MEDICAL GENETICS · LECTURE

Essential Genetic Testing Techniques

Karyotype · FISH · Chromosomal Microarray · Next-Generation Sequencing

Learning Objectives

By the end of this session you should be able to:

1. **Compare** the resolution, sensitivity, and clinical utility of karyotyping, FISH, chromosomal microarray (CMA), and next-generation sequencing (NGS).
2. **Select** the most appropriate genetic test for common clinical scenarios — ID/DD, congenital anomalies, recurrent pregnancy loss, oncology, and prenatal diagnosis.
3. **Interpret** a basic genetic test report, including ACMG variant classification, mosaicism, and copy number variants (CNVs).
4. **Recognize** the limitations of each technique, including variants of uncertain significance (VUS) and findings each platform cannot detect.
5. **Counsel** patients on incidental/secondary findings and the ethical considerations of genome-scale testing.

Why Genetic Testing Matters in Clinical Practice

Modern medicine increasingly depends on molecular and cytogenetic diagnosis. Identifying the genetic basis of disease changes management in every specialty — from pediatrics to oncology to reproductive medicine.

Five clinical impacts:

- Establishing a definitive diagnosis
- Tailoring therapy (pharmacogenomics, targeted oncology drugs)
- Predicting prognosis and disease course
- Cascade screening of at-risk family members
- Reproductive planning and prenatal diagnosis

~6,000+

Mendelian disorders catalogued in OMIM

1 in 20

Live births affected by a genetic or partly genetic condition

30–40%

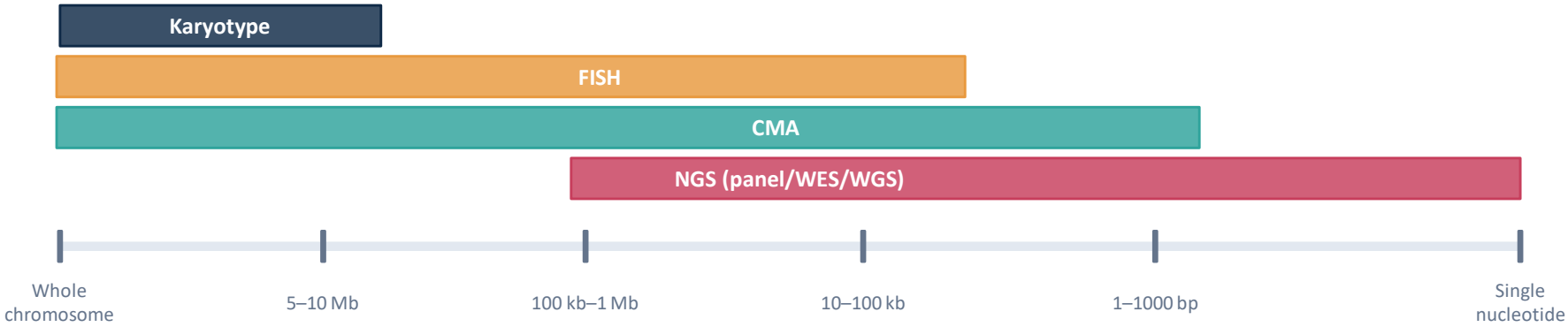
Diagnostic yield of WES/WGS in undiagnosed pediatric cases

10%

Of all cancers attributable to an inherited predisposition

The Resolution Spectrum

What size of genetic change does each test detect?



← *LARGER changes (cytogenetic)*

smaller changes (molecular) →

Key teaching point

No single test detects every kind of genetic change. Match the test to the suspected lesion — karyotype for large structural rearrangements, FISH for known targeted regions, CMA for unbalanced submicroscopic CNVs, NGS for sequence-level variants. Tests are increasingly used in tiered or parallel fashion.

PART 1

Karyotyping

The original whole-genome view — chromosome morphology at light-microscope resolution

Karyotype: Principle

Light-microscopic visualization of condensed metaphase chromosomes

1. **Sample** Peripheral blood lymphocytes, bone marrow, amniocytes, CVS, or solid tissue
2. **Culture** Cells stimulated to divide (e.g. phytohaemagglutinin for lymphocytes)
3. **Arrest** Colchicine arrests cells in metaphase, when chromosomes are most condensed
4. **Spread & stain** Hypotonic swelling, fixation, drop onto slide, G-banding with trypsin & Giemsa
5. **Analyze** Image, pair homologues, arrange by size & centromere position; ≥ 20 metaphases scored

Banding conventions

G-banding (Giemsa)
Standard; gives alternating light/dark bands. Resolution 400–550 bands; high-res up to 850.

ISCN nomenclature
Chromosome → arm (p/q) → region → band. Example: 7q31.2 = 7, long arm, region 3, band 1, sub-band 2.

Reporting
46,XX or 46,XY = normal female/male. Karyotype reads: total count, sex chromosomes, abnormalities.

Karyotype: What It Detects

Resolution ≈ 5–10 Mb (lesions smaller than a single band are invisible)

Aneuploidy

Trisomies 13, 18, 21; monosomy X; sex chromosome aneuploidies (47,XXY, 47,XYY, 47,XXX)

Translocations

Balanced reciprocal and Robertsonian; unbalanced derivative chromosomes — uniquely detected here

Large deletions / duplications

Cri-du-chat (5p–), Wolf-Hirschhorn (4p–) — but only if > 5–10 Mb

Inversions

Pericentric and paracentric; clinically relevant in infertility and recurrent miscarriage

Marker chromosomes

Small supernumerary chromosomes of unknown origin; need follow-up FISH/CMA

Mosaicism

Two or more cell lines (e.g. 45,X/46,XX); detectable if present in ≥ 10–20% of cells analyzed

Karyotype: Clinical Use, Strengths & Limits

Indications

- Suspected aneuploidy (Down, Edwards, Patau, Turner, Klinefelter)
- Recurrent pregnancy loss (≥ 2 miscarriages) — parental karyotype
- Infertility (male and female)
- Disorders of sex development
- Hematologic malignancies (leukemia/lymphoma) — clonal rearrangements (e.g. t(9;22) BCR::ABL1)
- Family member of a known translocation carrier

Advantages

- Whole-genome overview in one test
- Detects balanced rearrangements (translocations, inversions) — unique among routine tests
- Identifies mosaicism if multiple cell lines present
- Well-established; standardized nomenclature (ISCN)
- Relatively inexpensive
- Useful when family history suggests structural rearrangement

Limitations

- Low resolution: misses changes < 5–10 Mb
- Requires viable, dividing cells → slow (5–14 days)
- Cannot identify the gene content of CNVs
- Labor-intensive and operator-dependent
- Cannot detect uniparental disomy or single-gene mutations
- Mosaicism below ~10% often missed

Karyotype in Practice

Clinical vignette

Clinical scenario

A 32-year-old G3P0 woman is referred after three first-trimester miscarriages. Her partner is healthy. Routine work-up for thrombophilia and uterine anatomy is unremarkable. The team orders parental karyotypes.

Result	Female partner: 46,XX,t(11;22)(q23;q11.2) — a balanced reciprocal translocation. Male partner: 46,XY (normal).
Interpretation	Balanced carriers are phenotypically normal but at risk of producing unbalanced gametes. Live-born offspring can have partial trisomy/monosomy (e.g. Emanuel syndrome).
Why karyotype, not CMA?	CMA reads dosage only; it cannot distinguish a balanced translocation from a normal genome. This rearrangement is invisible to microarray.

PART 2

Fluorescence In Situ Hybridization

Targeted, fluorescent probes — fast answers about known regions

FISH: Principle

Fluorescently labelled DNA probes anneal to complementary chromosomal targets

A single-stranded DNA probe — labelled with a fluorophore — is denatured together with the patient's chromosomes (metaphase) or interphase nuclei. The probe hybridizes to its complementary genomic sequence; fluorescence microscopy reveals the location and copy number of the target.

1 Probe design
Labelled with fluorophore (e.g. SpectrumOrange/Green)

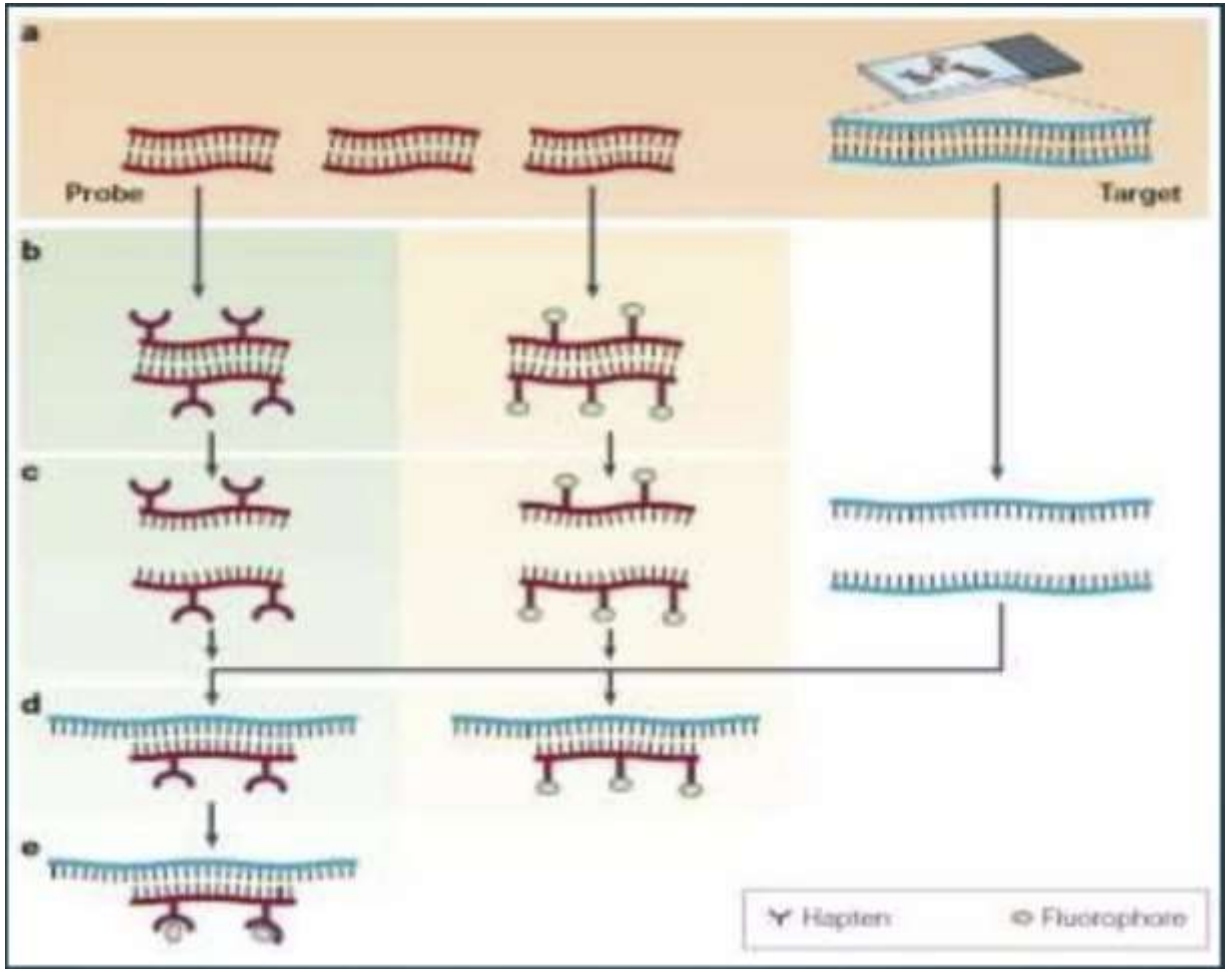
2 Denaturation
Both probe and target DNA → single-stranded

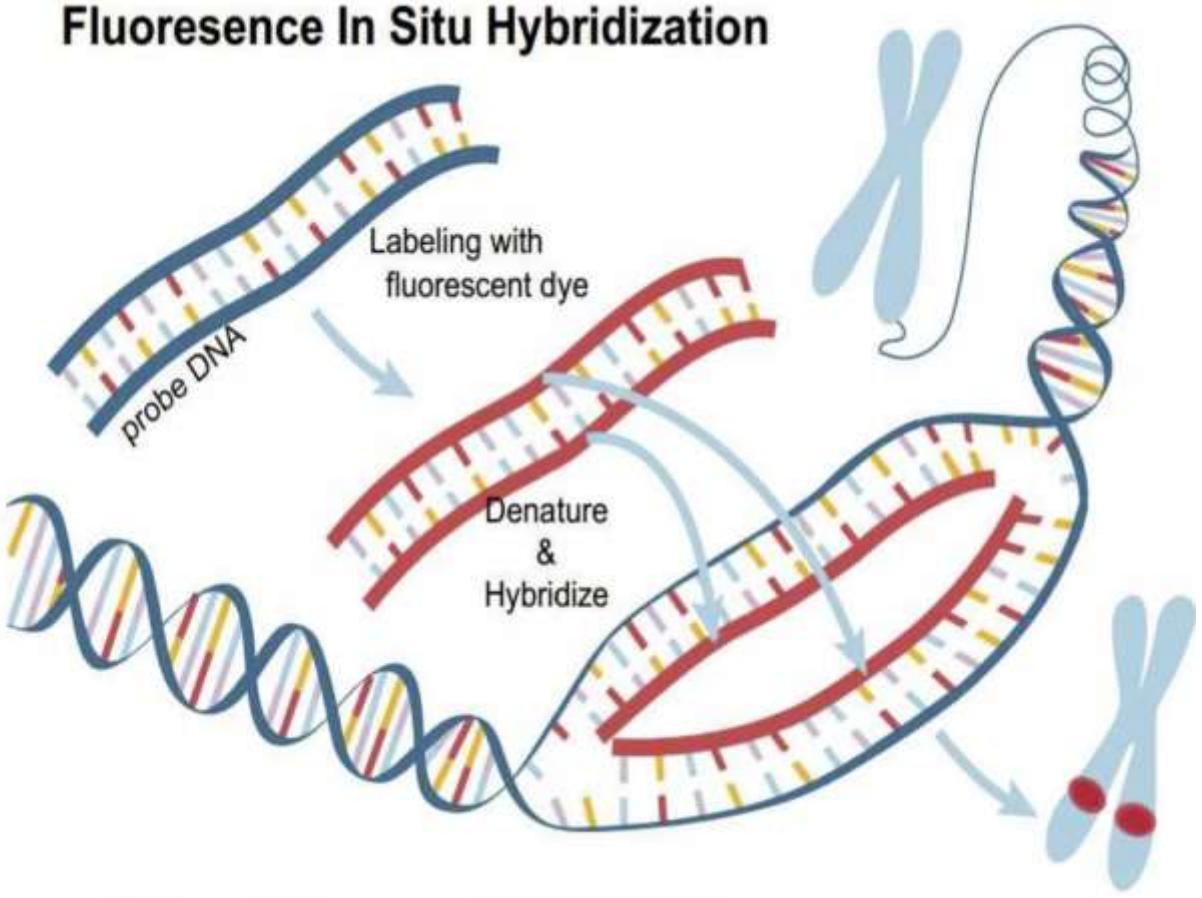
3 Hybridization
Overnight; complementary sequences anneal

4 Wash & DAPI
Remove non-specific binding; counterstain nuclei

5 Microscopy
Count fluorescent signals per nucleus or metaphase

Critical advantage: FISH works on **interphase nuclei** — no cell culture or division is required. This makes it the fastest cytogenetic test (results in 24–48 h), ideal for rapid prenatal aneuploidy screening on uncultured amniocytes or for HER2 status on a tumour section.


a
Probe
Target
b
c
d
e
Hapten
Fluorophore


Fluoresence In Situ Hybridization
probe DNA
Labeling with
fluorescent dye
Denature
&
Hybridize

Diagnosing DiGeorge syndrome by fluorescence *in situ* hybridization (FISH)

DiGeorge syndrome in humans:

- **Accounts for 5% of all congenital heart defects**
- **Affected people are heterozygous for a 22q11 deletion**

FISH on human metaphase chromosomes

Green dots; control probe for chromosome 22

Red dot; probe from 22q11 region

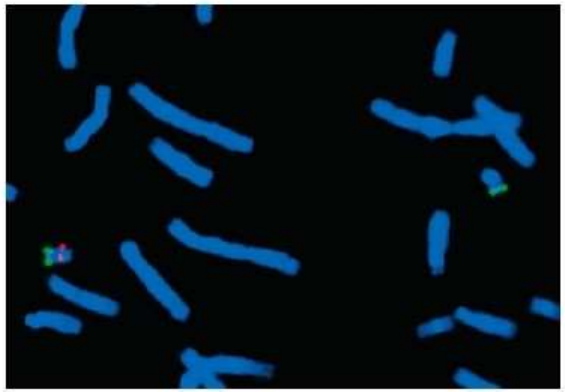
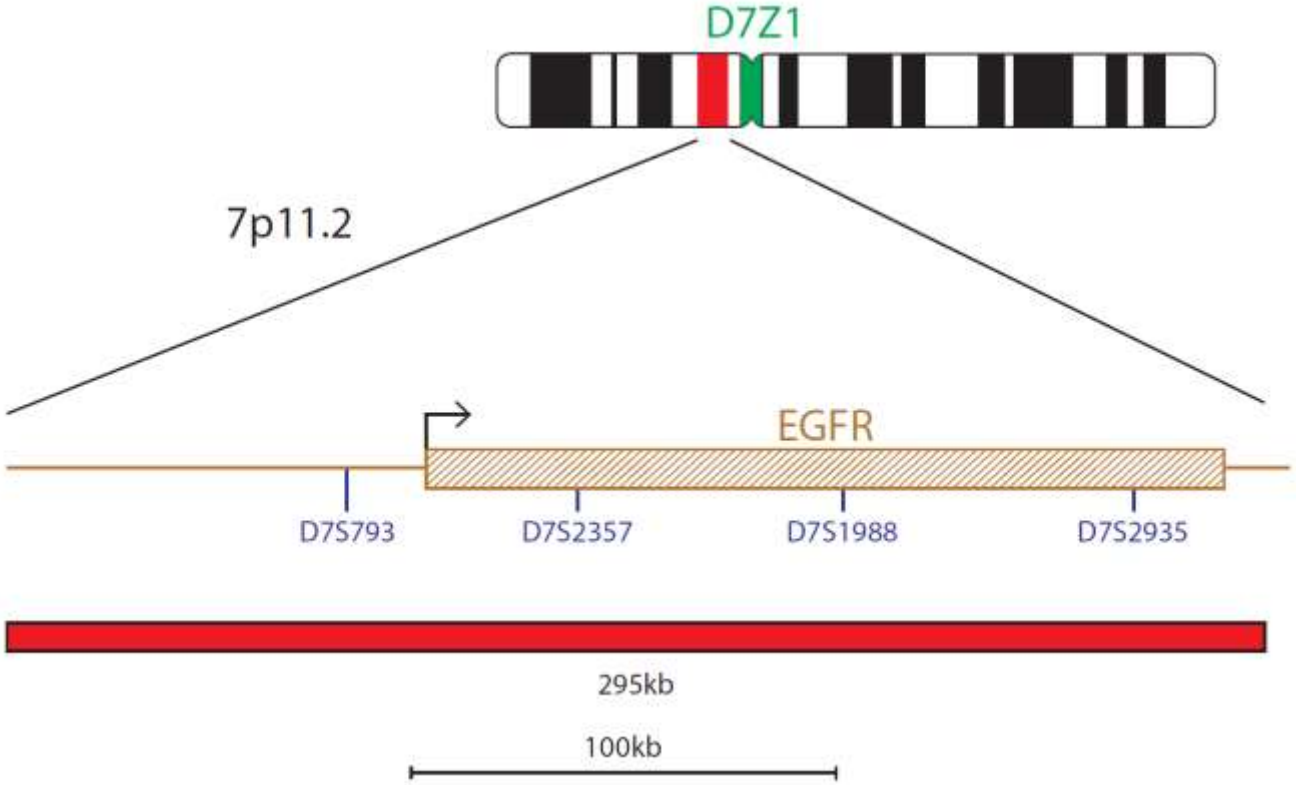
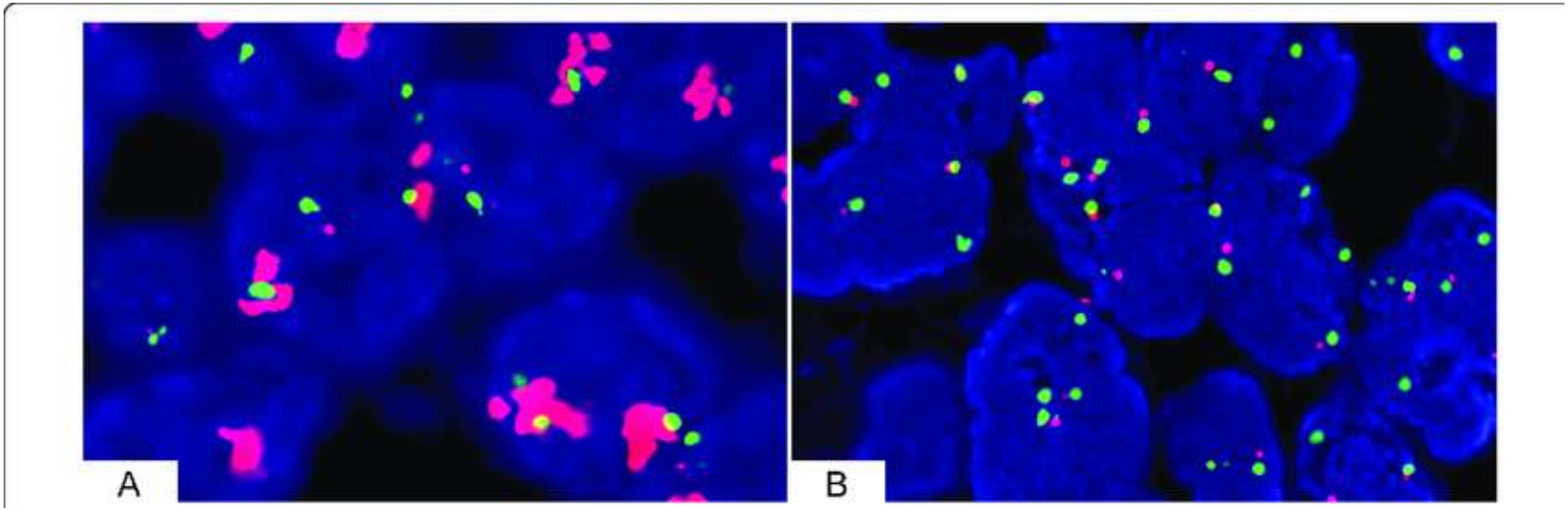


Fig. 13.10


D7Z1
7p11.2
EGFR
D7S793
D7S2357
D7S1988
D7S2935
295kb
100kb



Analysis of EGFR gene amplification and mutations based on FISH and RT-PCR analysis. A: FISH positive (amplification), B: FISH negative (low trisomy),

Types of FISH Probes

Locus-specific (LSI)

Targets a single gene or chromosomal locus. Used for microdeletions (22q11.2/DiGeorge, 7q11.23/Williams), gene fusions (BCR::ABL1), or single-gene loci (ERBB2/HER2 in breast cancer).

Centromeric (alpha-satellite)

Targets repetitive alpha-satellite DNA at chromosome centromeres. Used for rapid aneuploidy detection (e.g. trisomy 21 on interphase amniocytes) and to enumerate chromosomes.

Telomeric / subtelomeric

Probes for the unique sequences just proximal to the telomeres. Detects cryptic subtelomeric rearrangements — a recognized cause of unexplained intellectual disability.

Whole-chromosome paint (WCP)

A mix of probes covering an entire chromosome. Identifies the origin of marker chromosomes and complex translocations seen on karyotype.

FISH: Common Clinical Applications

Setting	Indication	Probe / target
Prenatal	Rapid aneuploidy screen on amniocytes	13, 18, 21, X, Y centromeric probes
Pediatrics	Suspected DiGeorge / VCFS — heart defect + hypocalcaemia + immune defect	22q11.2 LSI probe (TUPLE1/HIRA)
Pediatrics	Suspected Williams syndrome — supravalvular aortic stenosis, elfin face	7q11.23 LSI probe (ELN)
Pediatrics	Prader-Willi / Angelman	15q11–q13 LSI (now often replaced by methylation testing)
Oncology	CML — confirm Philadelphia chromosome	BCR/ABL1 dual-fusion probe
Oncology	Breast cancer — assess HER2 status when IHC equivocal (2+)	ERBB2 (17q12) LSI probe
Oncology	MDS / AML — recurrent deletions	5q, 7q, 20q LSI probes

FISH: Strengths & Limitations

Advantages

- Fast turnaround — interphase results in 24–48 h
- Works on non-dividing cells (uncultured amniocytes, paraffin-embedded tumour)
- Detects submicroscopic deletions/duplications (down to ~100 kb)
- Can identify low-level mosaicism (analyze hundreds of cells)
- Useful adjunct when karyotype is ambiguous or impractical

Limitations

- Targeted — you only see what you ask about; cannot screen the whole genome
- Requires prior clinical hypothesis to pick the right probe
- Each probe is a separate test → cumulative cost
- Does not detect point mutations or single-base changes
- Cannot distinguish balanced from unbalanced changes in many cases

PART 3

Chromosomal Microarray

Genome-wide screen for copy-number variation — first-line for unexplained ID/DD/ASD/MCA

CMA: Principle

Quantitative, genome-wide detection of copy-number variants (CNVs)

Patient DNA is hybridized to thousands of probes spanning the genome on a single chip. By measuring fluorescence intensity at each probe — relative to a reference (aCGH) or to an expected baseline (SNP array) — the assay reveals whether each genomic region is present in normal, increased, or decreased copy number.

Array-CGH

Comparative Genomic Hybridization

- Patient + reference DNA labelled with different fluorophores
- Co-hybridized; ratio of intensities → copy number
- Detects deletions and duplications only
- Cannot detect LOH or uniparental disomy (UPD)

SNP array

Single-Nucleotide Polymorphism array

- Only patient DNA; probes target known SNPs
- Provides genotype (AA, AB, BB) at each probe
- Detects deletions, duplications AND loss of heterozygosity
- Can flag UPD, consanguinity, triploidy

CMA: First-Line for ID/DD/ASD/MCA

ACMG, AAP, ACOG, and ISCA consensus recommendation

15–20%

Diagnostic yield of CMA in children with unexplained intellectual disability, developmental delay, autism, or multiple congenital anomalies

vs. 3% for routine karyotype in the same population

Order CMA when…

- Unexplained developmental delay or intellectual disability
- Autism spectrum disorder without obvious syndromic diagnosis
- Multiple congenital anomalies not fitting a recognizable syndrome
- Dysmorphic features without a clear clinical diagnosis
- Fetus with structural anomalies on ultrasound (often replaces karyotype prenatally)
- After stillbirth or anomalous fetal demise

Interpreting a CMA Result

ACMG 5-tier CNV classification

Pathogenic	Well-established deletion/duplication causing a known syndrome (e.g. 22q11.2 deletion, 7q11.23 duplication).
Likely pathogenic	Overlaps a known disease region or contains haploinsufficient gene(s); evidence suggests causation.
Uncertain significance (VUS)	Novel or rare CNV with insufficient evidence; may require parental testing or follow-up.
Likely benign	Reported in healthy controls or population databases at low frequency without phenotype.
Benign	Common copy-number variant in population (e.g. DGV, gnomAD-SV); no clinical relevance.

Reporting note: Classification uses size, gene content, inheritance pattern, and overlap with established databases (DECIPHER, ClinGen, ClinVar). De novo CNVs are more likely pathogenic than inherited ones.

What CMA Cannot Detect

Balanced rearrangements

Reciprocal translocations, inversions — no change in DNA dosage

Single nucleotide variants

Point mutations and small indels < probe resolution

Low-level mosaicism

Generally needs > 10–20% mosaicism to be reliably detected

Triploidy (aCGH only)

69,XXX or 69,XXY shows normal ratio against reference; SNP array does detect it

Methylation defects

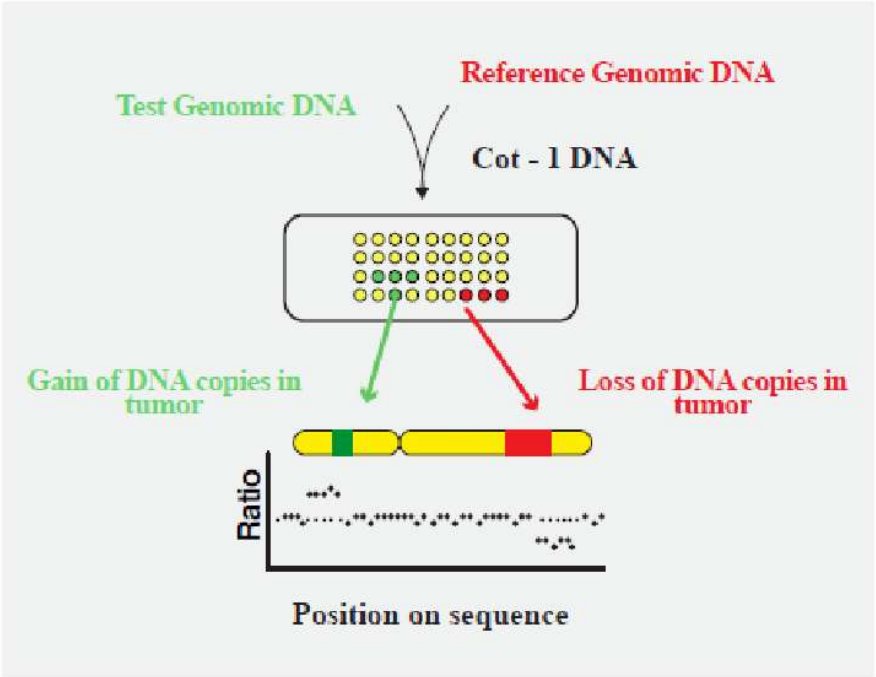
Imprinting disorders (Prader-Willi, Angelman) — needs methylation-specific testing

Repeat expansions

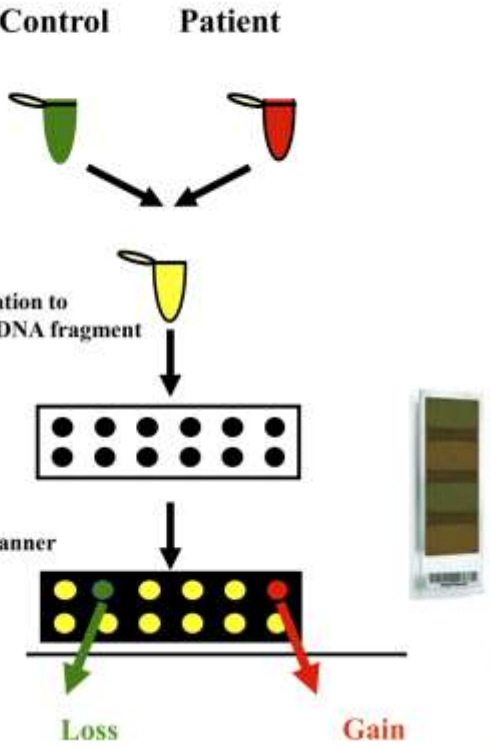
Trinucleotide repeat disorders (Fragile X, Huntington) — needs targeted methods

Microarray Comparative Genomic Hybridization (aCGH)

- Detects genome-wide copy number variations (deletions, duplications, gains, losses)
- Higher resolution than conventional chromosome banding
- Does not require prior knowledge of specific chromosomal abnormalities
- Uses labeled DNA from patient samples; no cell culture needed
- Hybridization occurs on glass/silicon slides printed with thousands of DNA probes

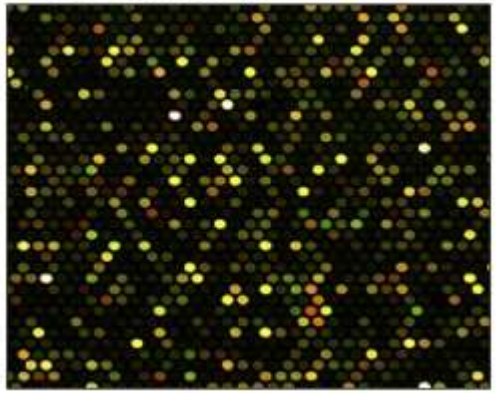

Reference Genomic DNA
Test Genomic DNA
Cot - 1 DNA
Gain of DNA copies in tumor
Loss of DNA copies in tumor
Ratio
Position on sequence

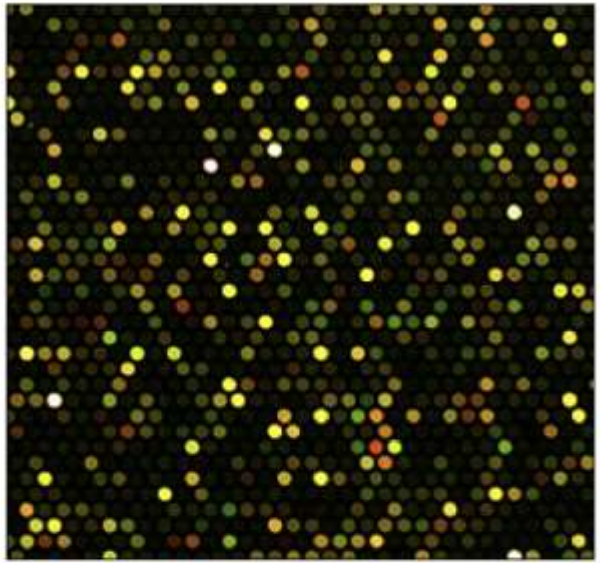
[A]

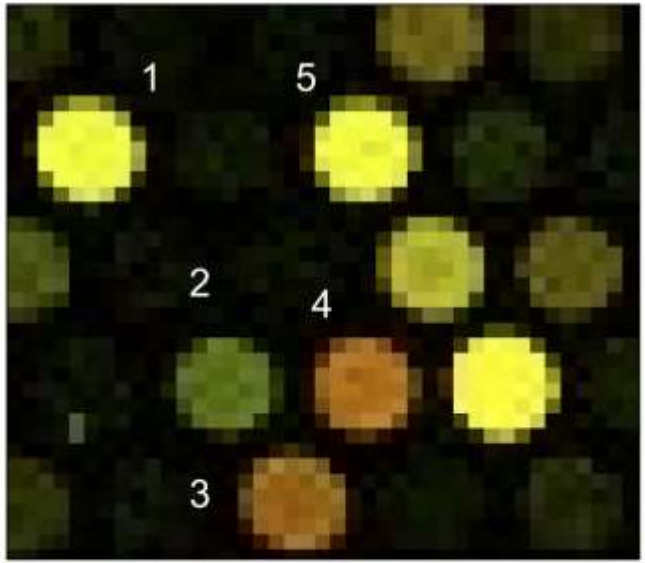


[B] Laser Scanner

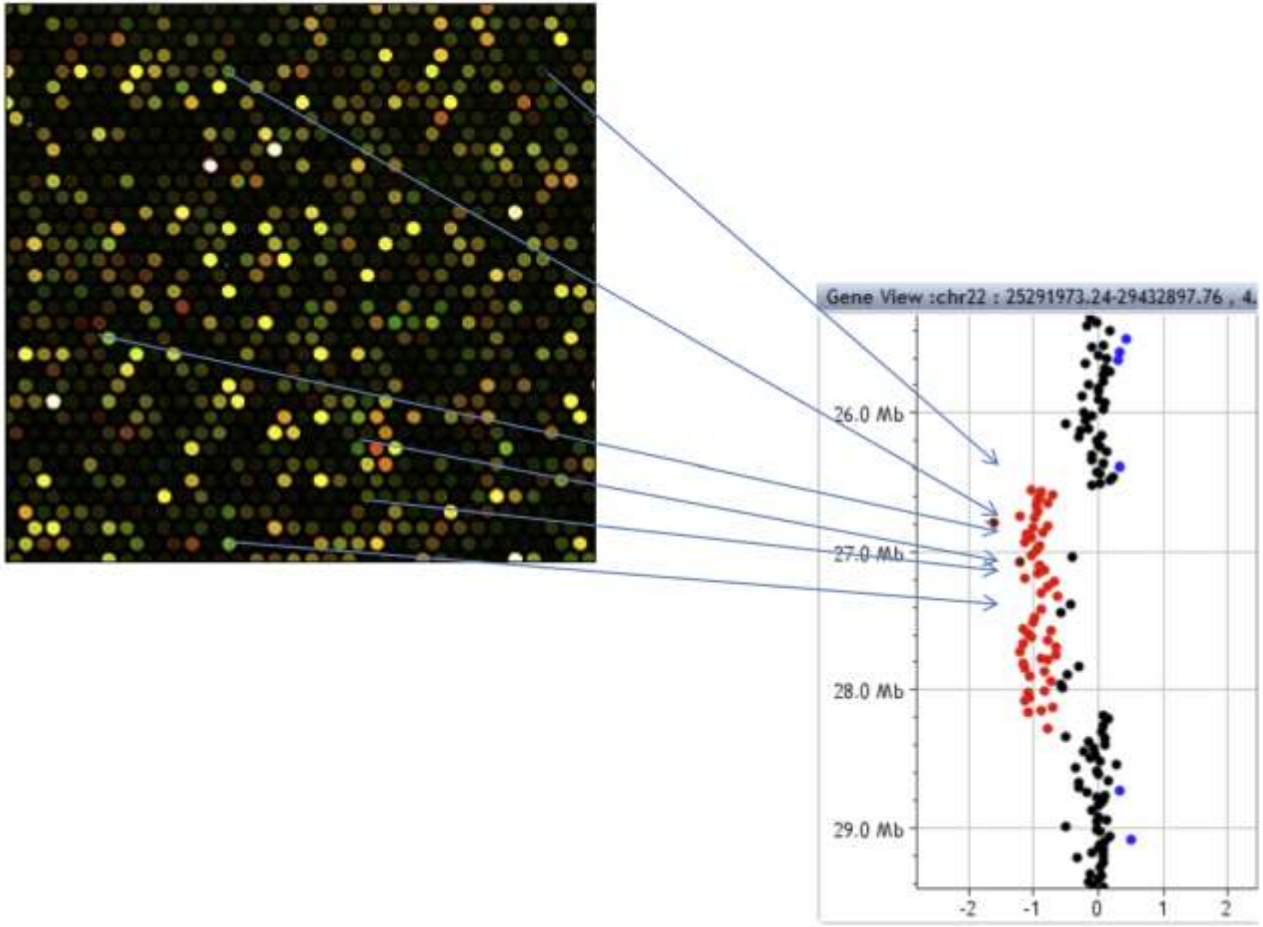
[C] Actual Array



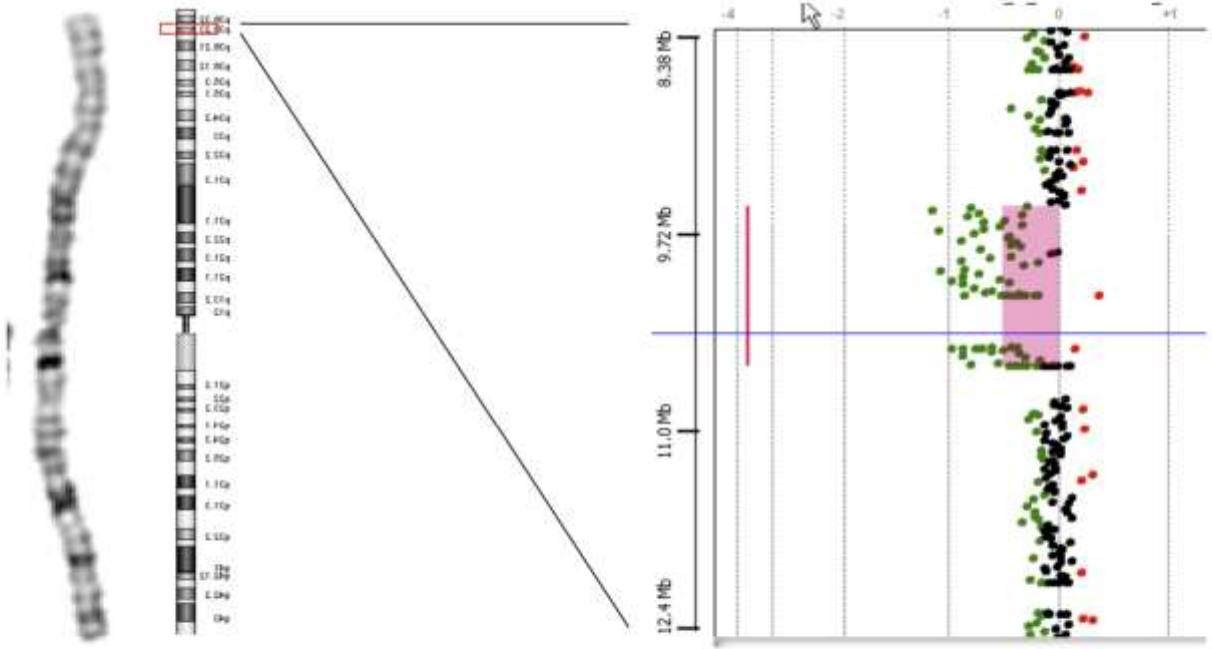




Spot	position	Intensity Red patient	Intensity Green control	Ratio	Log_2	In the threshold? -0.5-0.3	Interpretation
1	1p22	656	632	1.0	0.05	yes	normal
2	Xq28	302	583	0.5	-0.95	no	loss
3	3p26	850	621	1.4	0.45	no	gain
4	4p16	826	588	1.4	0.49	no	gain
5	22q11	586	601	1.0	-0.04	yes	normal


Gene View :chr22 : 25291973.24-29432897.76 , 4.
26.0 Mb
27.0 Mb
28.0 Mb
29.0 Mb
-2
-1
0
1
2

CMA can detect del/dup of ~>25-

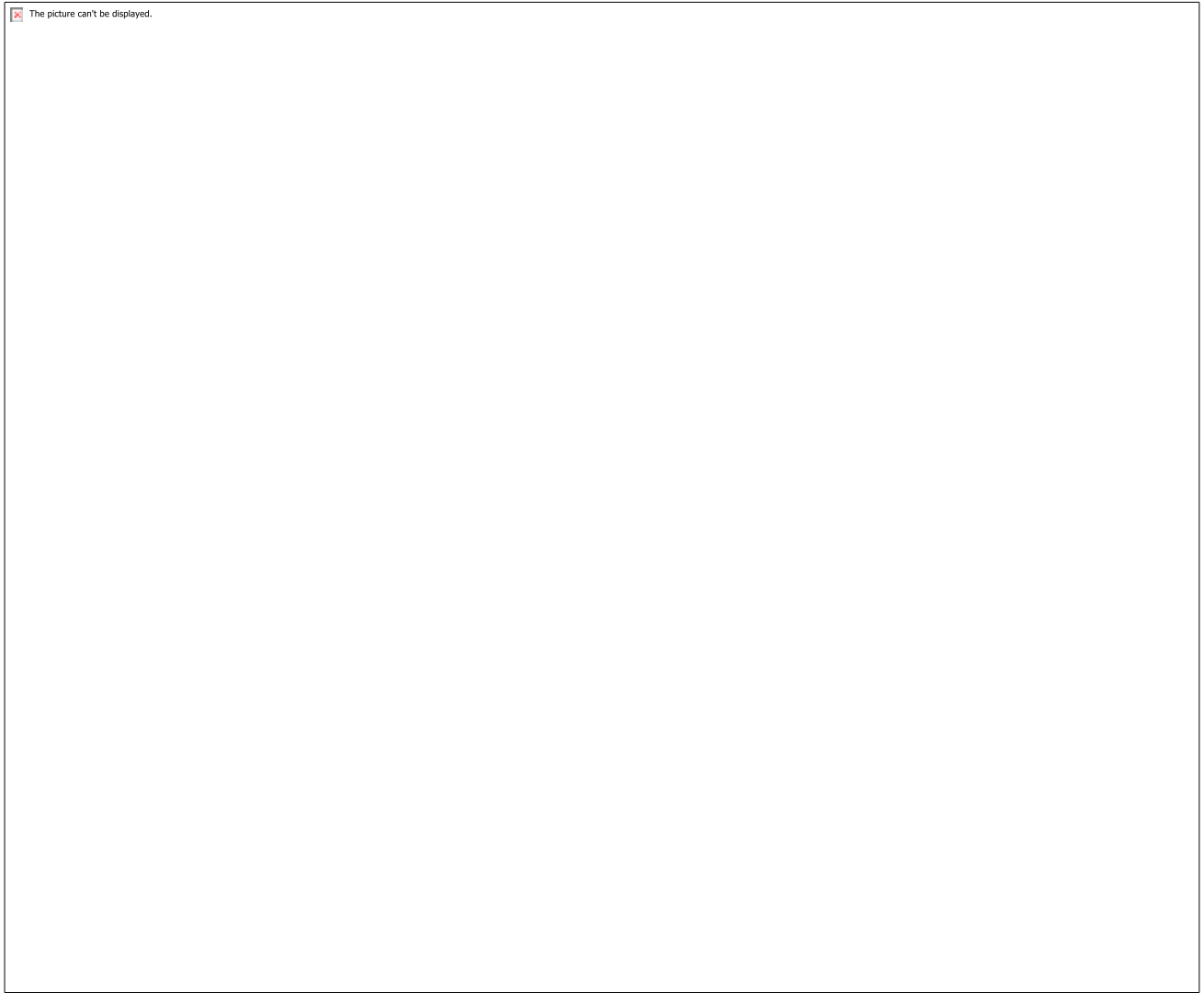


Chromosome 1

Limits of detection for G-banded chromosome analysis is 4-5 Mb

4 Mb region of microarray data showing a 1 Mb deletion encompassing ~70 oligos

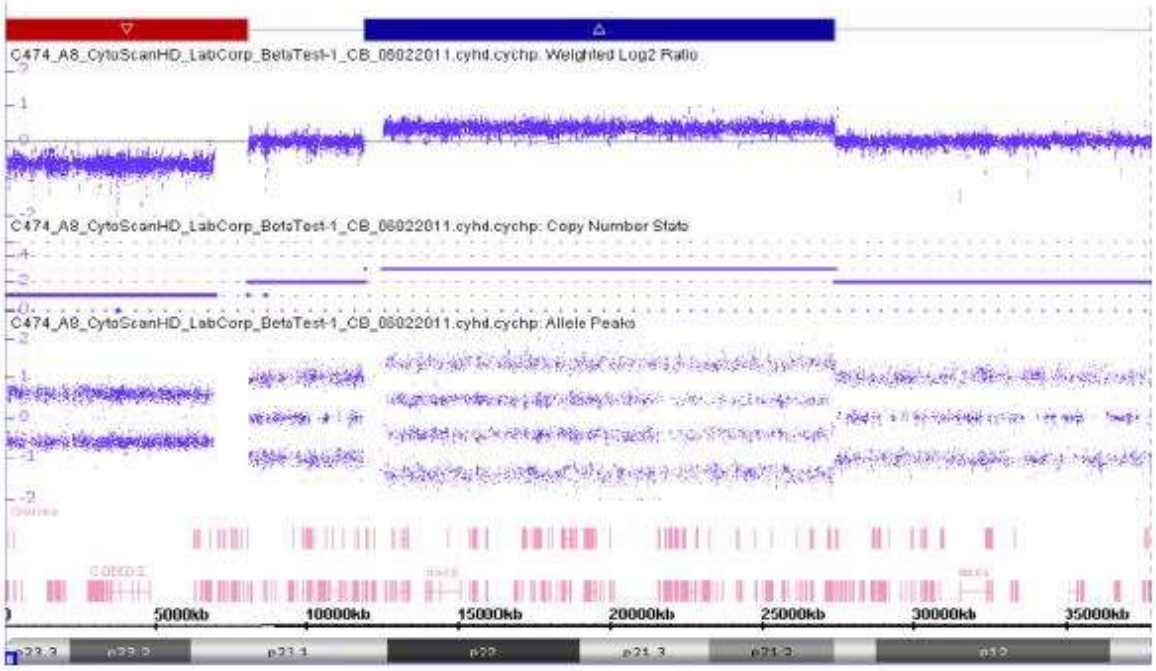
- Modern microarray platforms typically combine copy-number (CNV) probes with SNP probes.
- SNPs must be present in >1% of the population (most informative when present at ~50% frequency).
- SNPs may be located in coding regions, noncoding regions, or intergenic areas between genes.
- Each SNP's possible states are assigned arbitrary allele labels (A and B).
- SNP data can identify uniparental disomy (UPD) and regions of homozygosity (ROH).
- Different microarray platforms vary in their specifications; for example, some SNP-only arrays do not require a control sample.


The picture can't be displayed.

Copy number + SNP array

Copy #

Allele peaks



Interpretation of germline

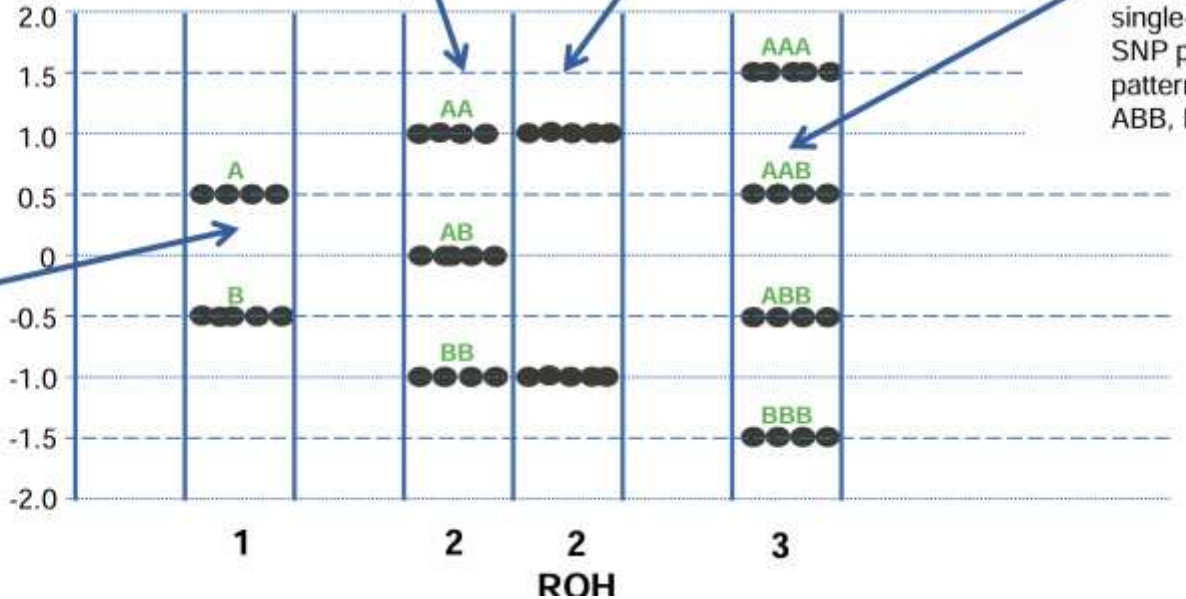
Each SNP probe present in a diploid region is genotyped as:
- AA (0 uncut copies), homozygous
- AB (1 uncut copy), heterozygous
- BB (2 uncut copies), homozygous

(normal copy number and SNPs = 3 lines)

Regions of Homozygosity (ROH) have no heterozygous alleles = AA or BB (2 lines)

In aberrant genomic locations harboring single-copy gains, SNP probes yield four patterns: AAA, AAB, ABB, BBB (4 lines)

In aberrant genomic locations harboring single-copy losses, SNP probes are either cut or uncut and the resulting genotype is simply A or B (2 lines)



In amplified regions (typically tumor samples), additional A or B alleles are detected by the SNP probes and the genotypes become increasingly more complex

Copy Number

PART 4

Next-Generation Sequencing

Massively parallel sequencing — panels, exomes, and whole genomes

From Sanger to Massively Parallel Sequencing

Sanger sequencing (1977) reads one DNA fragment at a time and remains the gold standard for confirming single variants. Next-generation sequencing reads millions of fragments simultaneously, making whole-exome and whole-genome analysis clinically feasible and affordable.

Feature	Sanger sequencing	Next-generation sequencing
Reads per run	1 fragment (~800 bp)	Millions of fragments in parallel
Throughput	Low	Very high
Cost / base	High	Very low (and falling)
Best use	Variant confirmation, single-gene tests	Panels, exomes, whole genomes
Sensitivity for mosaicism	~ 15–20%	≥ 1–5% (depending on depth)
Turnaround	Hours–days	Days–weeks (clinical pipelines)

The NGS Workflow

From sample to clinical report

1 Library preparation

Genomic DNA is fragmented; adapters with sample barcodes are ligated.

2 Target enrichment (optional)

Capture probes pull down the regions of interest — panel or exome. WGS skips this.

3 Cluster generation

Fragments amplified on a flow cell, generating clonal clusters of identical DNA.

4 Sequencing by synthesis

Fluorescent nucleotides incorporated one base at a time; each cycle imaged.

5 Alignment

Short reads mapped to a reference genome (GRCh38).

6 Variant calling

Differences from reference identified: SNVs, indels, CNVs, structural variants.

7 Annotation & filtering

Variants linked to gene/transcript, population frequency, prior reports.

8 Clinical interpretation

ACMG criteria applied; report issued; Sanger confirmation if needed.

Coverage matters: average read depth ≥ 30× for germline, ≥ 100× for somatic; uniformity is as important as the mean — gaps cause false negatives.

Panels, Exome & Genome

Three flavors of clinical NGS

Targeted panel	Whole exome (WES)	Whole genome (WGS)
Tens to hundreds of curated genes	*~ 20,000 protein-coding genes*	*Coding + non-coding sequence*
Coverage 5–500 genes	**Coverage** Exonic regions (~ 1–2% of genome)	**Coverage** Whole genome
Genome fraction < 0.1%	**Genome fraction** 1–2%	**Genome fraction** ~ 100%
Depth Very deep (200–1000×)	**Depth** Moderate (80–150×)	**Depth** Lower (30–40×) but uniform
Best for Well-defined phenotype (e.g. hereditary cancer, cardiomyopathy, hearing loss)	**Best for** Suspected Mendelian disease without phenotypic certainty	**Best for** Critically ill neonates; structural variants; non-coding regulatory variants
Yield Variable; high when phenotype is specific	**Yield** 25–40% in suspected genetic disease	**Yield** +5–10% incremental over WES
Cost Lowest	**Cost** Moderate	**Cost** Highest, but falling rapidly

Variant Types Detected by NGS

SNVs

Single-nucleotide variants — substitutions. The bread and butter of clinical NGS.

Small indels

Insertions/deletions of a few base pairs; may cause frameshift if not multiples of 3.

CNVs

Copy-number variants — increasingly callable from NGS depth-of-coverage data.

Structural variants

Larger inversions, translocations, complex rearrangements; better detected with WGS or long reads.

Mitochondrial variants

If mtDNA is included in the capture; heteroplasmy quantifiable at deep coverage.

Repeat expansions

Historically poorly detected by short-read NGS; emerging algorithms and long reads are changing this.

NGS: Strengths & Limitations

Strengths

- Detects single-base resolution variants across thousands of genes simultaneously
- Quantitative: variant allele fraction informs mosaicism, tumour purity, heteroplasmy
- Re-analysis possible as new gene–disease associations emerge
- Trio testing (proband + parents) dramatically improves yield and shortens diagnostic odyssey
- Same workflow scales from a 5-gene panel to a whole genome
- Falling cost — WGS now under US$1000 in many labs

Limitations

- Misses some structural variants and large CNVs (especially with short reads)
- Poor coverage of GC-rich regions, repeats, pseudogenes (e.g. PMS2, SMN1, CYP21A2)
- Trinucleotide repeat expansions and methylation defects need targeted assays
- Mitochondrial variants need a dedicated workflow
- VUS rate is high — particularly for under-studied populations
- Incidental / secondary findings raise complex consent and counselling issues

ACMG/AMP Variant Classification

Standardized framework for clinical sequence variants

Pathogenic (P)	Sufficient evidence the variant causes disease. Report and act on it.
Likely pathogenic (LP)	≥ 90% certainty of being disease-causing. Treated clinically as pathogenic.
Uncertain significance (VUS)	Insufficient or conflicting evidence. Do NOT use for clinical decisions; may be re-classified.
Likely benign (LB)	≥ 90% certainty of being benign.
Benign (B)	Sufficient evidence the variant is not disease-causing.

Evidence categories combined to assign tiers:

Population data (allele frequency) · **Computational** (in-silico predictions) · **Functional** (experimental studies) · **Segregation** (in families) · **De novo** occurrence · **Allelic** data · **Other databases** (ClinVar)

Variants of Uncertain Significance (VUS)

The most challenging result for the clinician

> *A VUS is a variant for which there is insufficient or conflicting evidence to classify as benign or pathogenic. It is NOT a positive result and should not be used to make clinical decisions.*

Common pitfalls

- Treating a VUS as if it were pathogenic — risk of overdiagnosis, unnecessary surgery, anxiety
- Cascade testing in relatives — confirms nothing and adds confusion
- Modifying surveillance or therapy based on a VUS
- Failure to re-evaluate the variant over time

Best practice

- Manage the patient on phenotype and family history — not the VUS
- Consider segregation testing if the family is informative
- Re-query the laboratory and ClinVar periodically (~ every 1–2 years)
- Submit variant evidence to public databases to aid reclassification
- Document clearly and counsel that the result is non-diagnostic

Secondary & Incidental Findings

Unintended discoveries from genome-scale testing

When you sequence an exome or genome, you may find clinically important variants outside the indication for testing. ACMG maintains a list of medically actionable genes (currently ~ 80) for which findings should be reported to the patient — unless they opt out.

Examples of ACMG actionable categories

Hereditary cancer

BRCA1, BRCA2, MLH1, MSH2, MSH6, PMS2, APC, TP53, MEN1, RET

Cardiomyopathies

MYH7, MYBPC3, TNNT2, TNNI3, LMNA, DSP

Arrhythmias

KCNQ1, KCNH2, SCN5A (long QT); RYR2 (CPVT)

Aortopathies

FBN1 (Marfan), TGFBR1/2 (Loeys-Dietz), ACTA2

Familial hypercholesterolemia

LDLR, APOB, PCSK9

Malignant hyperthermia

RYR1, CACNA1S

PART 5

Putting It All Together

Choosing the right test for the clinical question

Master Comparison Table

	Karyotype	FISH	CMA	NGS
Resolution	5–10 Mb	~ 100 kb	10–400 kb	1 bp (single base)
Genome view	Whole	Targeted (locus)	Whole (CNV only)	Whole (sequence)
Detects balanced rearrang.	Yes	Sometimes	No	WGS partially
Detects point mutations	No	No	No	Yes
Detects CNVs	If > 5 Mb	At targeted loci	Yes (sub-Mb)	Yes (variable)
Detects mosaicism	≥ 10–20%	Possible	≥ 10–20%	≥ 1–5% with depth
Cells must divide	Yes	No	No	No
Turnaround	5–14 d	1–3 d	5–10 d	1–6 wk (clinical)
Relative cost	$	$	$$	$$ – $$$$

Choosing the Right Test

A simplified clinical algorithm

Suspected aneuploidy, balanced translocation, or recurrent miscarriage?	→ **Karyotype ± FISH** *Karyotype is unique in detecting balanced rearrangements; FISH adds speed for known targets.*
Unexplained ID / DD / ASD / multiple congenital anomalies?	→ **Chromosomal microarray FIRST** *ACMG first-line recommendation; 15–20% yield vs ~ 3% for karyotype.*
Recognizable single-gene syndrome (Marfan, CF, Duchenne)?	→ **Targeted gene test / panel** *Highest yield when phenotype clearly points to a defined gene or pathway.*
Phenotype suggestive of a genetic disorder but non-diagnostic; first-tier tests negative?	→ **Whole exome (trio if possible) → Whole genome** *Trio WES yields 25–40% diagnosis; WGS adds non-coding and structural variants.*
Critically ill neonate in NICU with unexplained presentation?	→ **Rapid trio WGS (or WES)** *Results in days; can change management — antibiotics, surgery, withdrawal of care.*

Case 1: Pediatric Diagnostic Odyssey

Apply what you've learned

Vignette

A 4-year-old boy presents with global developmental delay, microcephaly, hypotonia, and dysmorphic features (broad forehead, low-set ears, widely spaced eyes). He has no recognizable syndrome. Hearing screen, metabolic panel, brain MRI, and Fragile X testing are all normal. Parents are non-consanguineous and healthy.

Q: What is your first-line genetic test?

A	**Karyotype**	*Low yield (~ 3%) in this setting; misses sub-microscopic CNVs that explain most of these phenotypes.*
B	**Chromosomal microarray**	*First-line per ACMG/AAP guidelines for unexplained ID/DD/ASD/MCA. Yield 15–20%.*
C	**Whole-genome sequencing**	*Powerful, but reserved for after first-tier work-up (and trio testing) when CMA negative — except in critical care settings.*
D	**Targeted Fragile X repeat expansion**	*Already done in this case (normal) — and would not explain the broader phenotype.*

Case 2: Hereditary Cancer

Family history changes everything

Vignette

A 38-year-old woman is diagnosed with triple-negative breast cancer. Family history: mother with ovarian cancer at 52, maternal aunt with breast cancer at 41, maternal grandfather with pancreatic cancer at 60. Ashkenazi Jewish ancestry.

Which testing strategy is most appropriate?

Multi-gene hereditary cancer panel by NGS

Why a panel and not just BRCA1/2? Phenotype overlaps with several syndromes — BRCA1/2 (HBOC), PALB2, CHEK2, ATM, TP53 (Li-Fraumeni), Lynch syndrome (MLH1/MSH2/MSH6/PMS2). A 20–80 gene panel covers the differential in a single, cost-effective assay.

Founder mutations: Ashkenazi heritage prompts targeted analysis of BRCA1 c.68_69delAG, c.5266dupC and BRCA2 c.5946delT — three variants account for ~ 90% of HBOC in this population.

Case 3: Prenatal Diagnosis

Layering tests as information evolves

Vignette

A 28-year-old woman, G1P0, at 20 weeks gestation. Anatomy scan shows a conotruncal cardiac defect (tetralogy of Fallot) and an absent thymus. Non-invasive prenatal testing (cell-free DNA) at 12 weeks was low-risk for trisomies 13/18/21.

Tiered diagnostic approach

1. **Diagnostic amniocentesis —** Provides fetal cells; consent for genetic testing.
2. **Rapid FISH on uncultured amniocytes —** Probes for 13, 18, 21, X, Y AND a 22q11.2 LSI probe. Conotruncal defect + absent thymus is highly suggestive of 22q11.2 deletion (DiGeorge). Results in 24–48 h.
3. **Chromosomal microarray —** If FISH negative: CMA defines the full extent of any deletion/duplication and screens for other CNVs.
4. **Consider WES / WGS —** If first-tier tests negative and phenotype suggests a Mendelian disorder (RASopathies — Noonan; CHARGE — CHD7).