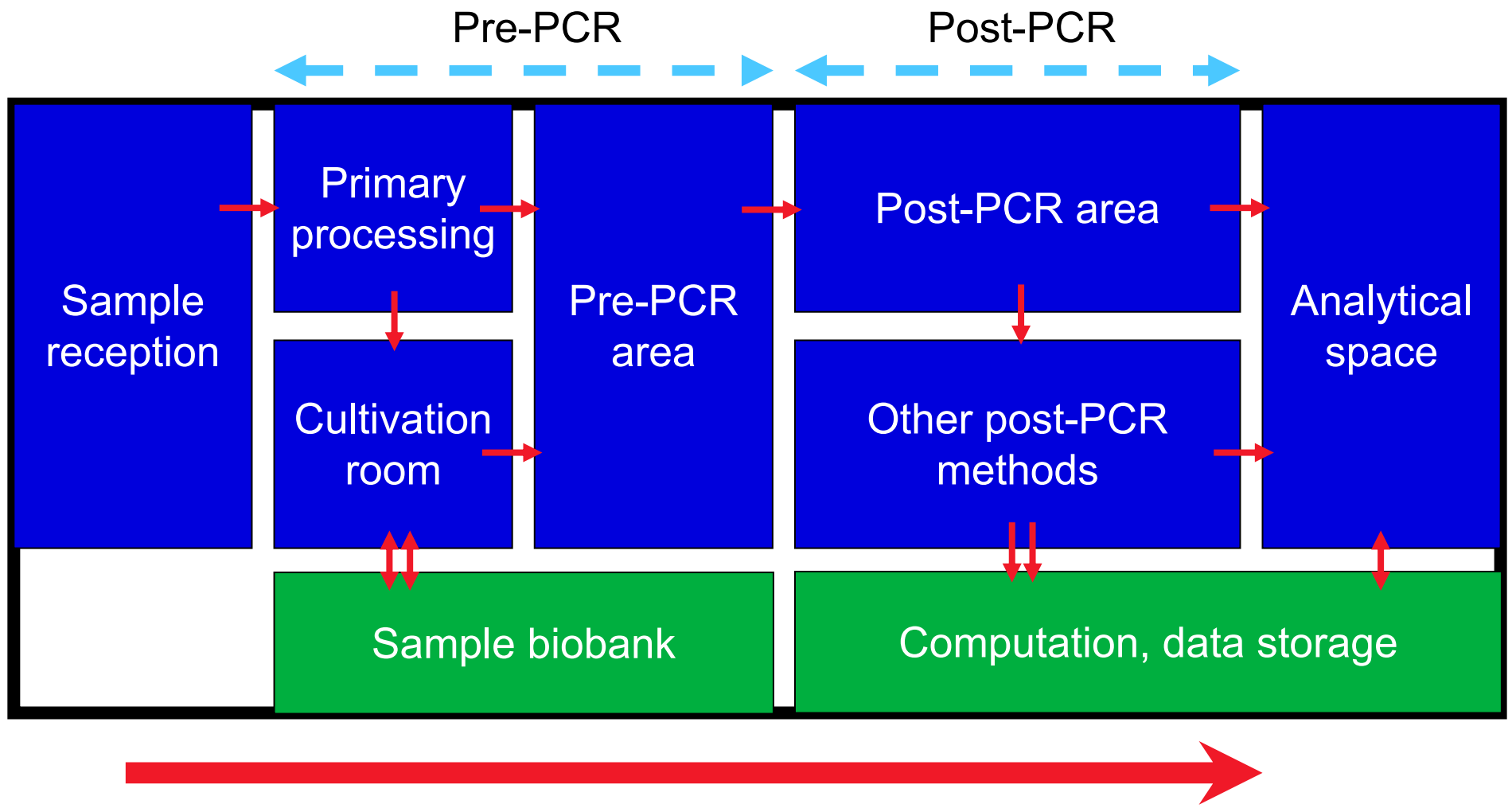
The report is an essential part of any laboratory test

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

How does it look the MG lab?



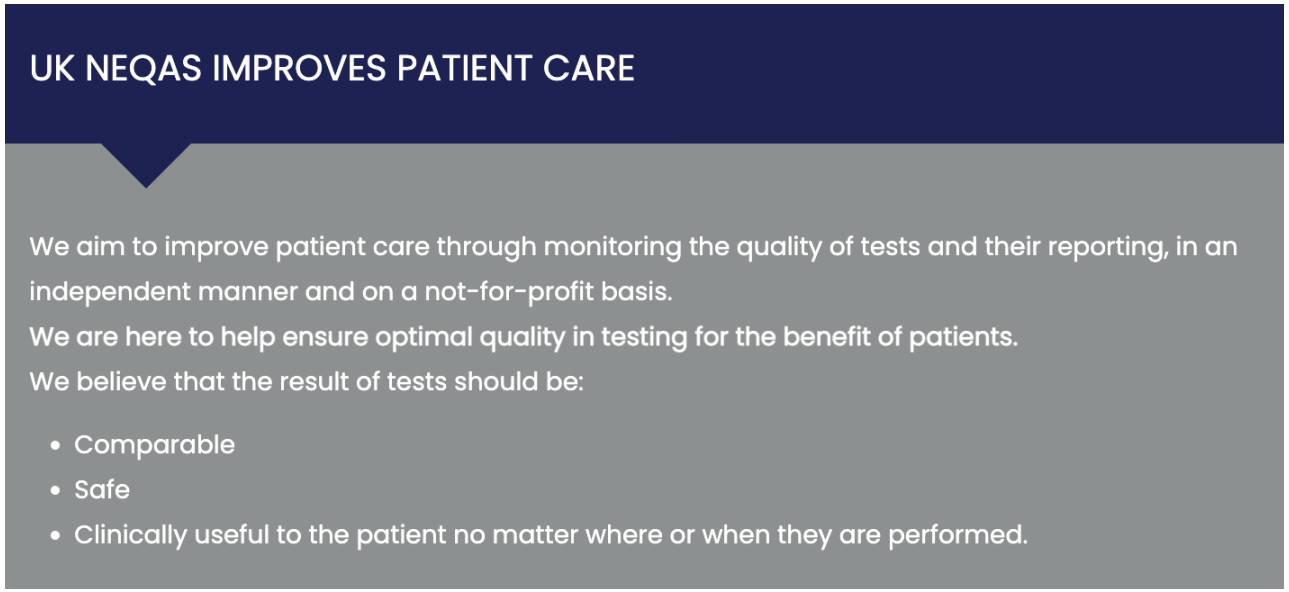
Quality control requirements

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

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

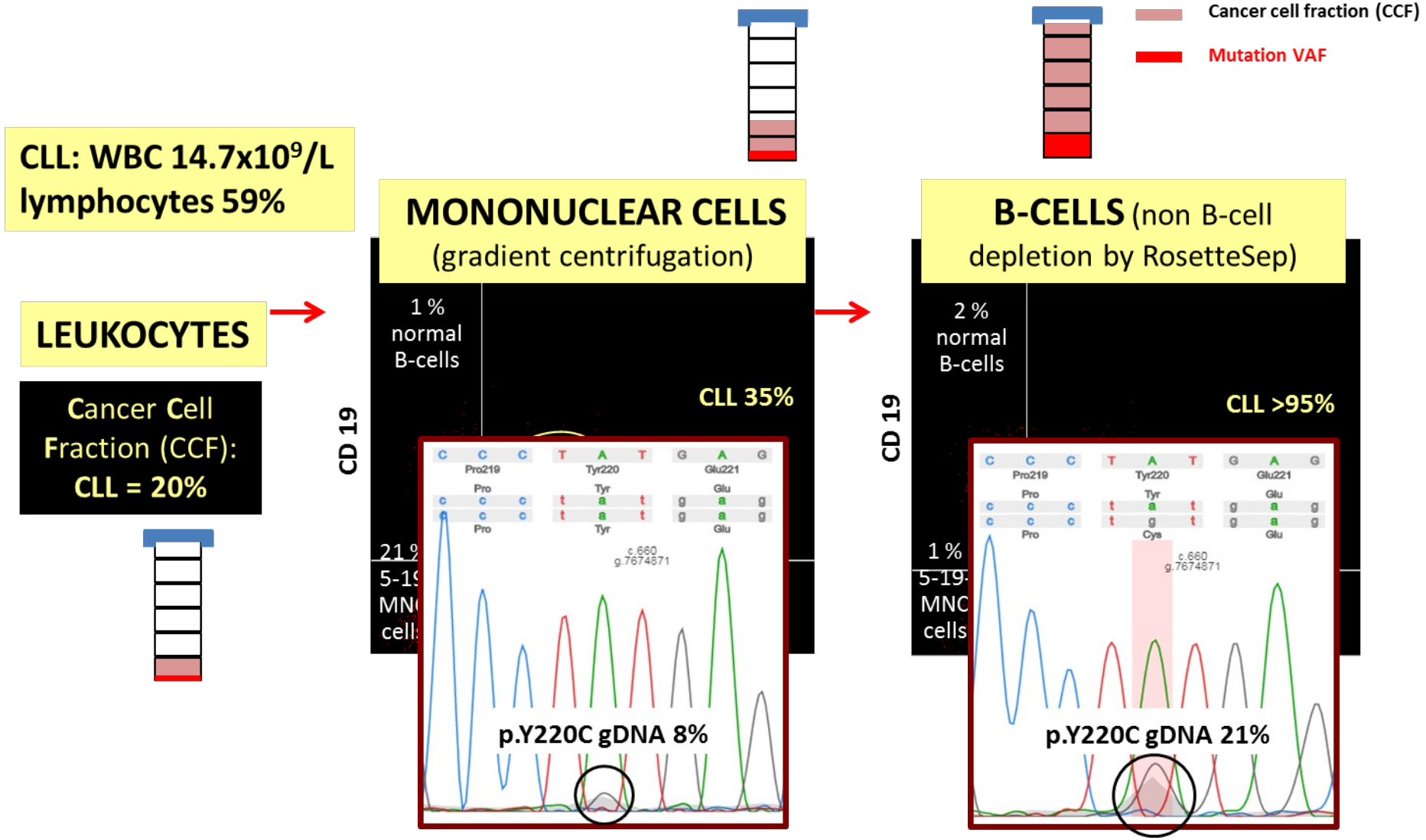
Granulocytes

Lymphocytes

Specific cell subpopulations

Why to perform cell separation?

Example of *TP53* gene testing in chronic lymphocytic leukemia

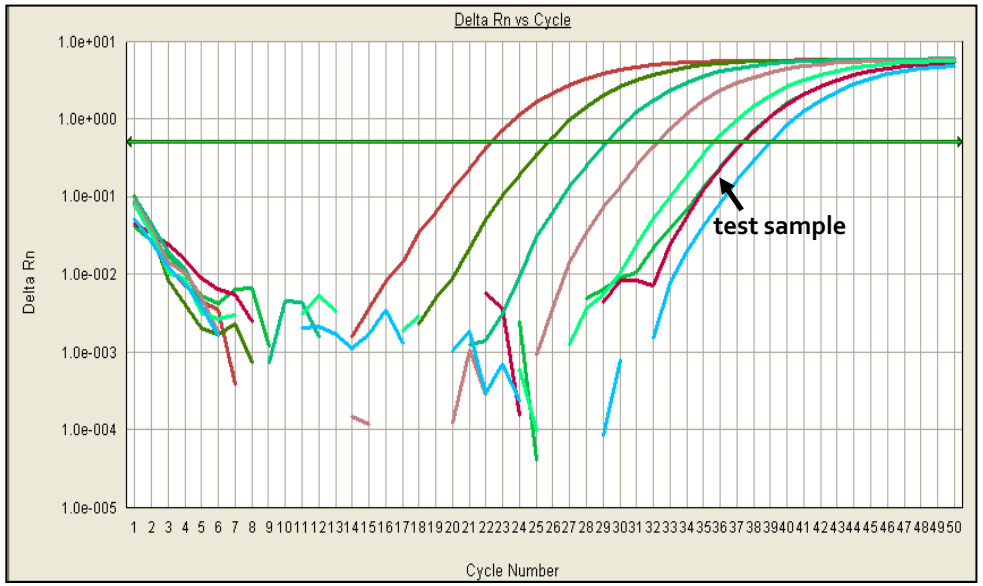


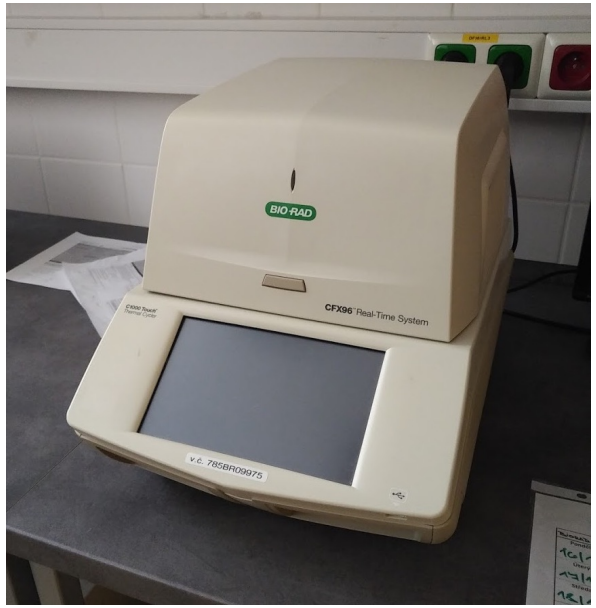
Polymerase chain reaction (PCR)

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



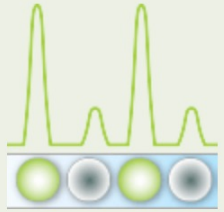


Droplet digital PCR (ddPCR)

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

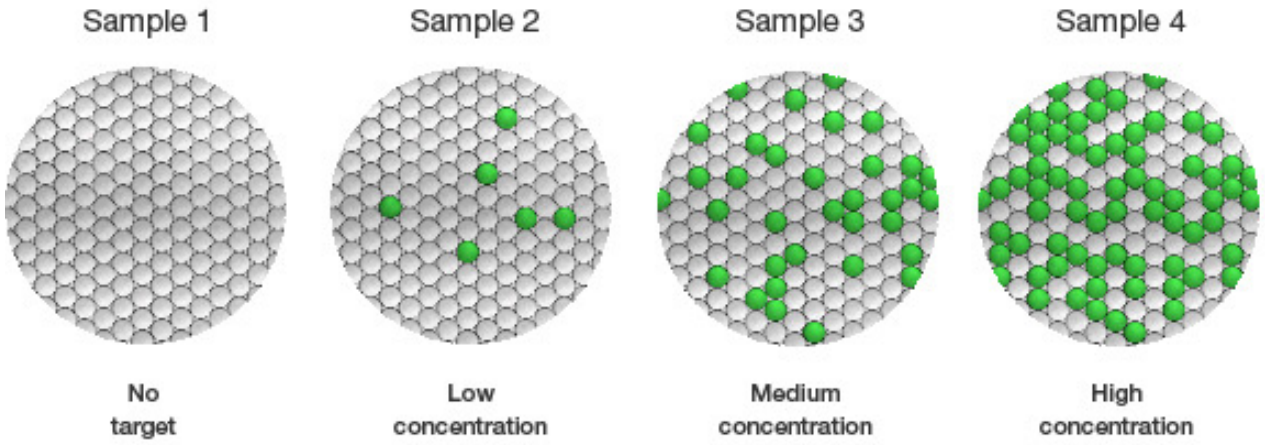


Positive droplets are counted to give precise quantification of target sequences in sample

Alternative method for marker absolute quantification

Highly precise

Need for specific instrumentation



Next-generation sequencing (NGS)

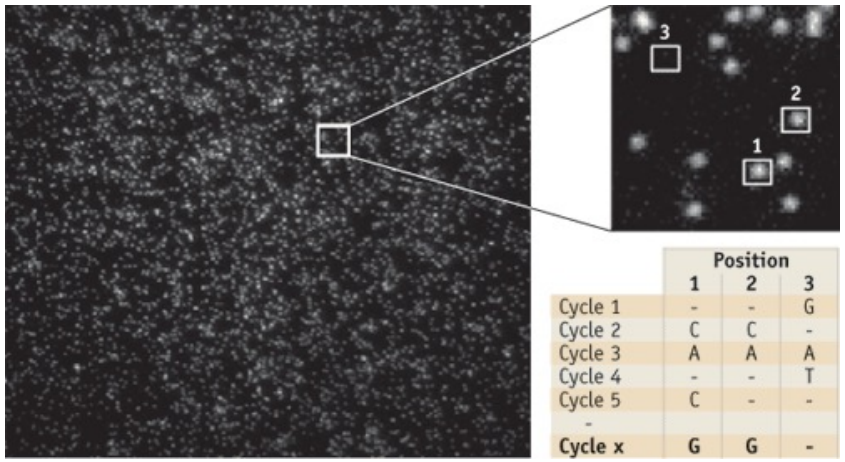
~ massively parallel sequencing (MPS)

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

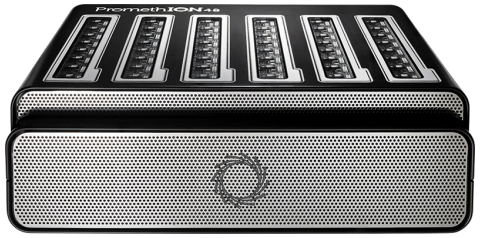
iSeq

0.01 genome/rin,
1.2 GB/run

New sequencing machines on the market

Element Biosystems

Complete Genomics



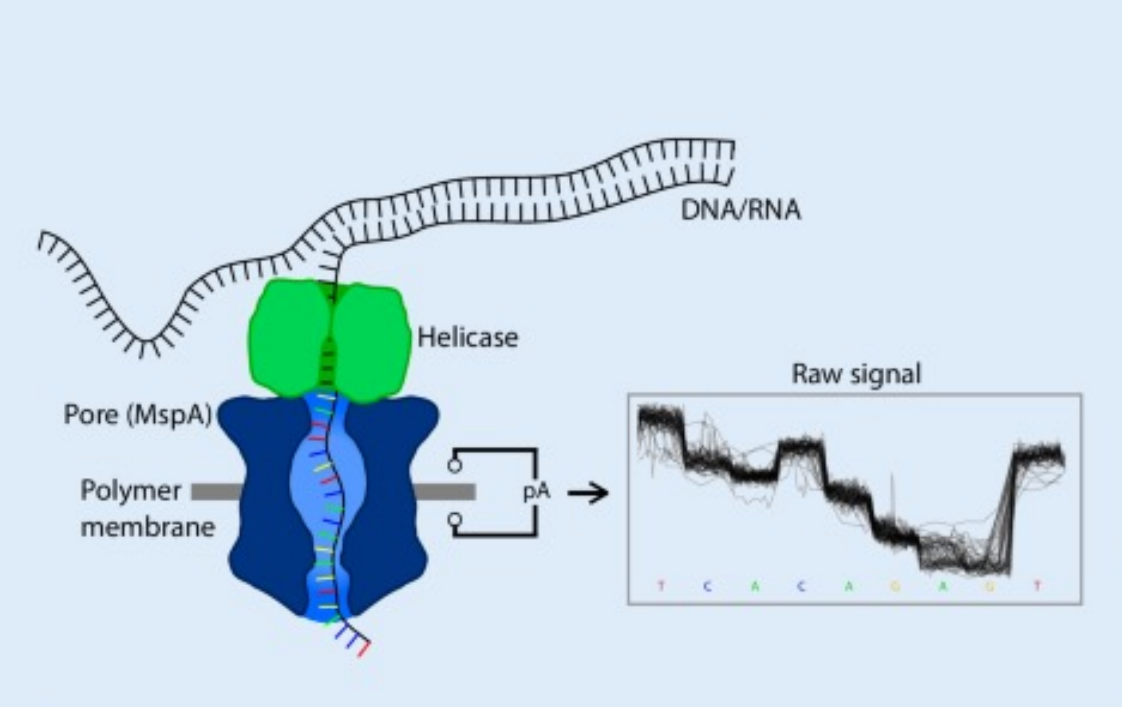
Oxford Nanopore Technologies

Singular Genomics

Sequel System

PacBio

Short-read vs. long-read sequencing



Kraft & Kurth, Medizinische Genetik 2019

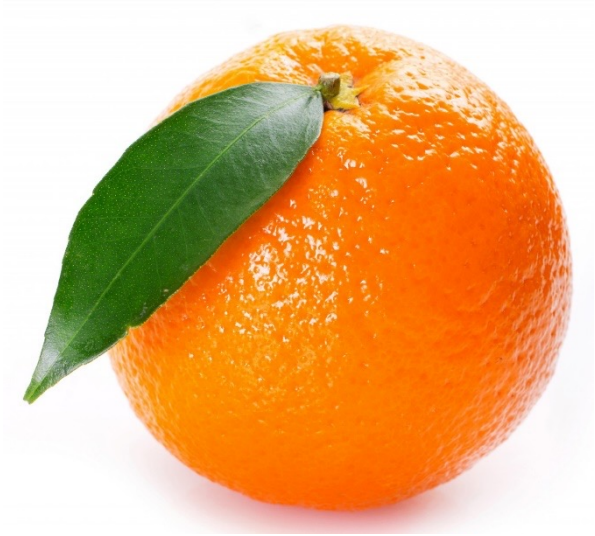
B Whole-genome short read data

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

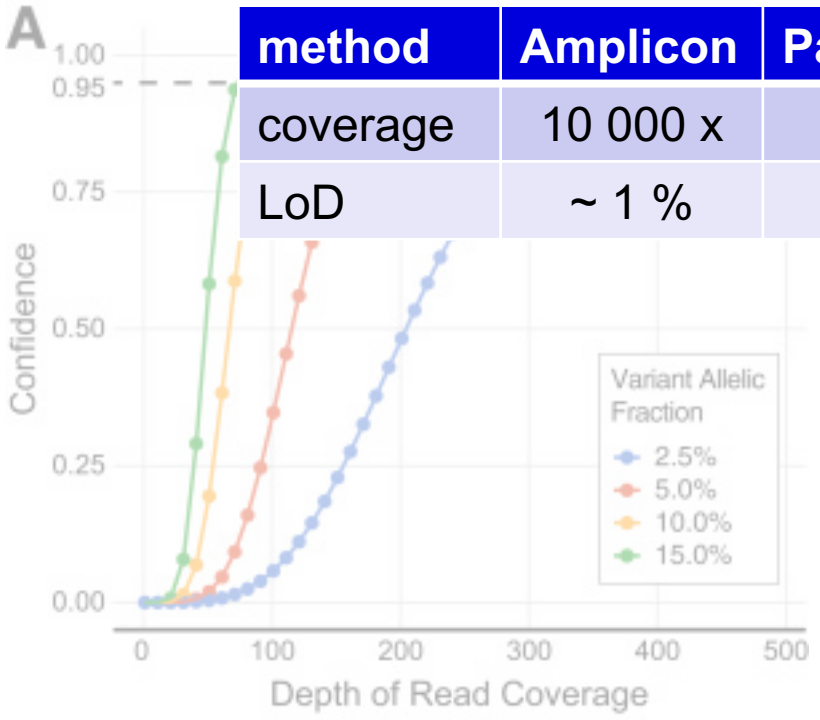
< 100 genes
≥ 1000 x read depth

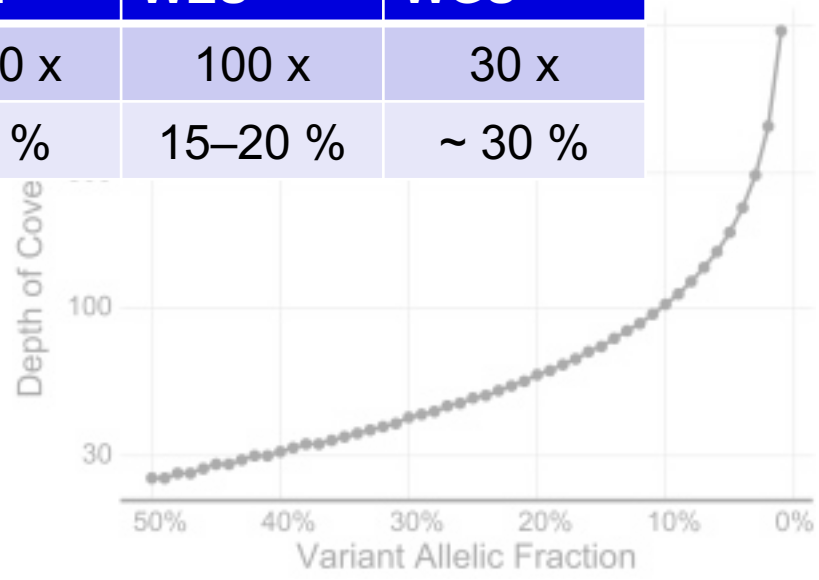
Practical example

Limit of detection (LoD) of various NGS methods

In theory… (binomial sampling statistics)

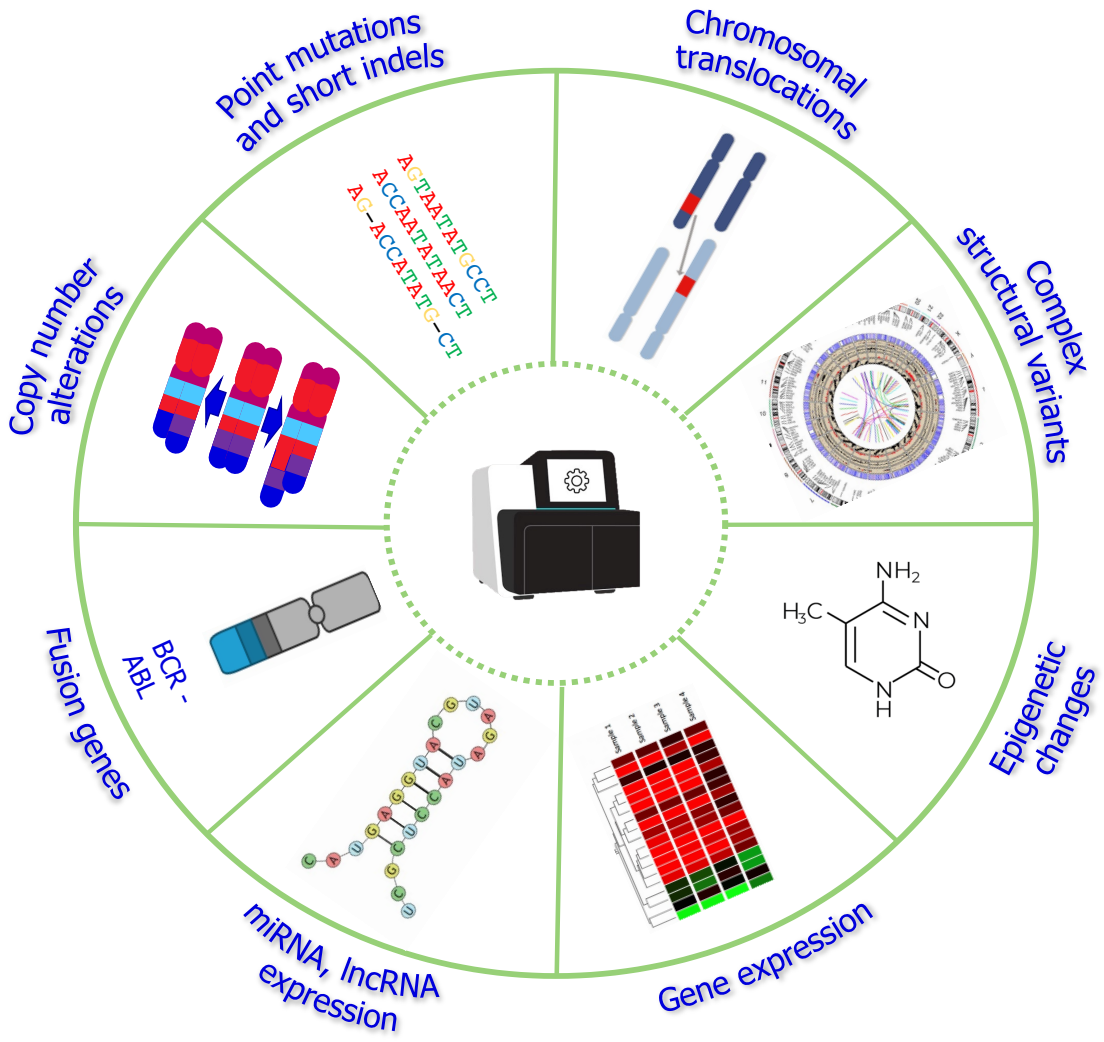
method	Amplicon	Panel	WES	WGS
coverage	10 000 x	1000 x	100 x	30 x
LoD	~ 1 %	~ 5 %	15–20 %	~ 30 %





McNulty et al, J Mol Diagn 2020

NGS – diverse targeted markers



Panel NGS

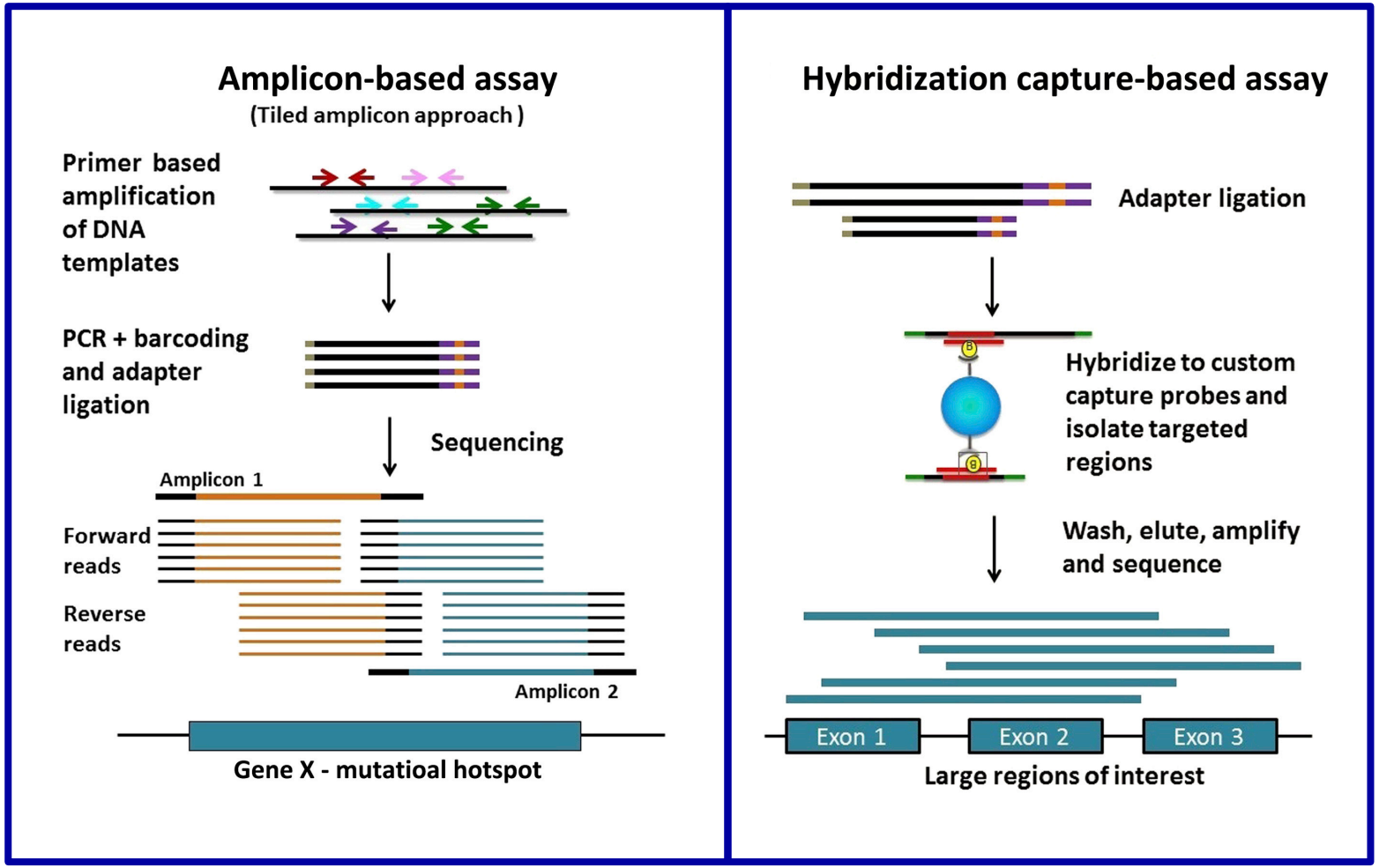
Sets of selected regions of interest

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



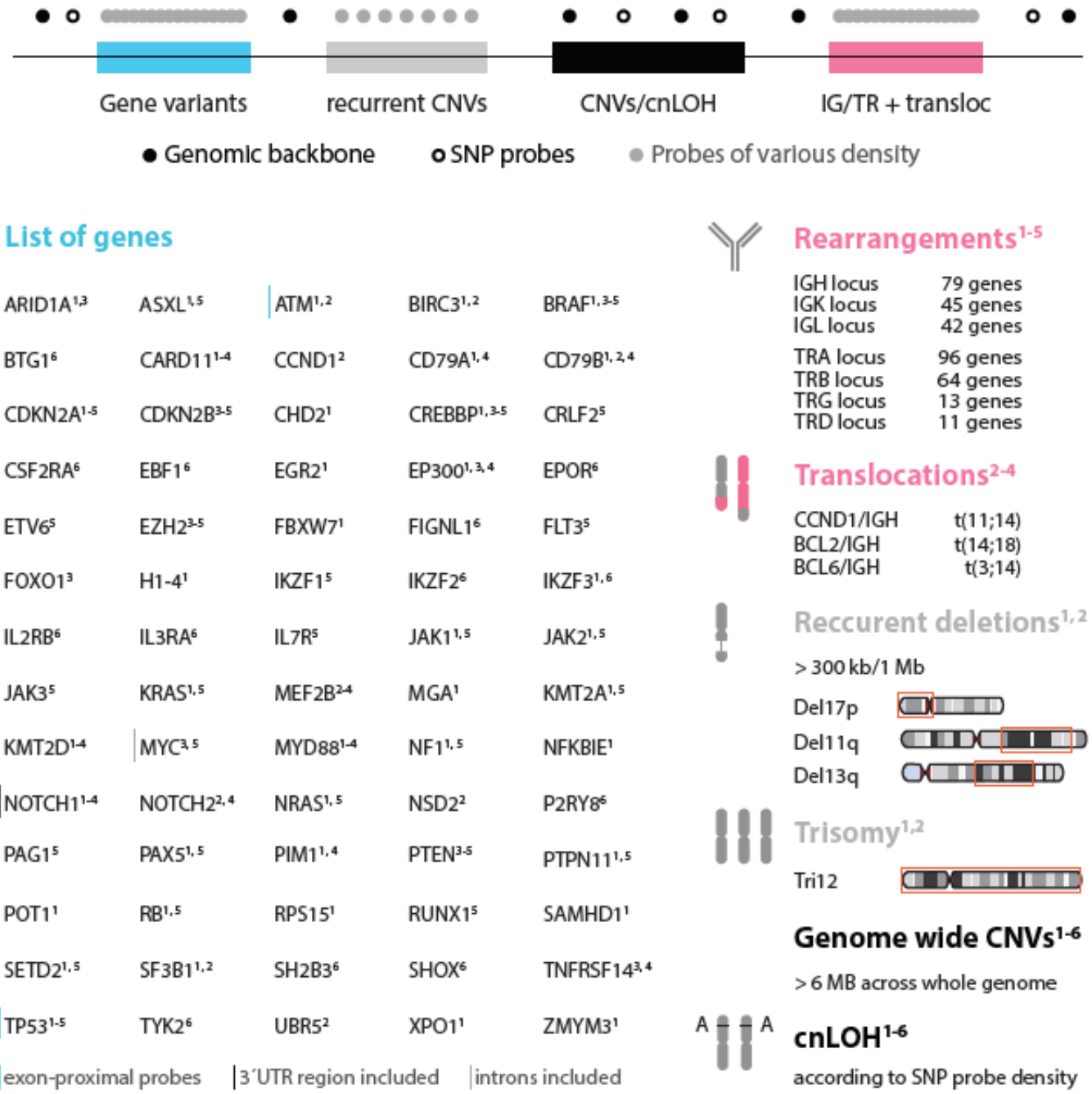
Jennings et al, J Mol Diagn 2017

Practical example

LYNX panel

– diagnostics of molecular markers in lymphoid malignancies

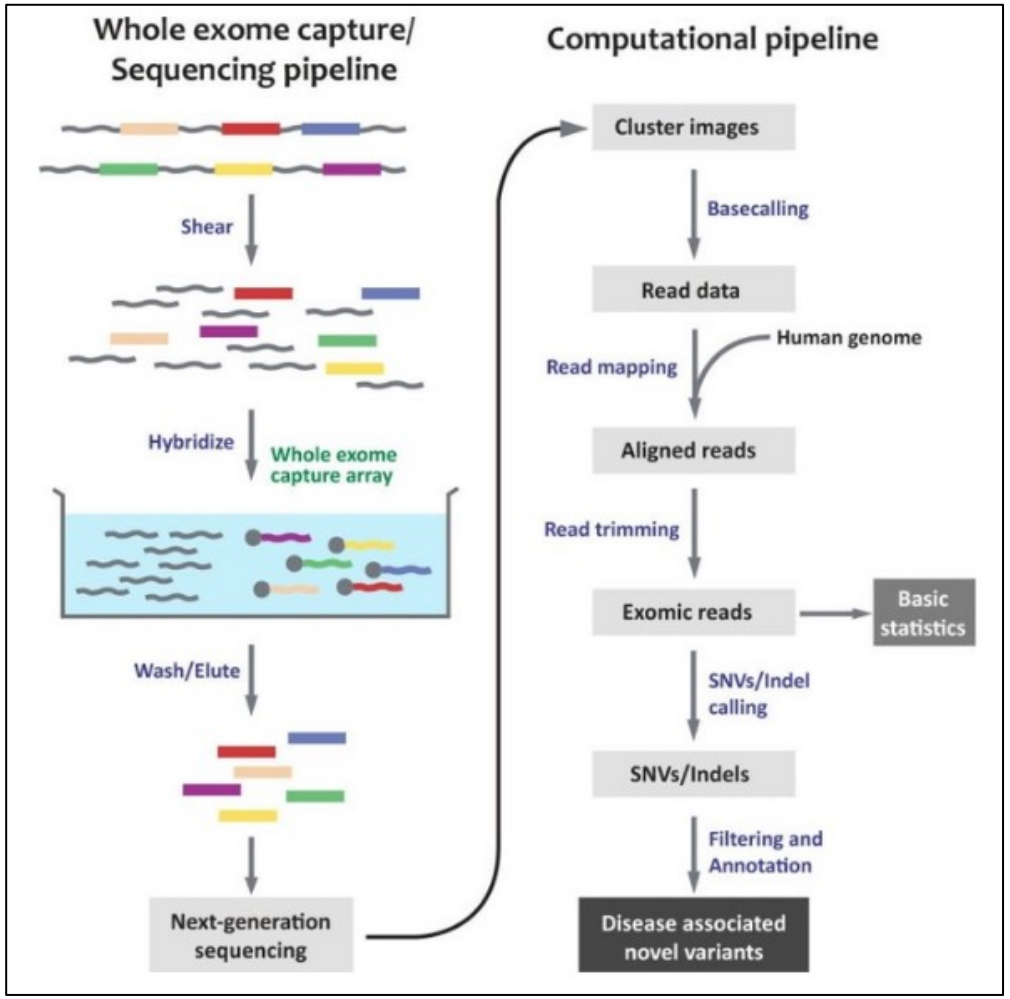
[1]CLL, [2]MCL, [3]FL, [4]DLBCL, [5]ALL, [6]Ph-like ALL



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)

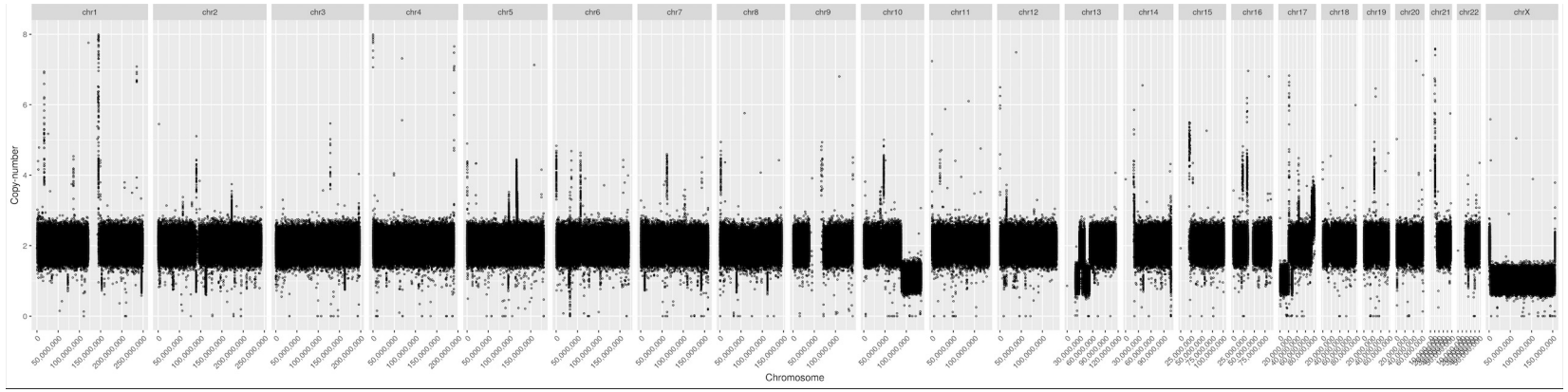
Mainly experimental method for exploring unknown variants

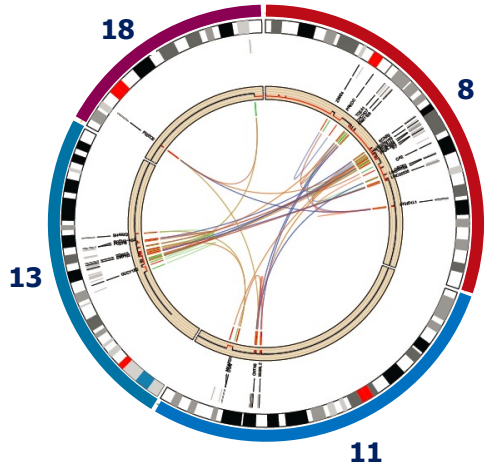
Applications similar to WES, additional information about non-coding regions and chromosomal abnormalities

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

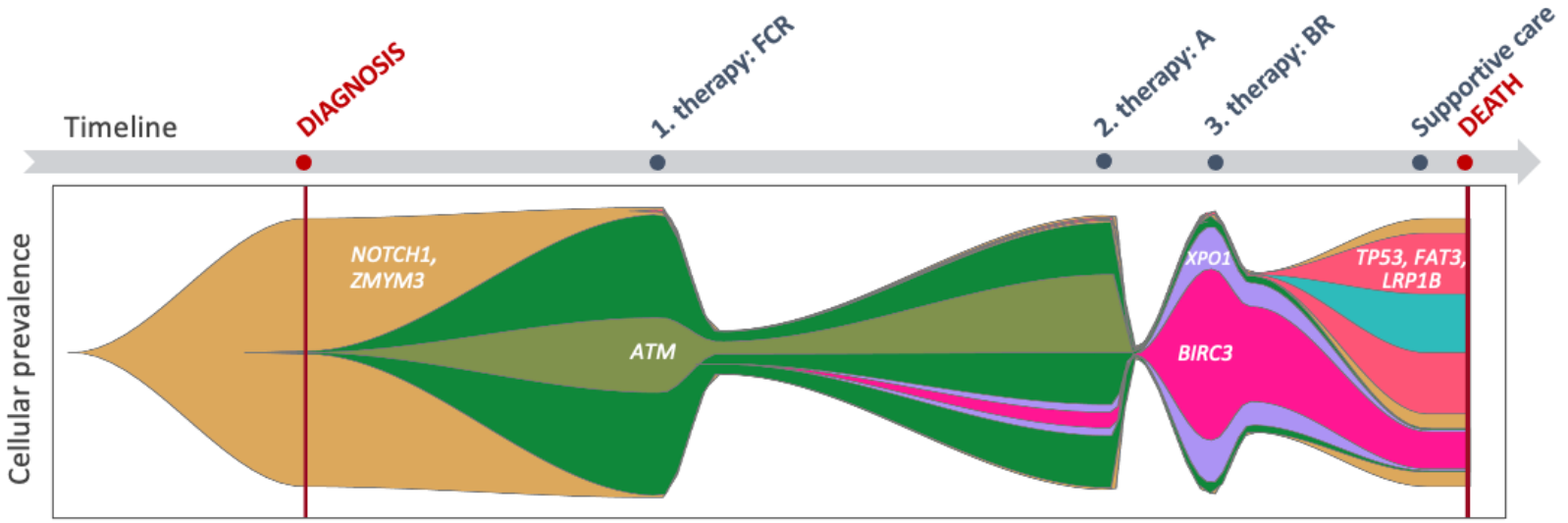




Practical example

Benefits of NGS in cancer diagnostics and monitoring

Reconstruction of clonal architecture and cancer evolution

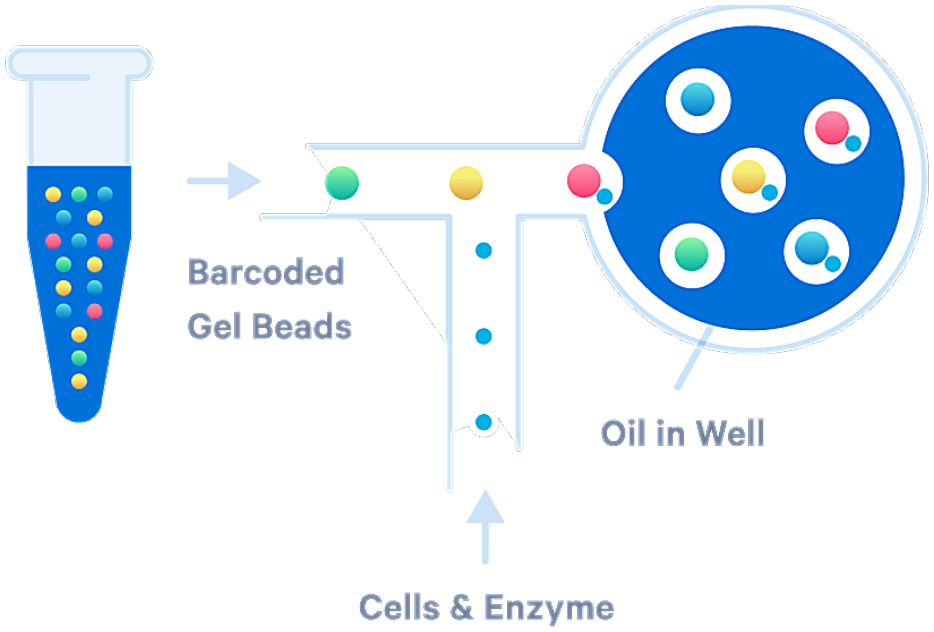


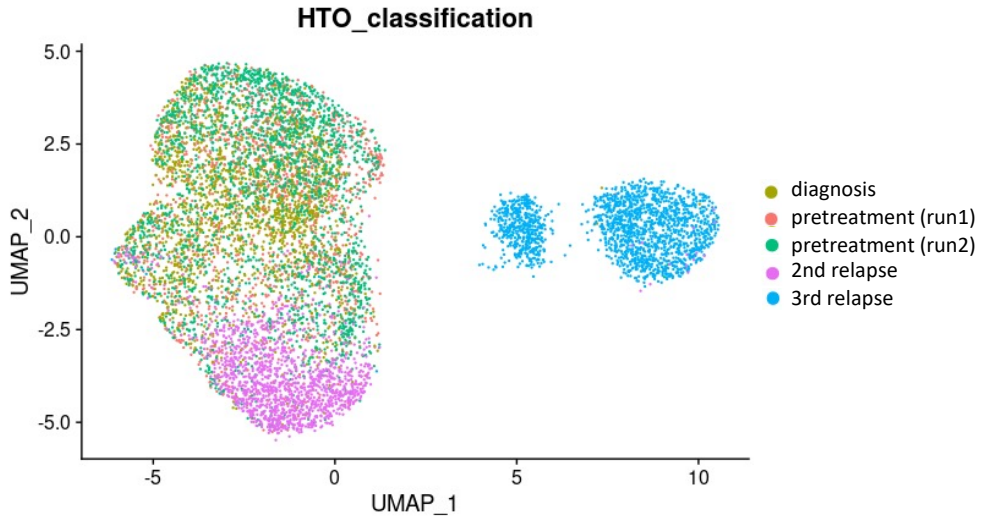
Need for multidisciplinary team

– biologists, geneticists, computational scientists, bioinformaticians, statisticians

Single-cell technologies

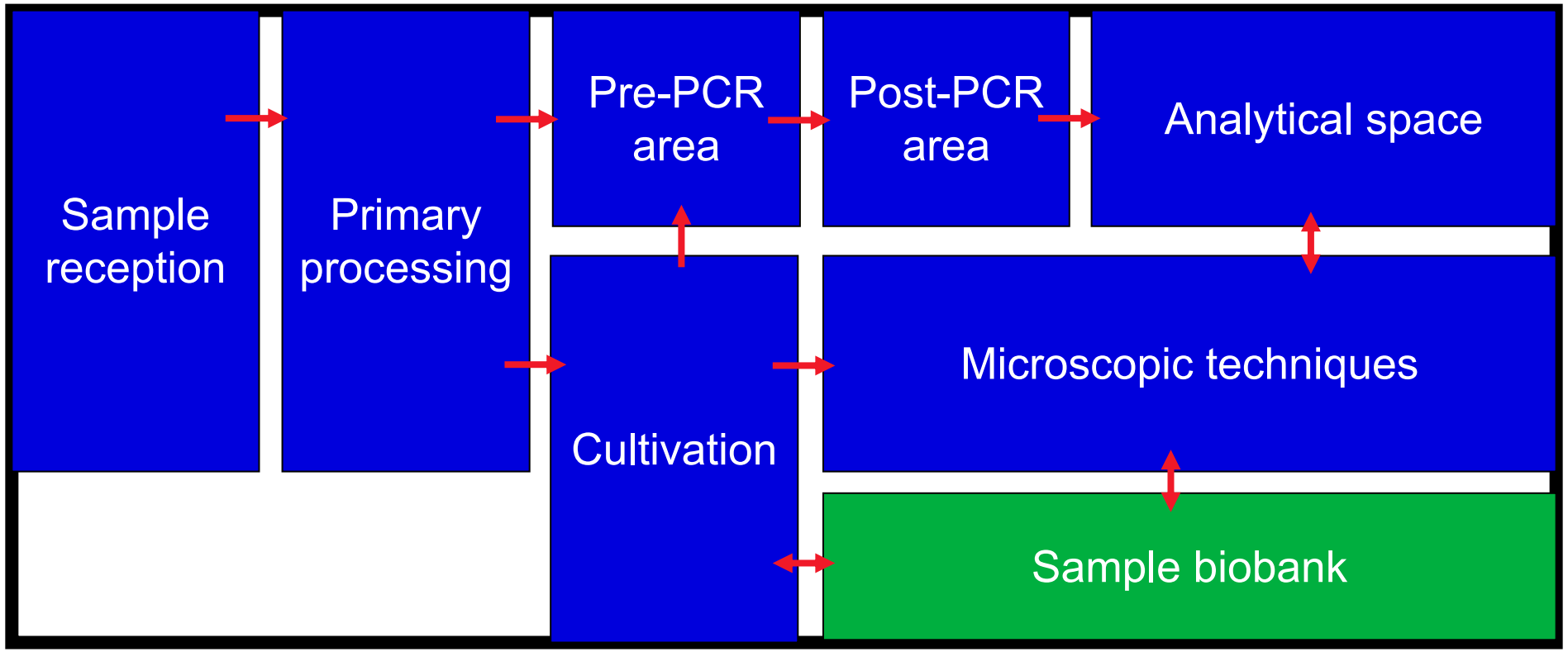
Experimental methods
Applications in cancer research, immunology, developmental biology, …
Analysis of DNA variants, chromatin activity, RNA expression profiles, protein expression, …
Coexistence of cellular features in single cells





https://www.10xgenomics.com/

Cytogenomics lab



Cytogenomics methods

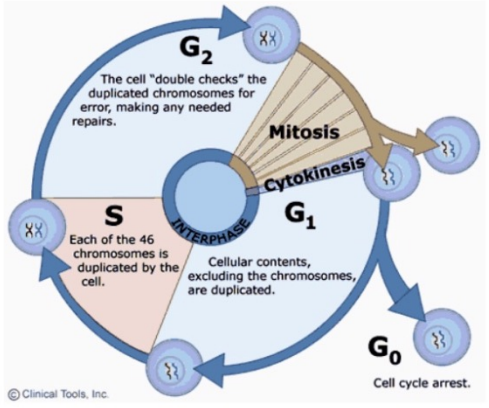
Comparison of sensitivity of the techniques

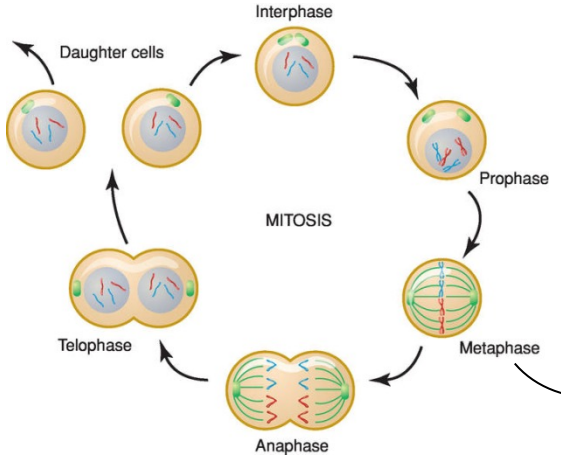
	Aneu-ploidy	CNA	Poly-ploidy	Clonal heterogeneity	Focal amplification	Balanced rearrangements	Unbalanced rearrangements	cn-LOH
Classical cytogenetics	+++	+	+++	+++	++	+++	+++	-
Interphase FISH	+++	++	+	+++	+++	+++	++	-
ArrayCGH	+++	++	-	+	+++	-	++	-
CGH+SNP array	+++	+++	+	+	+++	-	++	++
SNP array	+++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

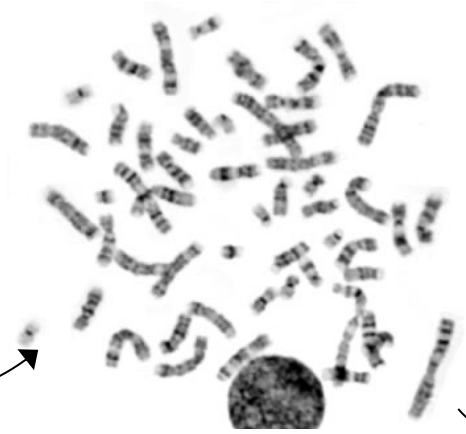
CNA – copy number alteration, gains or lossess of genetic material
cn-LOH – copy neutral loss of heterozygozity

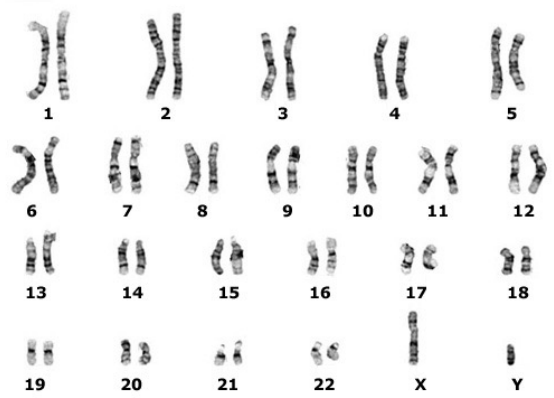
Schoumans J et al, 2016

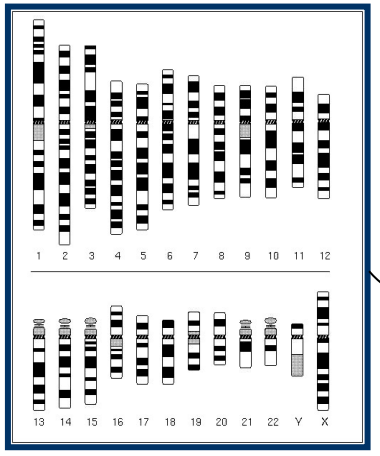
Classical cytogenetics
chromosome banding techniques

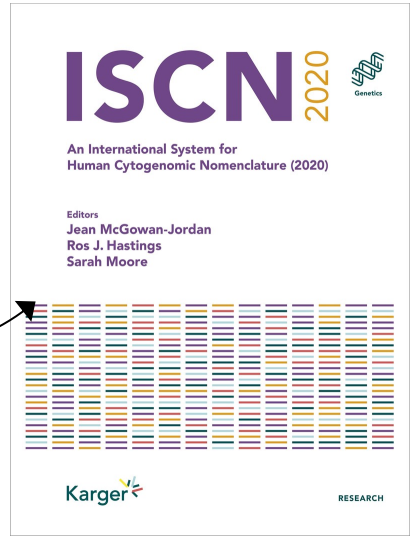












Methods not requiring PCR
Imaging methods
Cheap
Clonal composition assessment

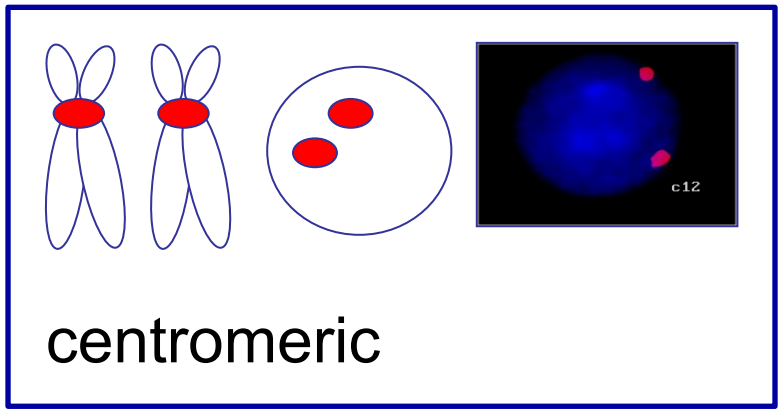
Molecular cytogenetics

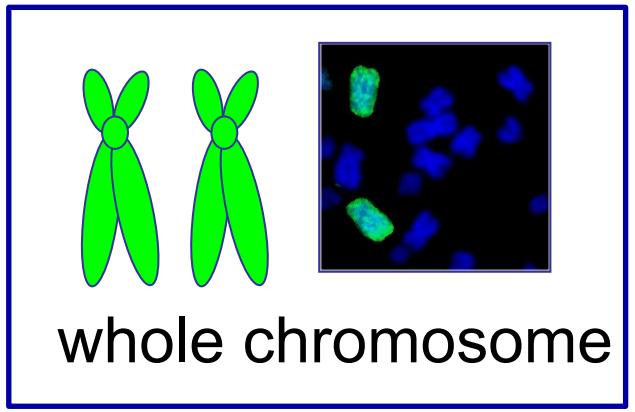
Fluorescent in situ hybridization (FISH)

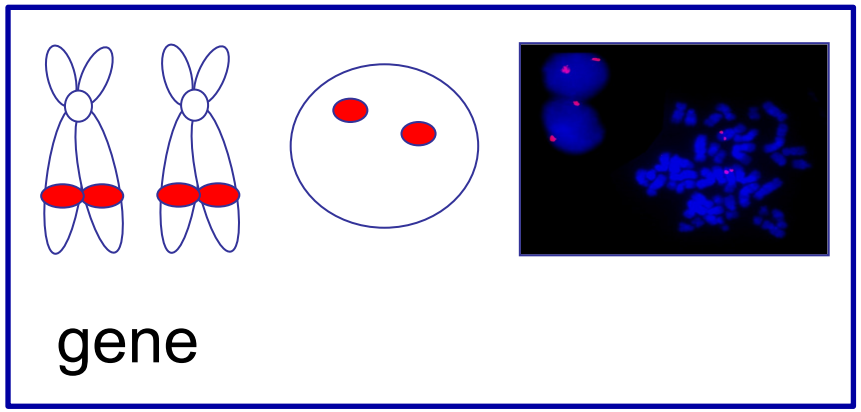
Targets specific regions based on DNA sequence

Detection of chromosomal abnormalities with diagnostic, prognostic and predictive value

Probe types:

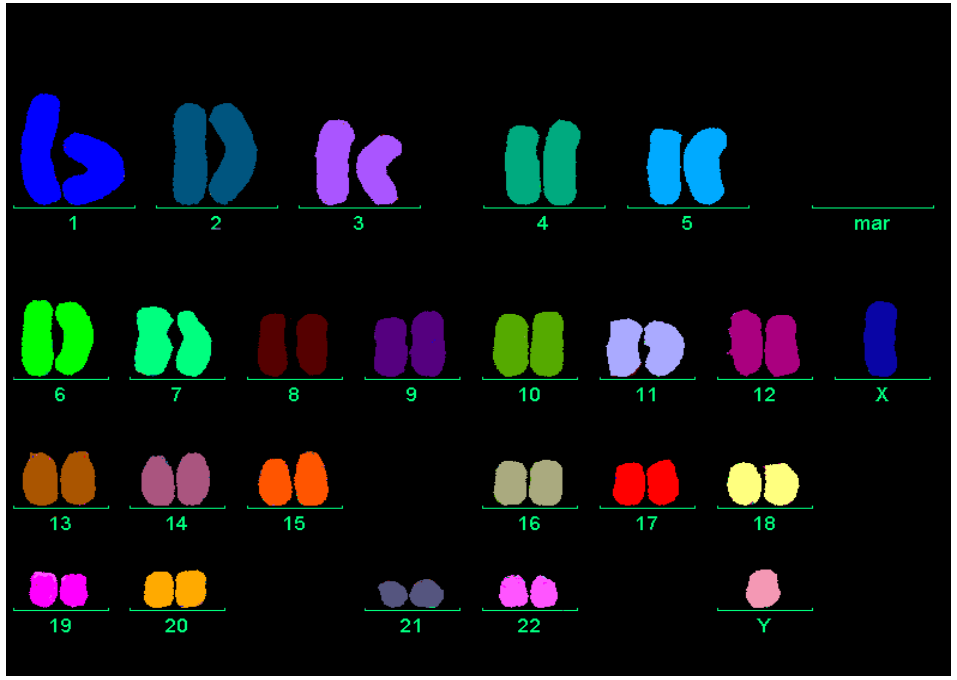




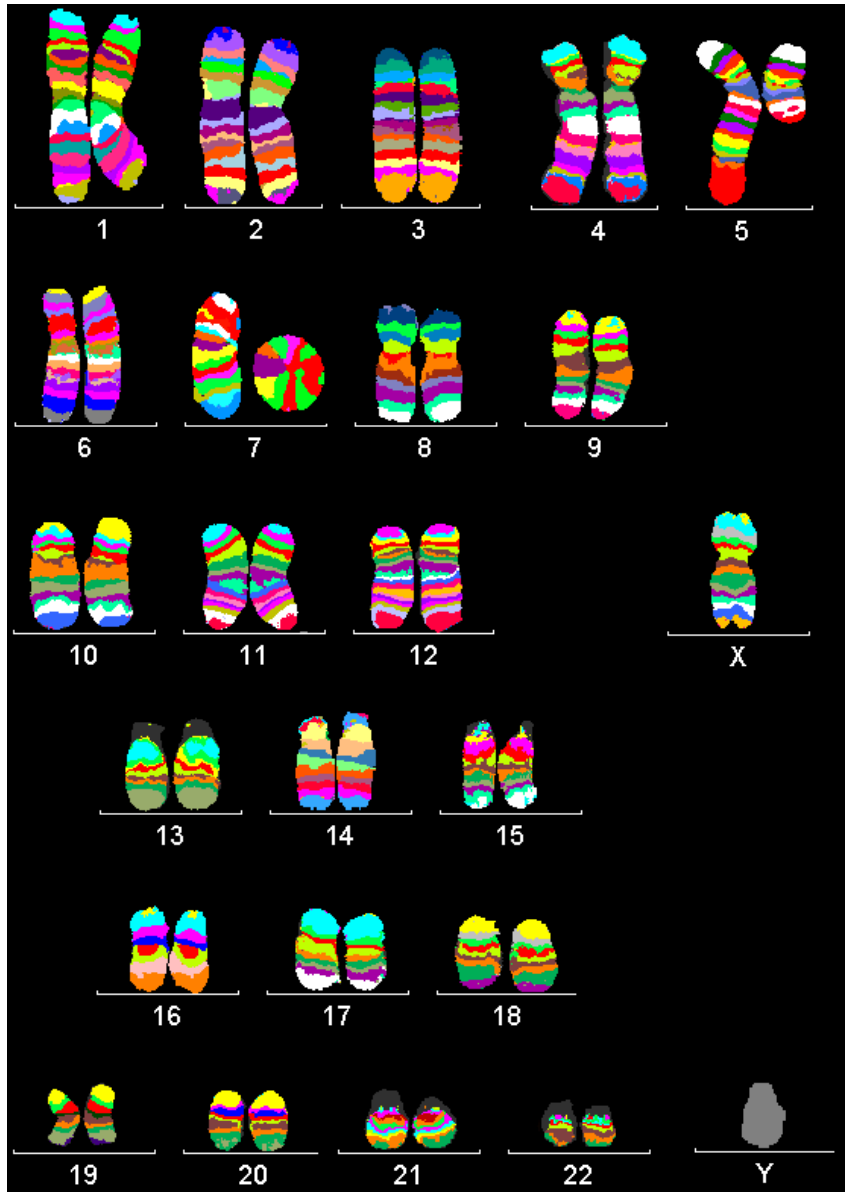


Molecular cytogenetics

FISH methods for genome-wide analysis



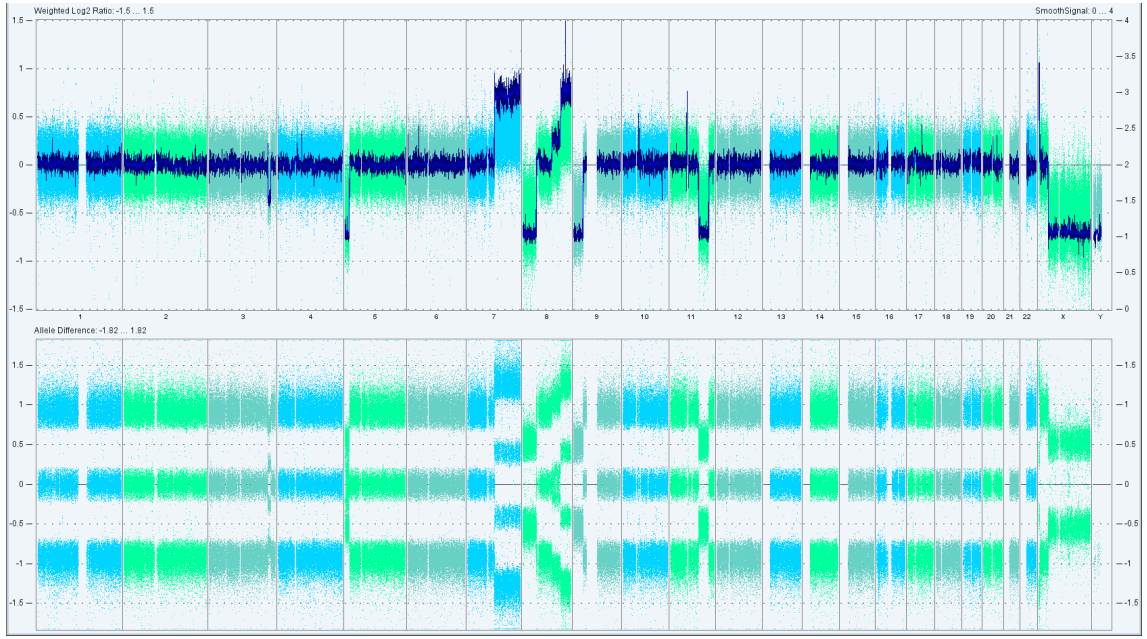
mFISH



mBAND

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



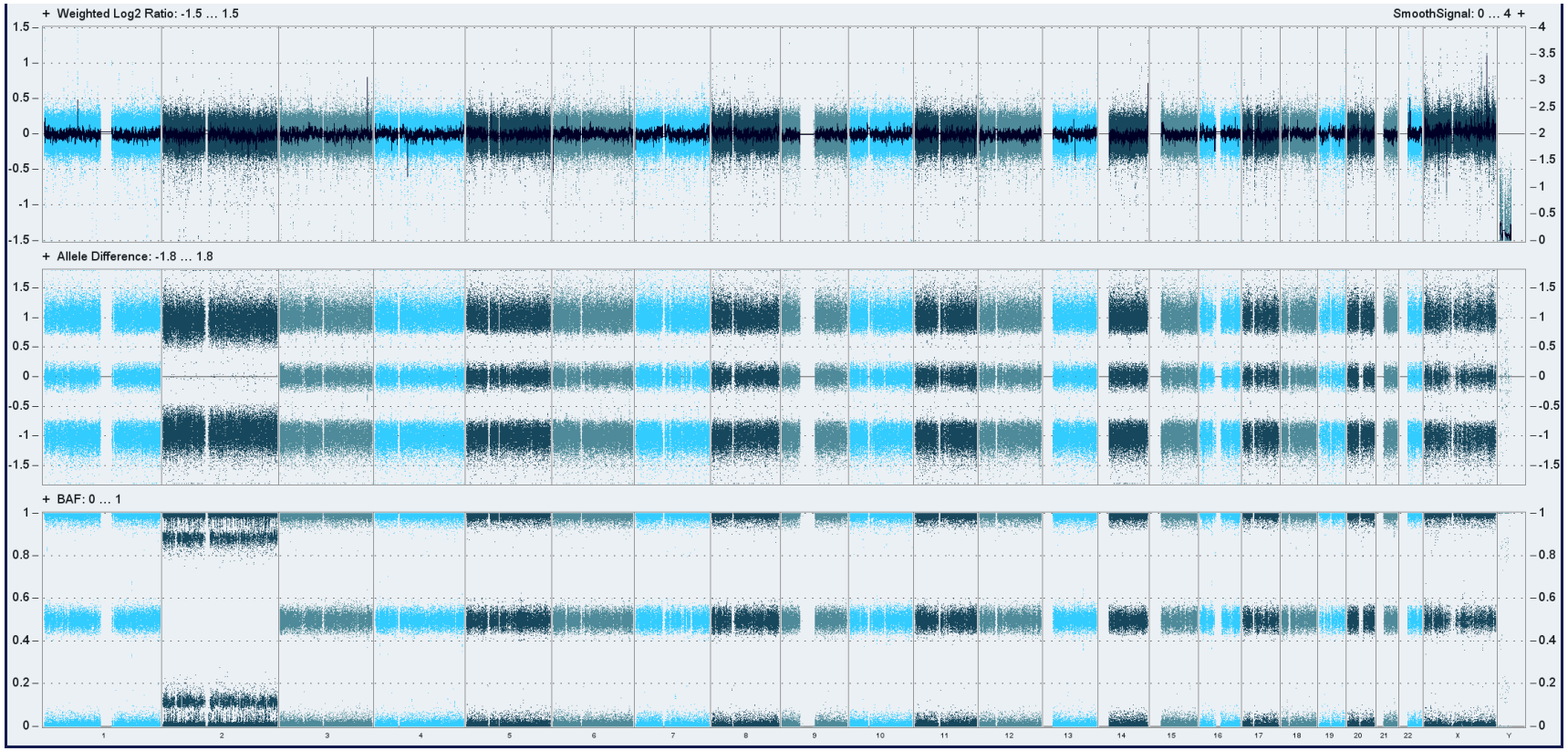
Equipment for genomic arrays

Affymetrix GeneChip System

Practical example

Muscular dystrophy

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



Take-home messages

- Discuss
- Standardize
- Integrate

The end…

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Thank you for your attention!