

Fluorescence in situ hybridization

Molecular cytogenetics: principles, interpretation, and diagnostic applications

Pathology teaching lecture

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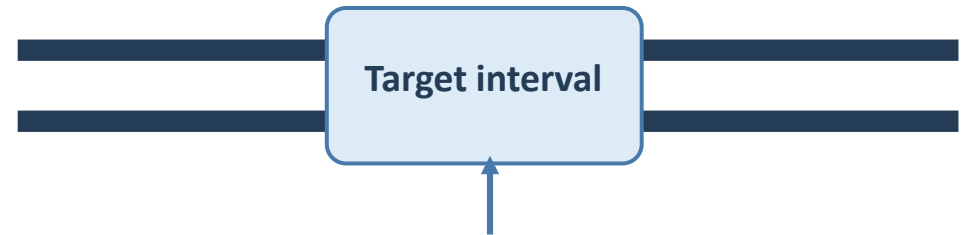
Learning objectives

- Explain how a labeled probe identifies a complementary DNA interval in a nucleus
- Interpret qualitative patterns for aneuploidy, deletion, amplification, and rearrangement
- Select suitable specimens and recognize analytical limitations
- Relate FISH results to selected constitutional and neoplastic disorders

FISH is a targeted DNA-localization assay

- Fluorescently labeled DNA probes bind their homologous genomic sequences
- Signals are viewed at their chromosomal or nuclear locations
- The output is a spatial signal pattern, not a nucleotide-by-nucleotide readout

Probe maps to a defined locus

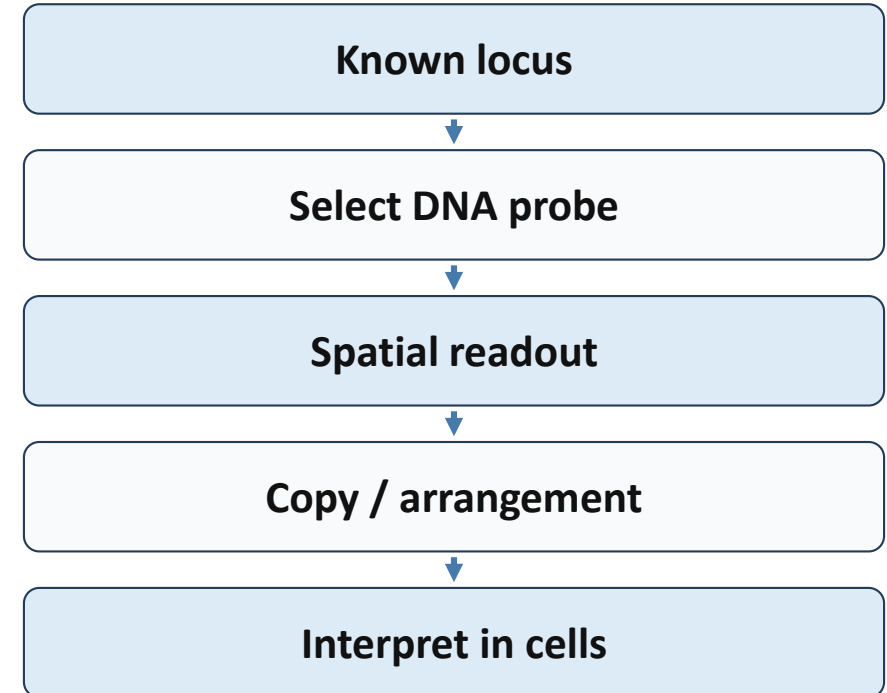


Locus-specific probe

Schematic — relative lengths not scaled

Why large structural lesions need other methods

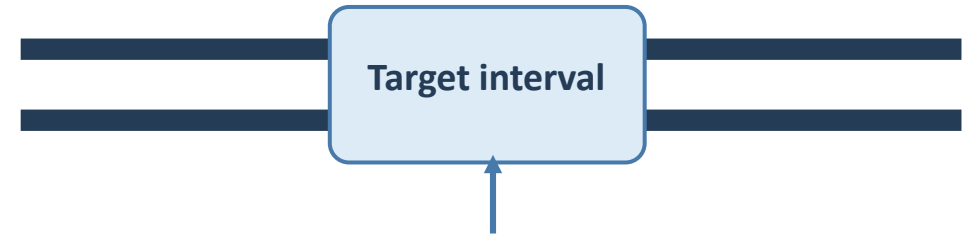
- Large deletions, duplications, and complex rearrangements may be difficult to characterize by short-target PCR
- FISH visualizes a chosen chromosome interval rather than amplifying one short sequence
- Its use is strongest when the suspected locus or abnormality is already known



The diagnostic question determines the probe

- A copy-number question requires a probe at the locus of interest
- A deletion question benefits from a separate retained control region
- A rearrangement question requires probes whose geometry changes when DNA is reorganized

Probe maps to a defined locus



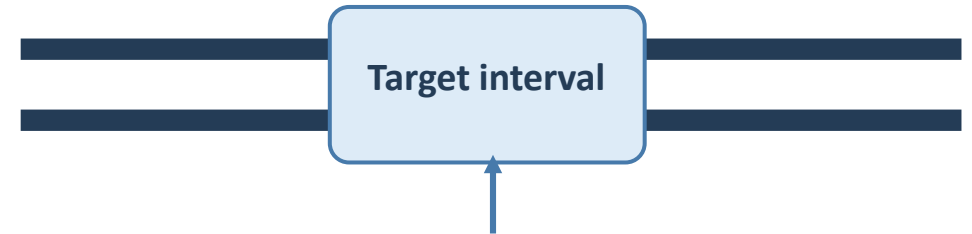
Locus-specific probe

Schematic — relative lengths not scaled

Probes recognize chromosome intervals

- Labeled genomic fragments spanning as much as approximately 200 kb
- Each probe hybridizes to a complementary region of the specimen genome
- Probe position and span determine what the assay can and cannot interrogate

Probe maps to a defined locus

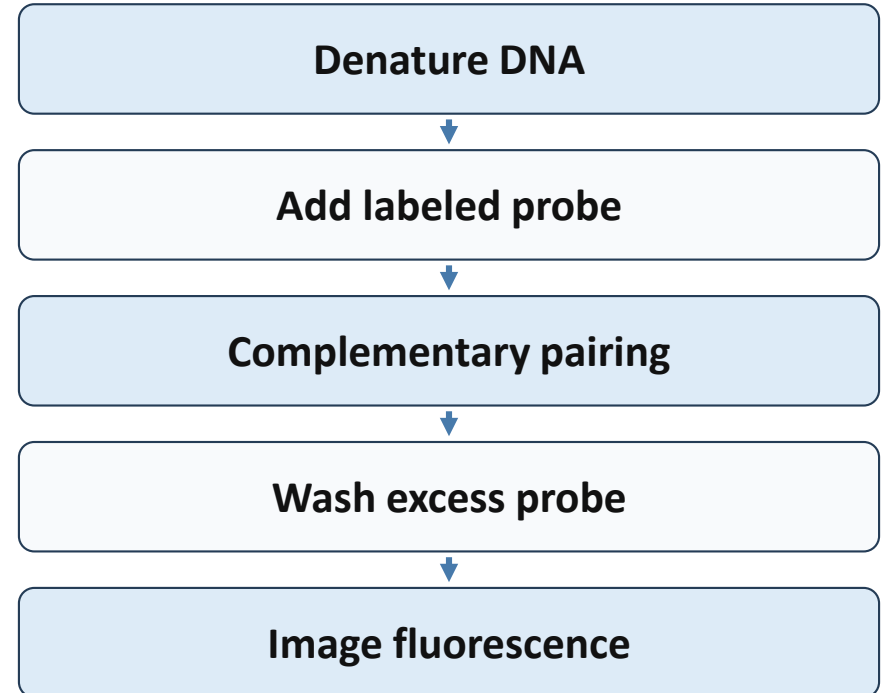


Locus-specific probe

Schematic — relative lengths not scaled

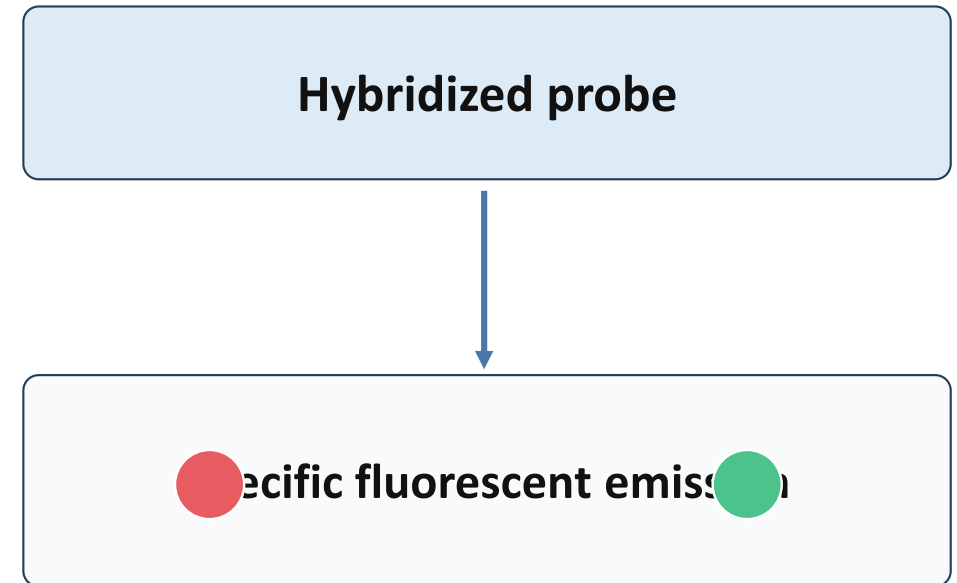
Complementarity provides specificity

- Heat or chemical treatment separates genomic DNA strands
- Single-stranded probe DNA binds the homologous specimen sequence
- Unbound or nonspecific probe is removed before fluorescence microscopy



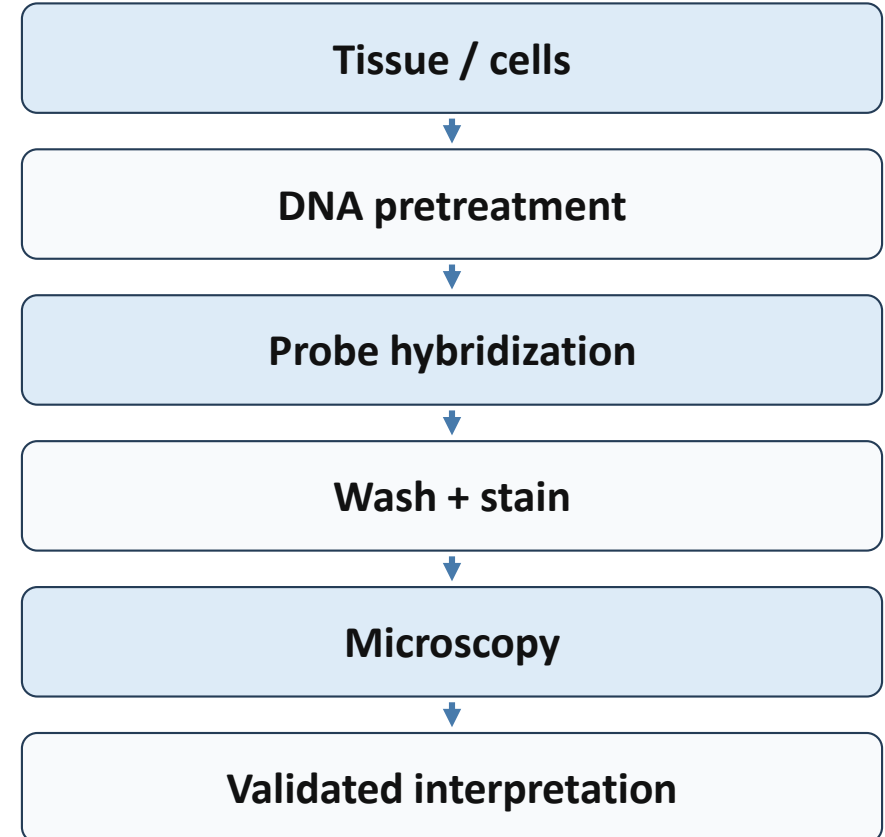
Fluorophores convert binding into signals

- The label emits light under suitable excitation
- Different dyes permit simultaneous visualization of two or more loci
- Signal number, relative position, and intensity are interpreted in assay context



FISH workflow: From specimen to interpretation

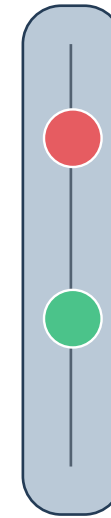
- Prepare cells or fixed tissue while preserving interpretable nuclei
- Denature DNA, hybridize probe, wash, and visualize
- Score informative cells using a validated probe-specific interpretation scheme



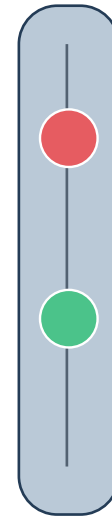
Metaphase FISH places signals on chromosomes

- Condensed metaphase chromosomes reveal the location of hybridization
- Useful when chromosome identity or rearrangement topology matters
- Requires an adequate metaphase preparation

Probe binding on homologs



Homolog 1

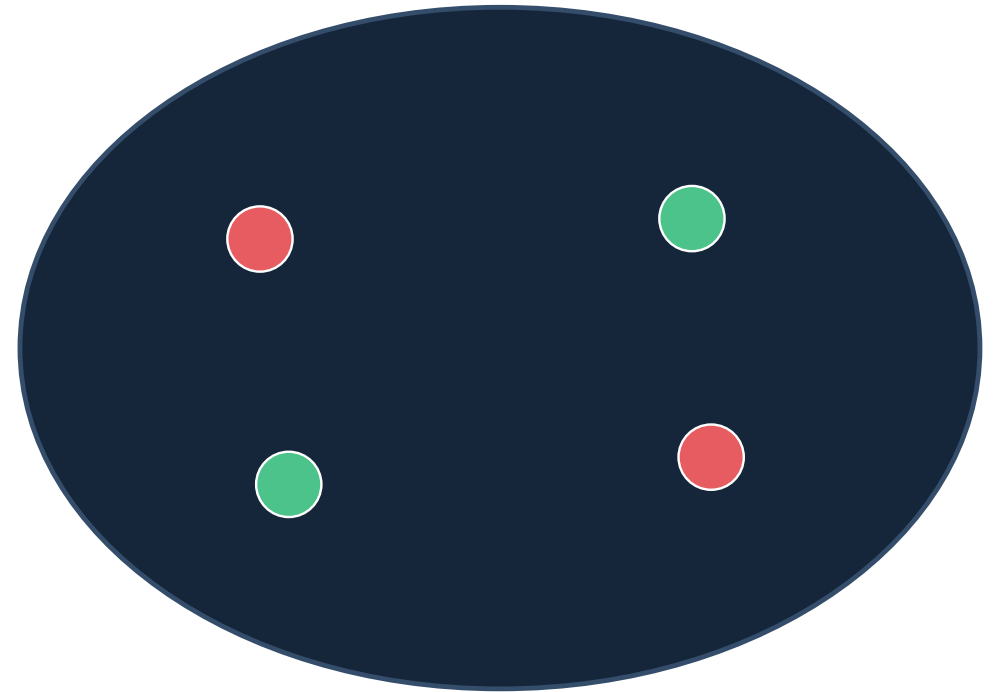


Homolog 2

Interphase FISH does not require metaphase

- Probes bind loci in nondividing interphase nuclei
- Signals can be assessed directly in suitable tissue or cell preparations
- Chromosome-wide architecture is less apparent than in a metaphase spread

Two-copy reference pattern



Schematic — illustrative signal pattern

● Target A ● Target B / control

Metaphase and interphase answer different questions

Feature	Metaphase	Interphase
Template	Condensed chromosomes	Nuclei without metaphase
Strongest readout	Chromosomal localization	Copy and spatial pattern
Constraint	Needs metaphase cells	Less structural context

Editable comparison table

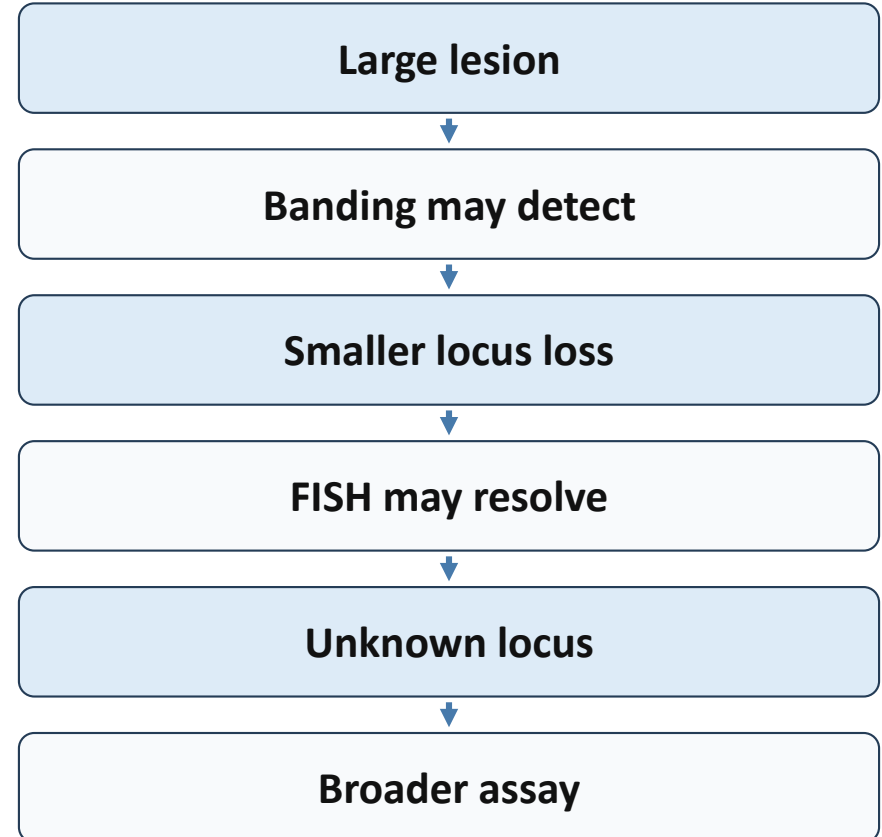
The preparation can come from different tissues

Material	Use in FISH	Important context
Prenatal samples	Selected constitutional targets	Appropriate prenatal workflow
Peripheral blood	Constitutional / hematologic cells	Target population present
Biopsy touch imprint	Accessible tumor nuclei	Tumor-cell selection
Fixed tissue sections	Archived histopathology	Truncation artifacts

Editable comparison table

Targeted resolution is a principal advantage

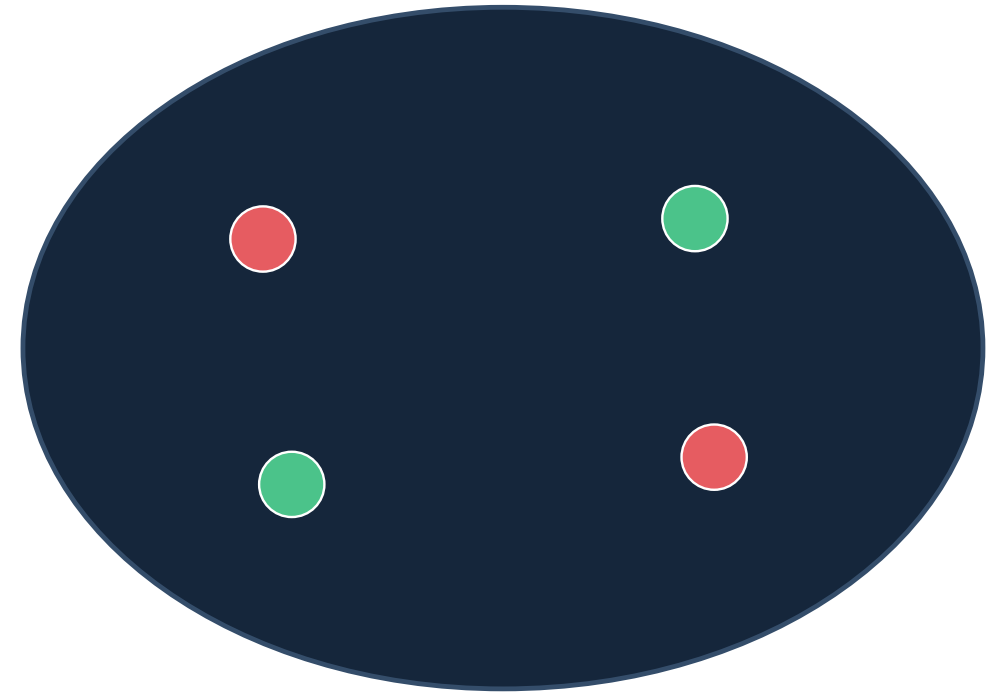
- Conventional chromosome banding requires large alterations to become visibly apparent
- Locus-specific FISH may disclose lesions below standard karyotype resolution
- Resolution depends on probe span, spacing, and the structural abnormality



Aneuploidy changes chromosome copy number

- Normal diploid cells generally contain two copies of an autosomal locus
- A trisomic cell may show an additional locus signal
- Signal enumeration must consider nuclear truncation, overlap, and assay controls

Two-copy reference pattern



Schematic — illustrative signal pattern

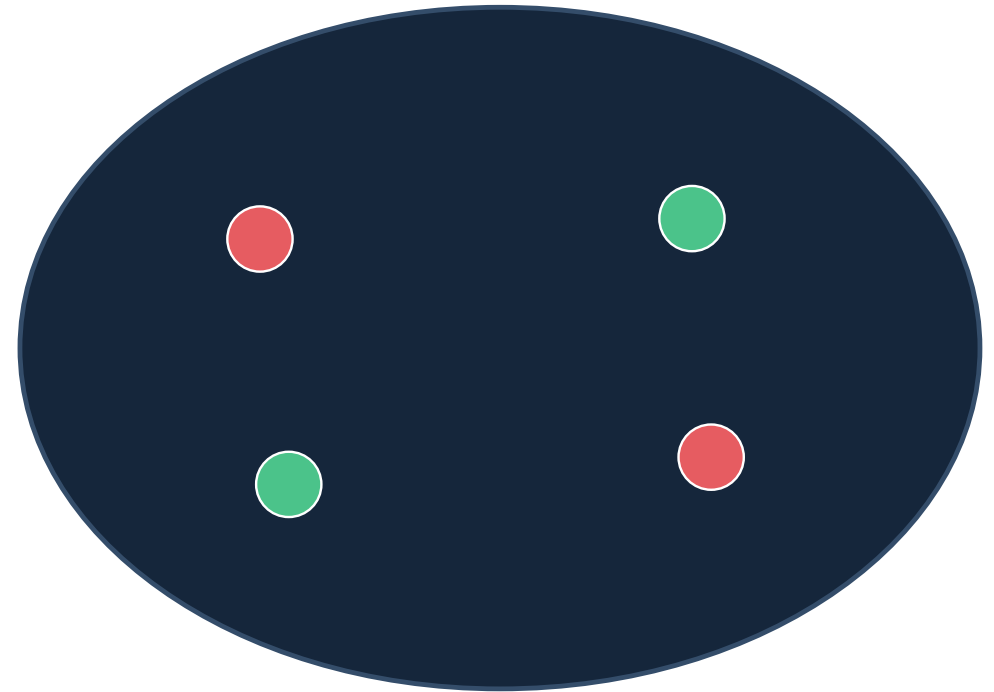
● Target A

● Target B / control

Normal two-color enumeration pattern

- A disomic reference nucleus is expected to show two signals per autosomal target in an idealized preparation
- Color identifies which chromosome or locus each signal represents
- An individual nucleus is illustrative; Diagnostic scoring evaluates a validated collection of cells

Two-copy reference pattern



Schematic — illustrative signal pattern

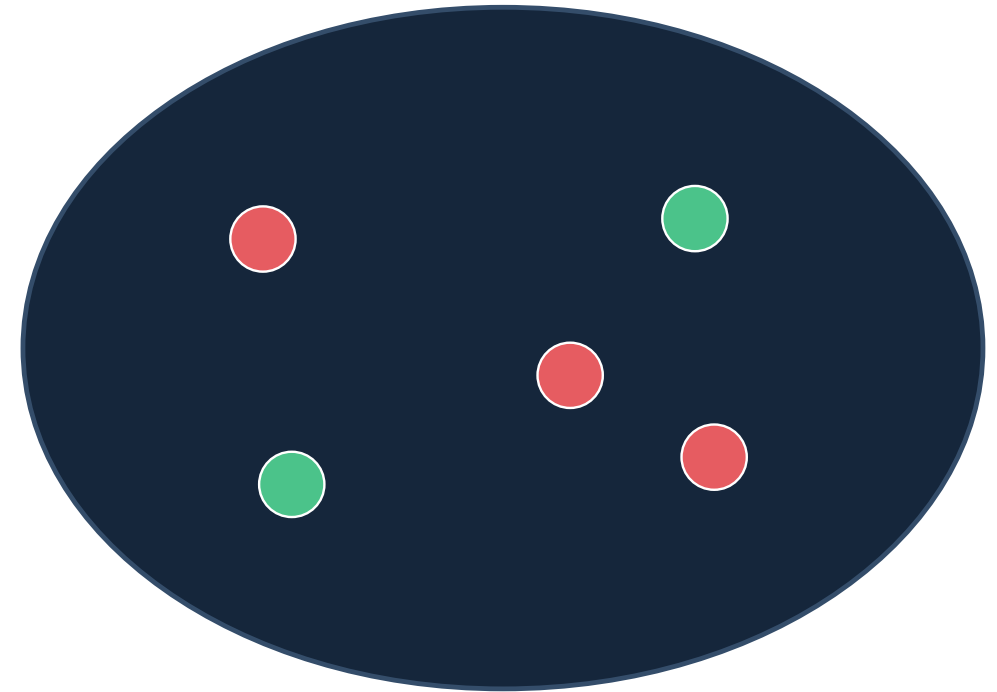
● Target A

● Target B / control

Trisomy 21: An extra locus signal

- Chromosome 21–specific probes identify an additional chromosome-21 target copy
- Robbins Figure 5.21 depicts three chromosome-21 signals with two chromosome-13 signals
- This demonstrates numeric chromosome imbalance at the interrogated loci

Three target / two reference signals



Schematic — illustrative signal pattern

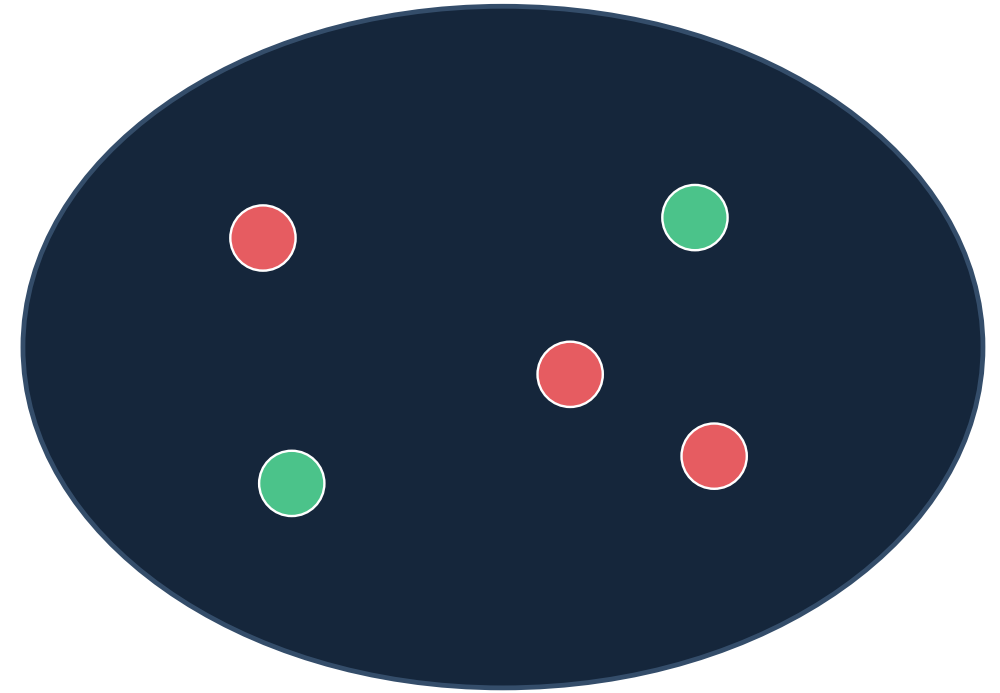
● Target A

● Target B / control

Read the trisomy signal pattern precisely

- Three target signals are consistent with an extra chromosome-21 locus
- A two-signal control supports adequate detection at the reference locus
- The pattern should be integrated with specimen identity and other cytogenetic information

Three target / two reference signals



Schematic — illustrative signal pattern

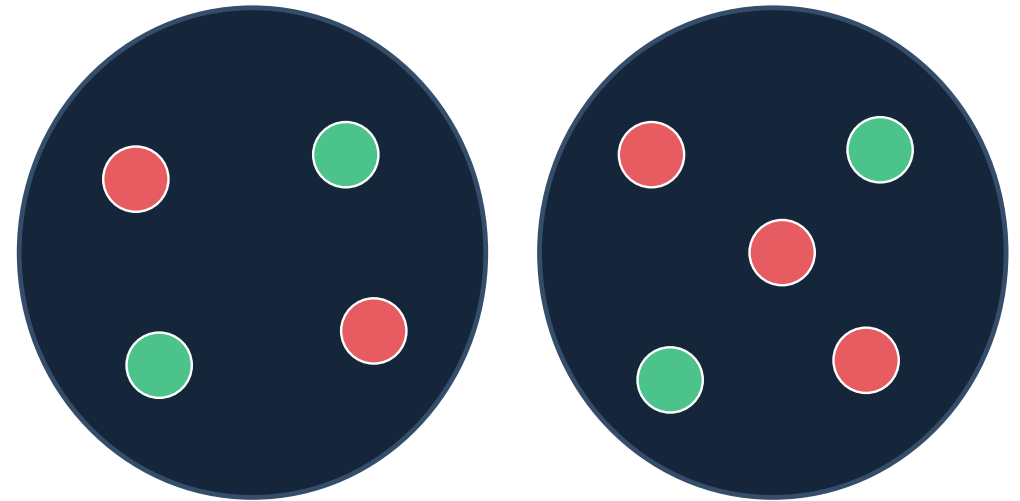
● Target A

● Target B / control

Mosaicism creates more than one cell population

- Different nuclei may display different locus counts within one specimen
- A mixture of two-signal and three-signal populations suggests possible mosaicism
- Biologic mosaicism must be distinguished from technical signal loss or scoring artifacts

Two cell populations in one specimen



Disomic example

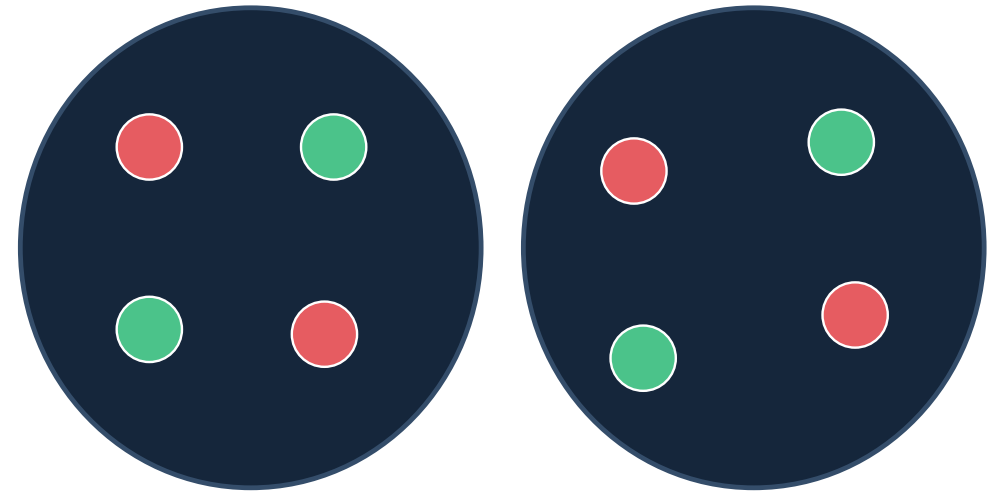
Trisomic example

Interpret the proportions across multiple nuclei

Signal enumeration requires multiple nuclei

- Single-cell patterns can be distorted by overlap, splitting, or incomplete nuclei
- Assess a sufficient number of interpretable nuclei under the laboratory protocol
- Report the observed distribution rather than extrapolating from one striking field

Score interpretable nuclei, not one field



Cell A

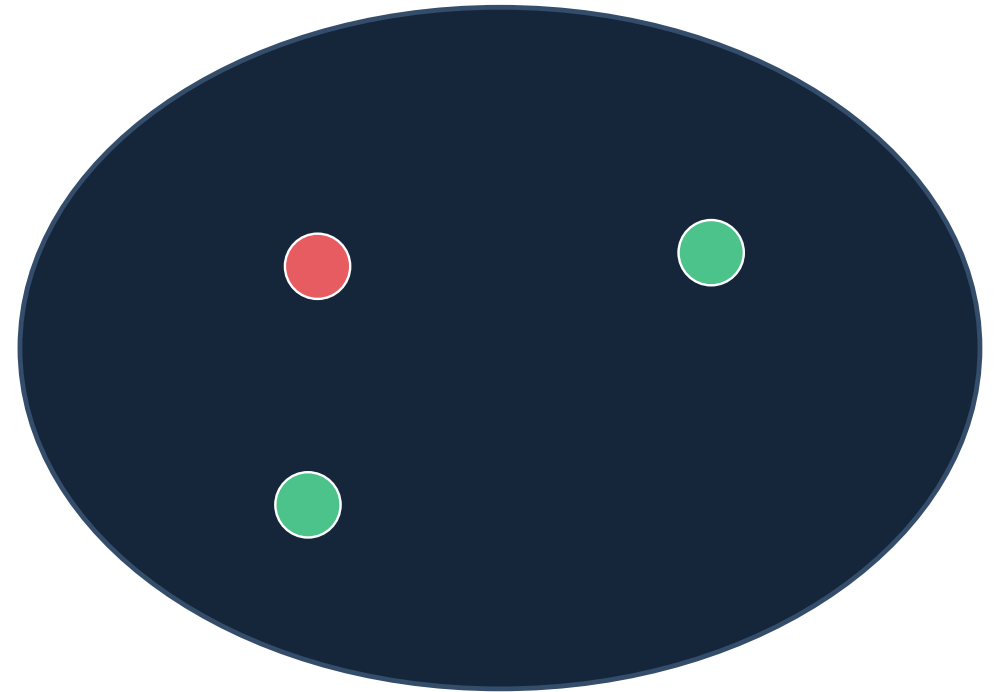
Cell B

Validated cell counts + exclusion rules

A deletion removes a targeted DNA interval

- A locus-specific probe fails to bind when its complementary interval is absent
- A separate chromosome control helps show that the chromosome or nucleus is present
- A normal control does not exclude loss of the interrogated target

Single-locus copy loss



Schematic — illustrative signal pattern

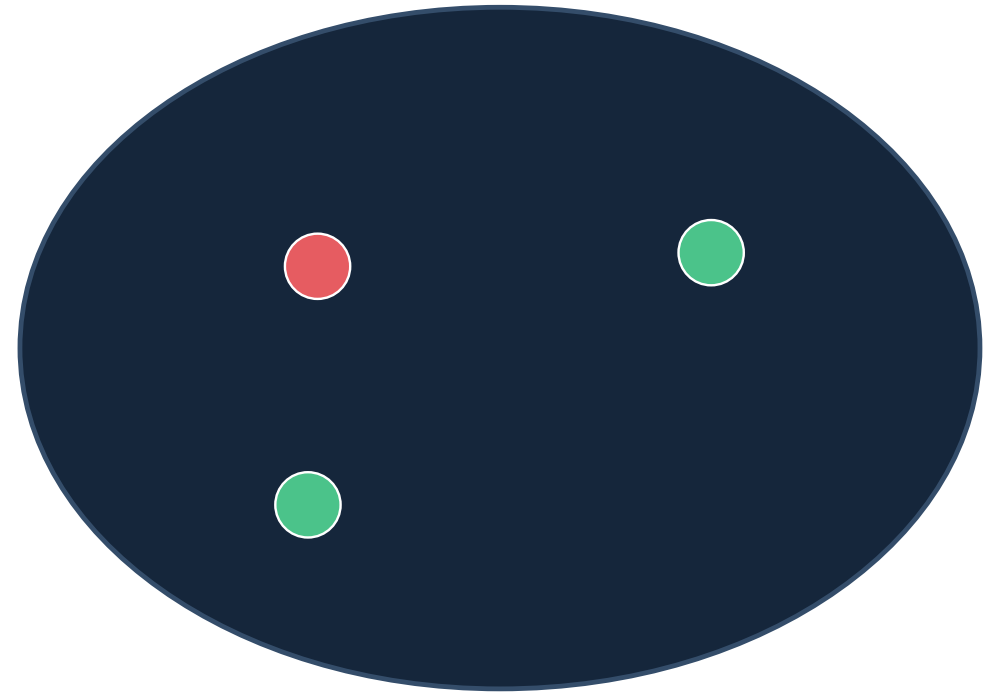
● Target A

● Target B / control

Microdeletions may escape routine banding

- Some pathogenic chromosomal deletions are too small for routine karyotypic visualization
- Targeted FISH can test a suspected microdeletion directly
- A negative FISH result does not survey unprobed regions of the chromosome

Single-locus copy loss



Schematic — illustrative signal pattern

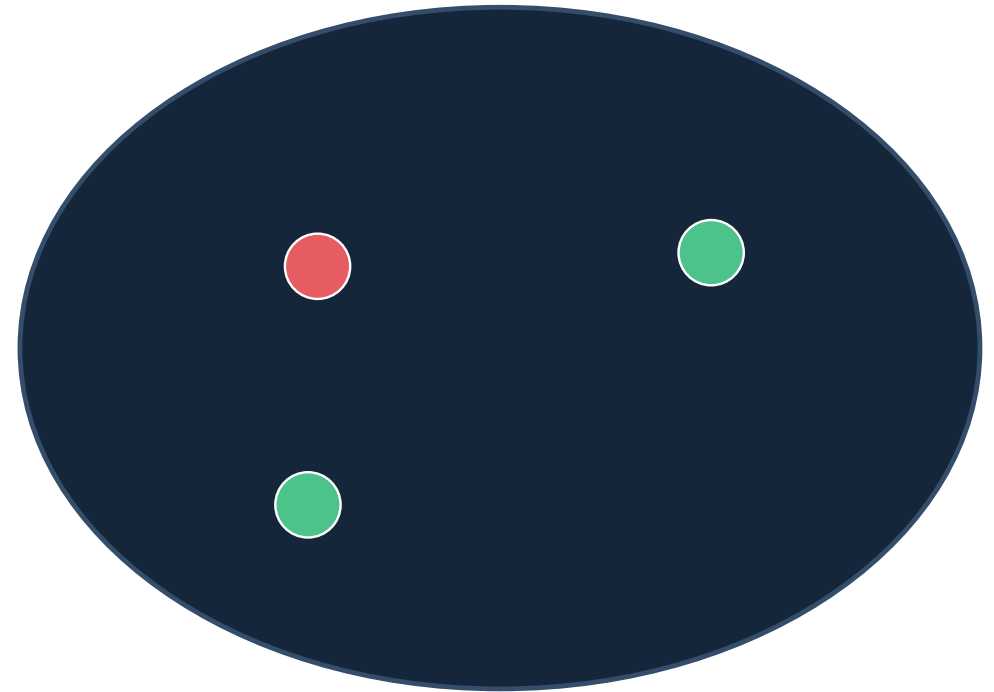
● Target A

● Target B / control

The 22q11.2 example uses an internal control

- Robbins Figure 5.23 uses a red probe at 22q11.2
- A green probe targets another chromosome-22 region for control
- Loss of one red signal with preservation of green signals supports a 22q11.2 deletion

22q11.2 deletion pattern

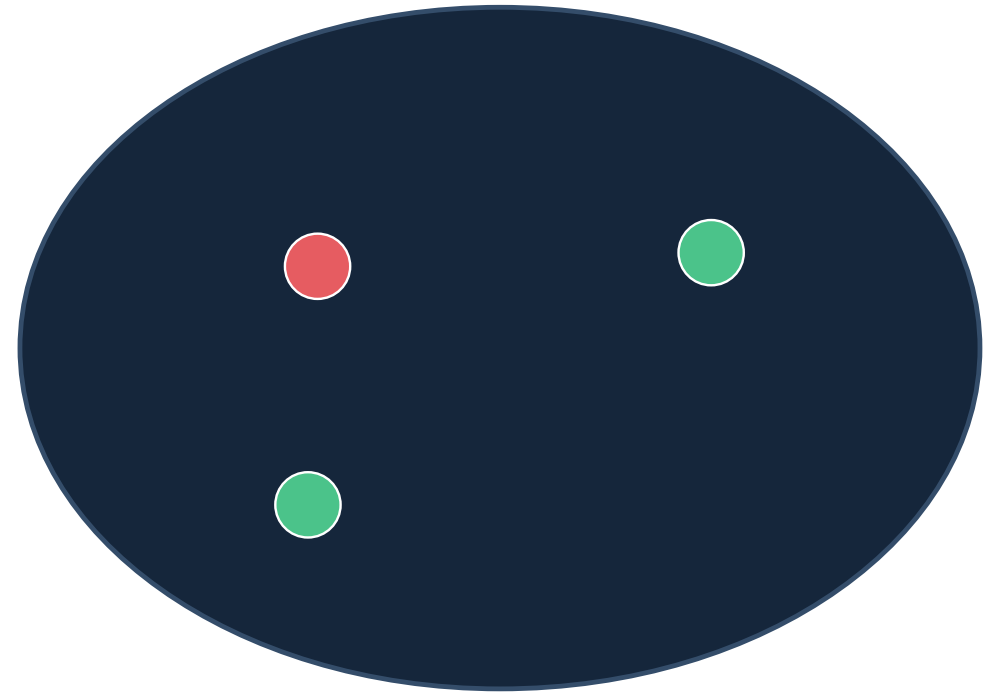


Schematic illustrative signal pattern
One 22q11.2 target **Two chr22 controls**

Deletion interpreted in an interphase nucleus

- An idealized deletion pattern shows one target signal and two control signals
- The control indicates that both homologous chromosomes contribute detectable signal
- Apparent loss still requires exclusion of truncation and hybridization failure

22q11.2 deletion pattern

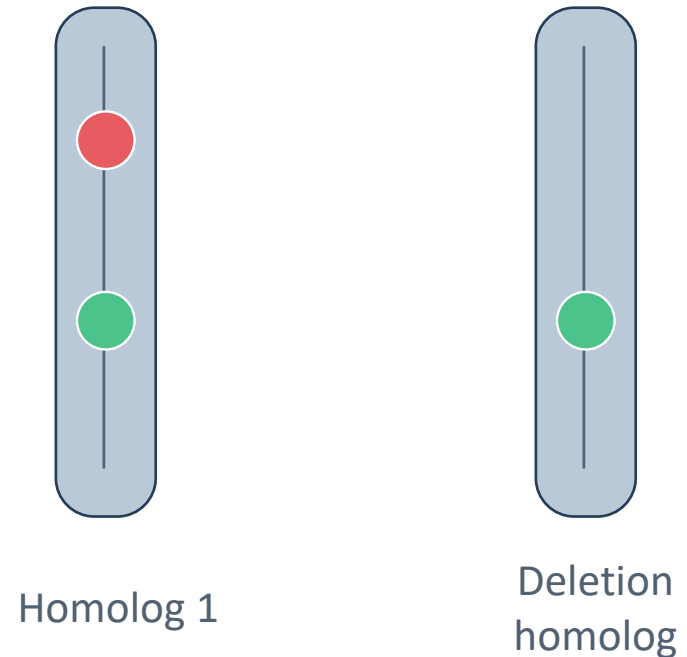


Schematic illustrative signal pattern
One 22q11.2 target **Two chr22 controls**

Metaphase FISH localizes the missing interval

- A normal homolog carries both target and control signals
- The homolog with a deletion retains control but lacks the target signal
- Matching interphase and metaphase patterns can support a coherent interpretation

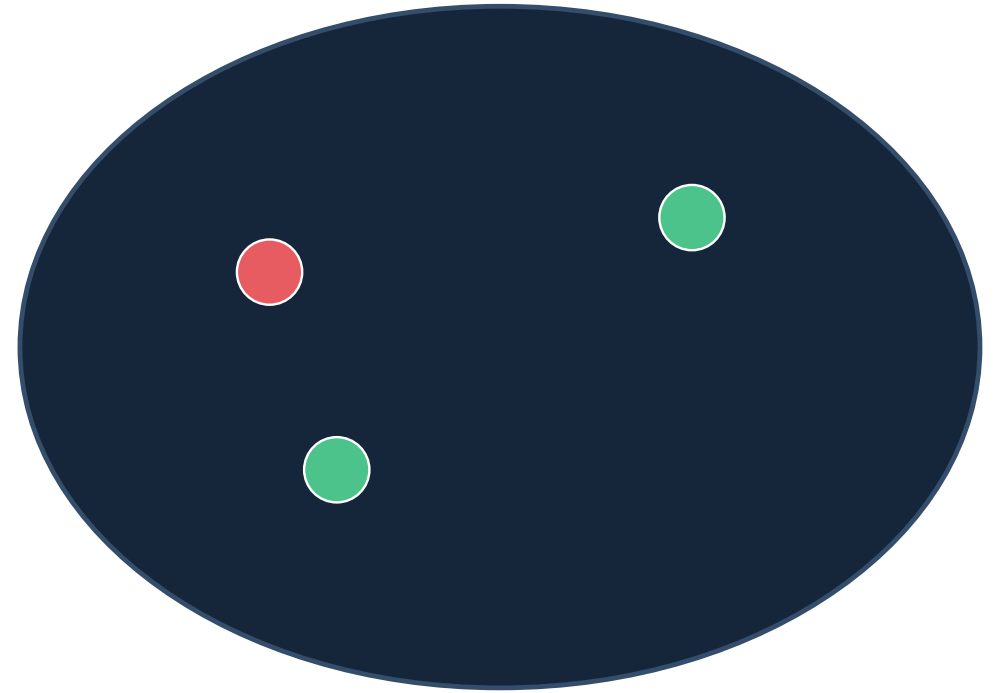
Chromosome 22 homolog comparison



A control probe addresses alternative explanations

- Without a control, a missing target may reflect chromosome loss or technical failure
- Preserved reference signals provide a check on the imaged nucleus
- Control strategy depends on the assay and suspected lesion

Target loss with retained reference



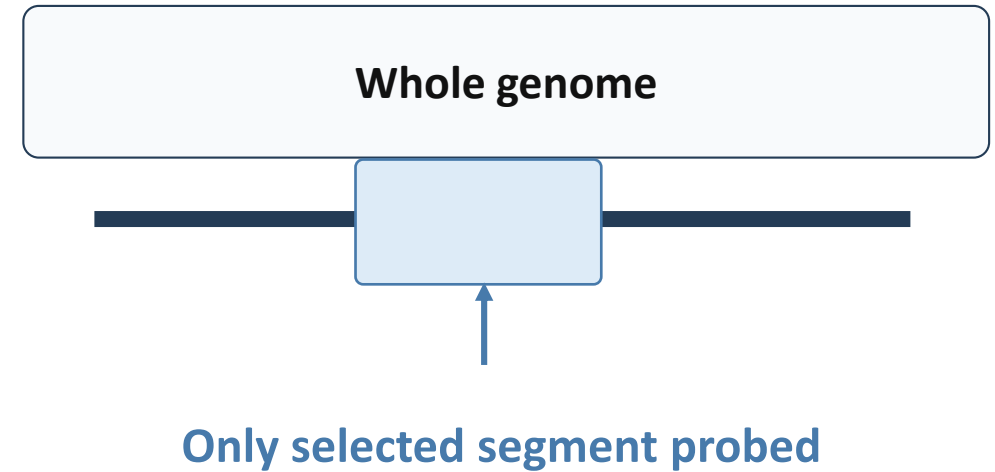
Schematic — illustrative signal pattern

● Target A

● Target B / control

A focused probe is not a genome-wide screen

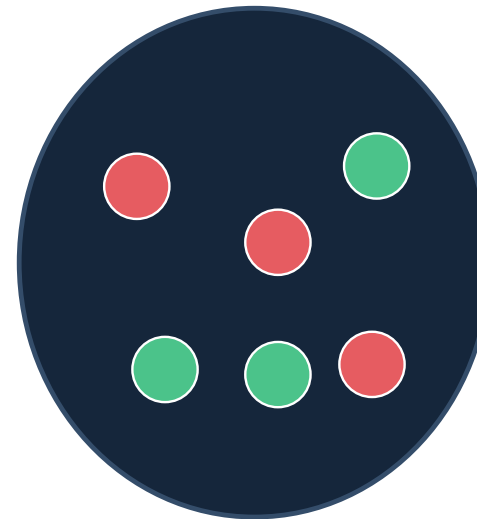
- FISH directly interrogates only intervals represented by the selected probes
- Unanticipated alterations elsewhere may remain invisible
- A broader assay is appropriate when the abnormal locus is unknown



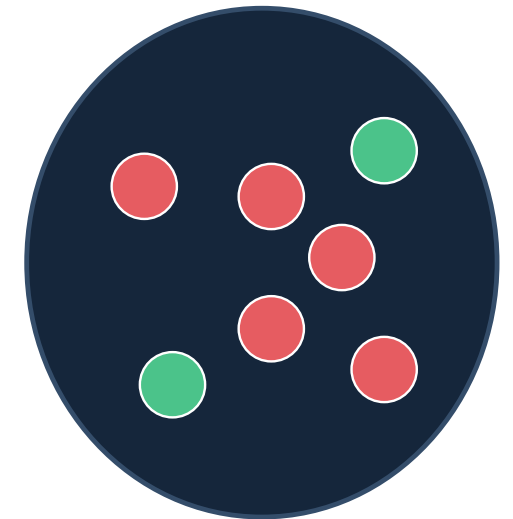
Copy-number gain and amplification are distinct ideas

- A modest increase in a locus signal can reflect increased chromosome content
- Focal gene amplification generates disproportionate gain of a genomic interval
- The signal pattern and a suitable comparator determine the interpretation

Whole-chromosome gain vs focal gain



Co-gain concept

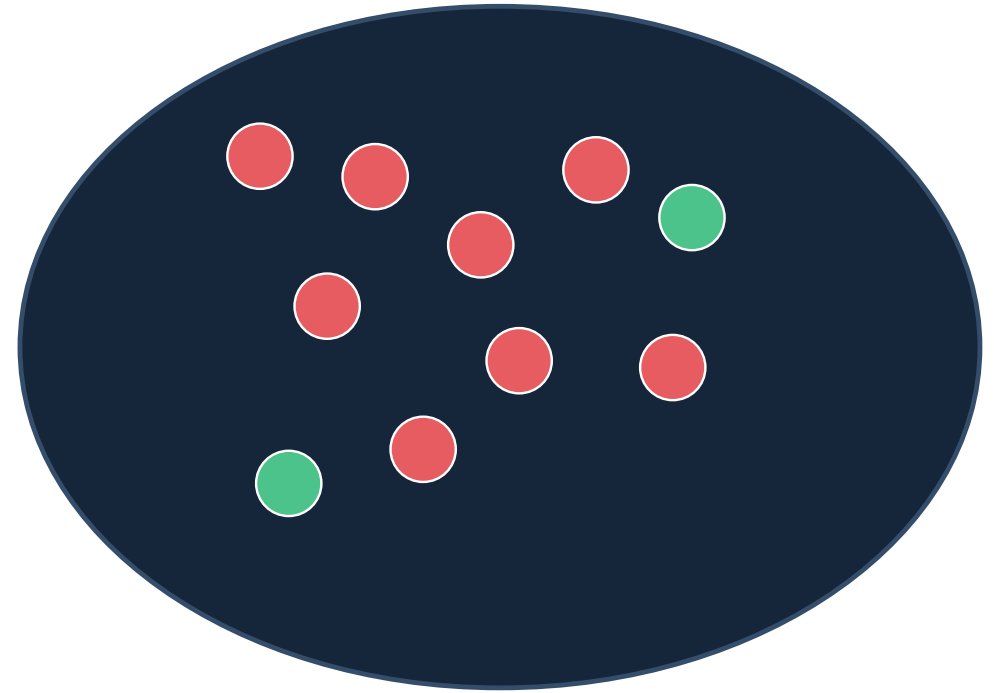


Locus-enriched concept

Gene amplification produces extra probe signals

- An amplified target can form multiple discrete signals or clustered signal groups
- Signal intensity and clustering are assay- and preparation-dependent
- A visual impression alone is not a validated amplification call

Disproportionate target signal gain



Schematic — illustrative signal pattern

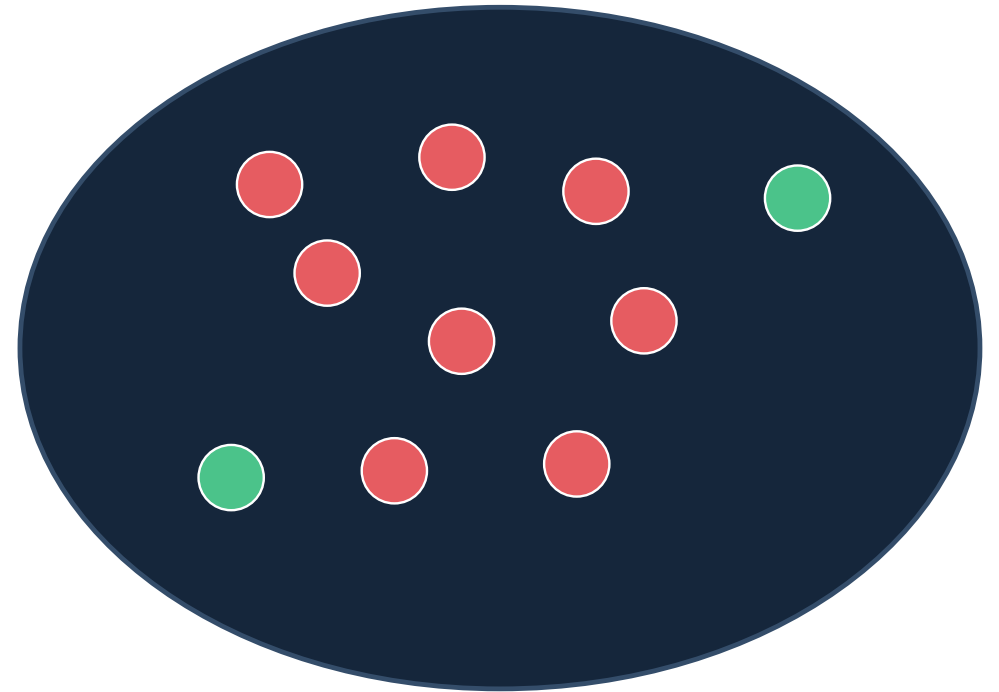
● Target A

● Target B / control

HER2 amplification is an actionable example

- Breast cancer testing can detect ERBB2 (HER2) copy-number amplification by FISH
- Robbins Figure 23.19 illustrates HER2 probes together with chromosome-17 control probes
- Gene amplification provides molecular context for HER2-positive disease

ERBB2/HER2 target and chr17 control



Schematic — illustrative signal pattern

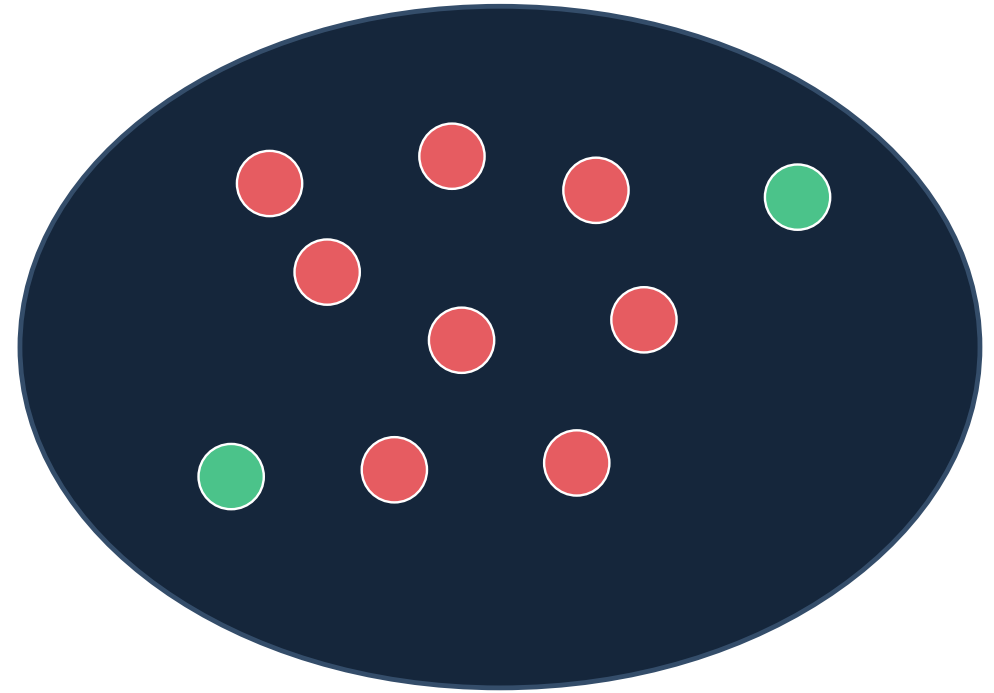
Red = HER2 target

Green = chr17 control

Why the reference chromosome probe matters

- A locus count must be interpreted relative to overall chromosome content where appropriate
- A chromosome-17 centromeric probe supplies comparative information in HER2 FISH
- Clinical classification requires the applicable assay standard; No universal cut-off is shown here

ERBB2/HER2 target and chr17 control



Schematic — illustrative signal pattern

Red = HER2 target

Green = chr17 control

Do not equate all signal gain with amplification

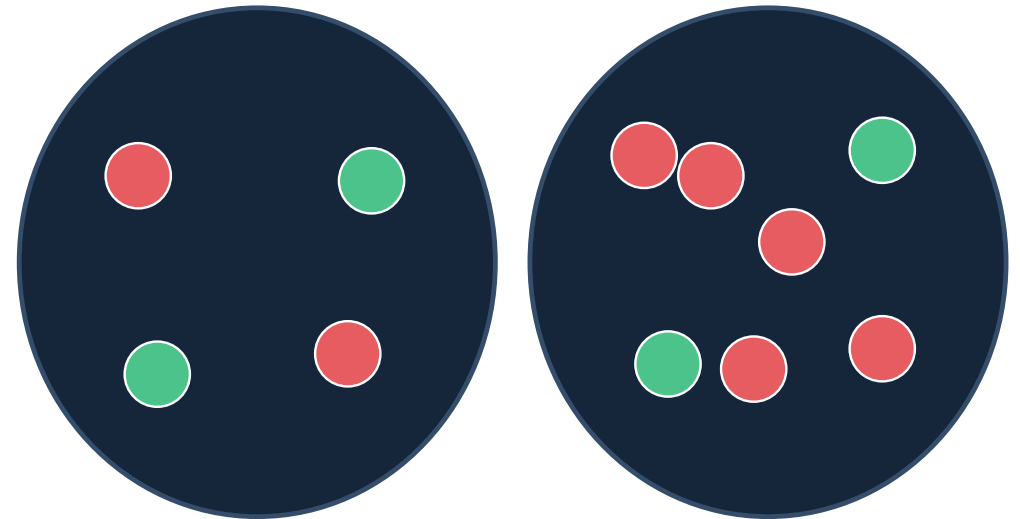
Pattern	Interpretive concept	Qualification
Target and reference co-gain	Broader copy-number increase	Interpret assay context
Target-enriched gain	Possible focal amplification	Use validated criteria
Mixed cells	Tumor heterogeneity	Representative scoring

Editable comparison table

Tumor heterogeneity changes what is sampled

- Genomic copy-number patterns may differ between tumor regions
- A selected field may not represent the entire lesion
- Morphologic selection and validated sampling procedures are essential

Intermingled tumor-cell populations



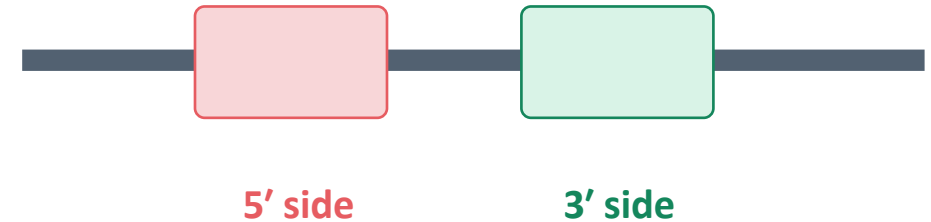
Lower-copy cell

Higher-copy cell

Cell identity and selection matter

Rearrangements alter chromosome architecture

- Translocations and other rearrangements reorganize genomic intervals
- A probe arrangement can be designed to change its spatial signal pattern
- FISH identifies the chosen rearrangement signature without sequencing its breakpoint



Two probes straddle one locus

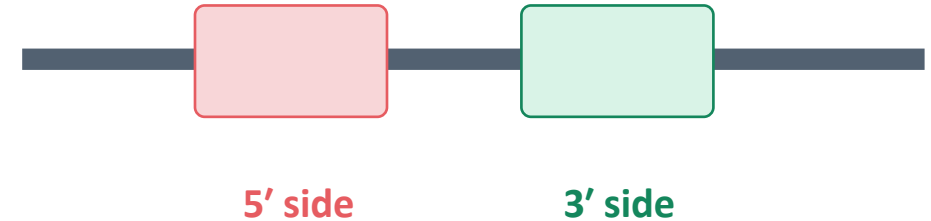
Two common rearrangement strategies

Design	Question	Interpretation
Locus-specific	Target present / how many?	Signal enumeration
Deletion + control	Is one locus lost?	Relative target loss
Break-apart	Is this locus rearranged?	Probe separation
Fusion-oriented	Are two loci juxtaposed?	Co-localized probe pattern

Editable comparison table

Break-apart probes flank a single locus

- Different fluorophores label sequences on opposite sides of the target gene
- An intact locus produces adjacent or overlapping colors
- A rearrangement involving the locus can separate the colors

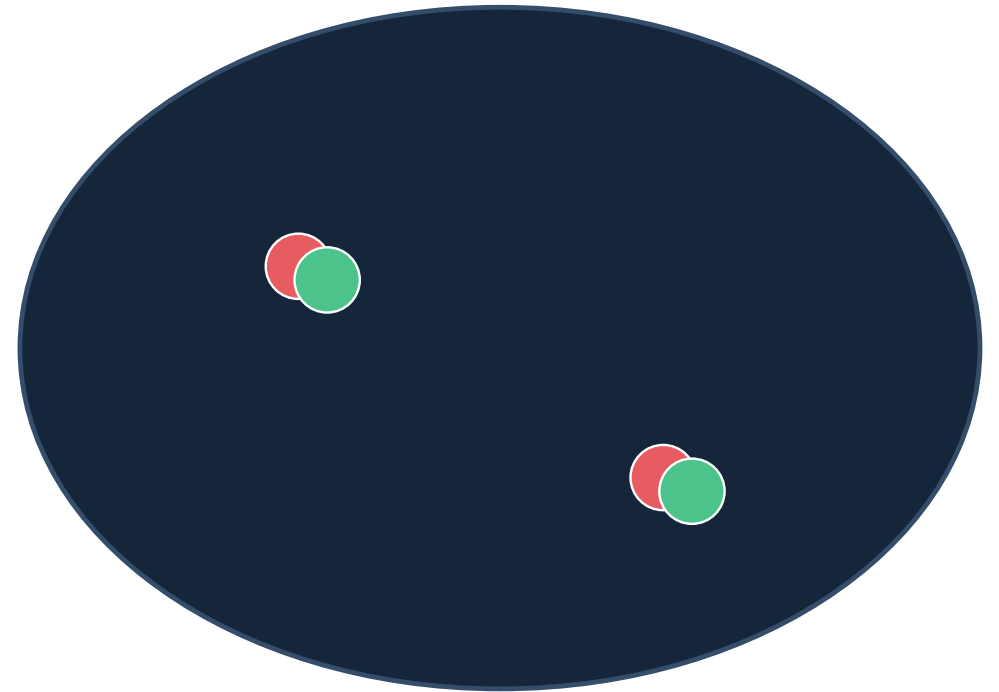


Two probes straddle one locus

An unarranged locus shows paired colors

- Two intact homologs generate two paired red/green configurations in an idealized diploid cell
- Close red and green signals may appear fused at optical resolution
- Pairing alone does not identify exact nucleotide structure

Both alleles retain paired flanking probes

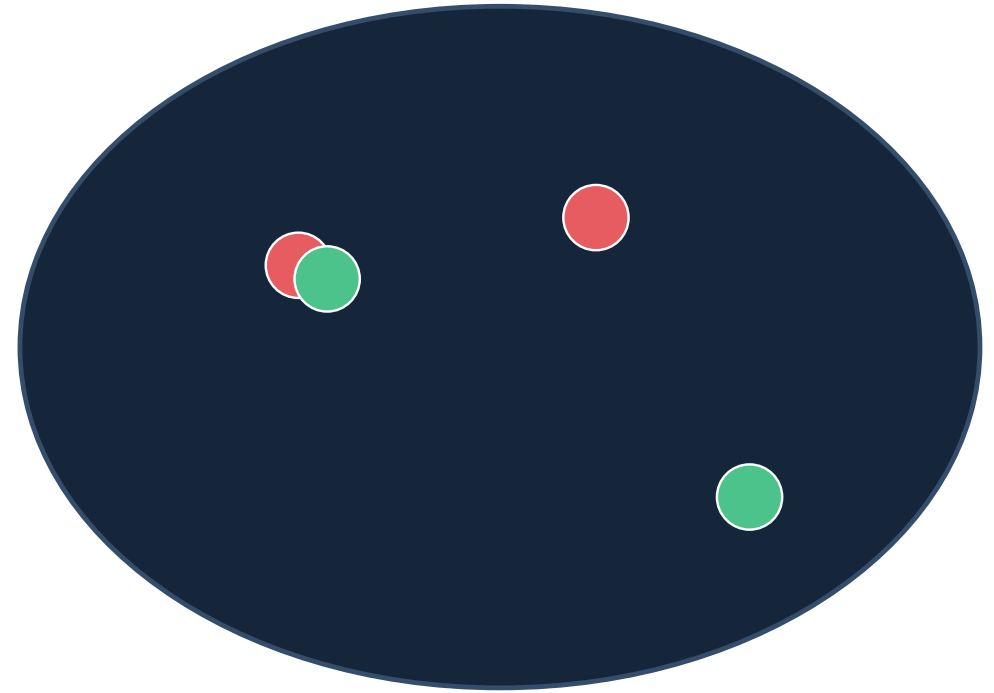


Schematic — illustrative signal pattern
Paired red/green on both homologs

A rearranged locus can split paired colors

- A locus-spanning break can separate the flanking probes
- One homolog may show a split while the other remains paired
- The validated assay determines what separation is considered abnormal

One paired allele and one split allele



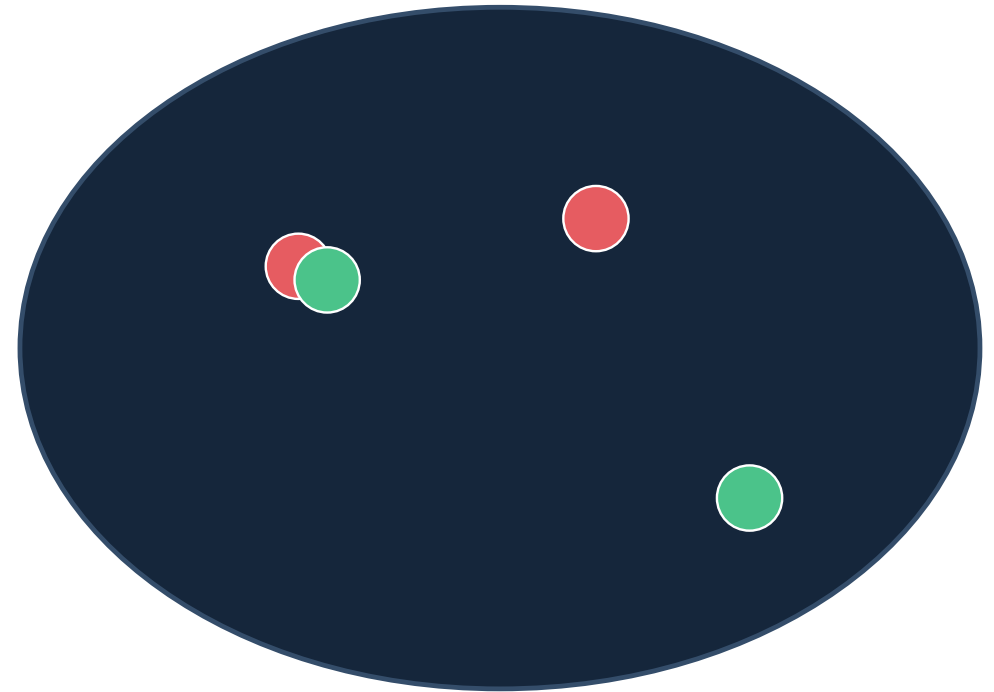
Schematic — illustrative signal pattern

Partner cannot be identified from split alone

MYC break-apart FISH detects partner-variable events

- MYC break-apart probes for gene rearrangements
- A split pattern can flag a MYC locus rearrangement without naming its partner
- Further work is necessary when the translocation partner changes diagnosis or management

One paired allele and one split allele

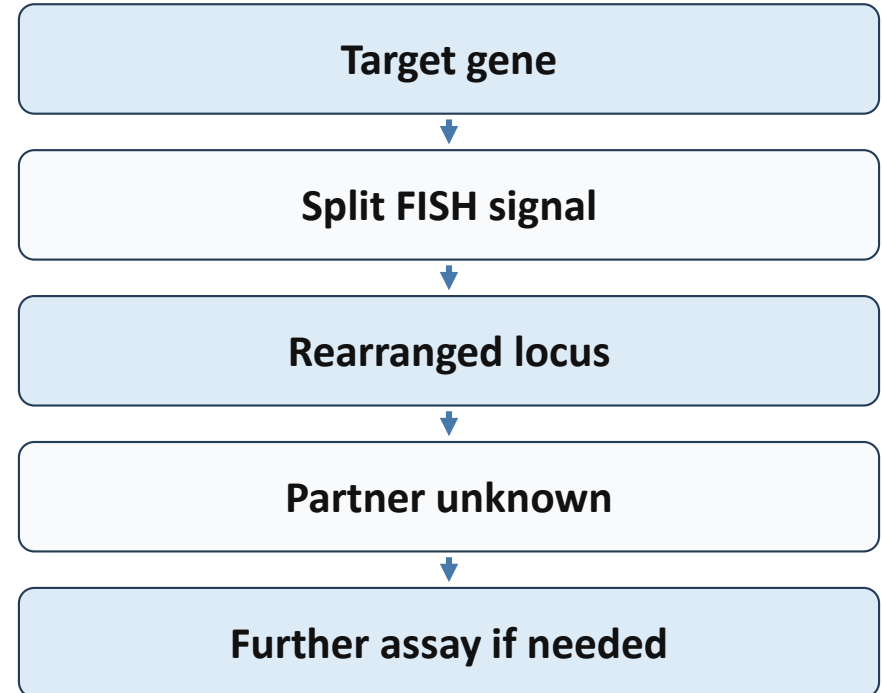


Schematic — illustrative signal pattern

Partner cannot be identified from split alone

What a break-apart result does not establish

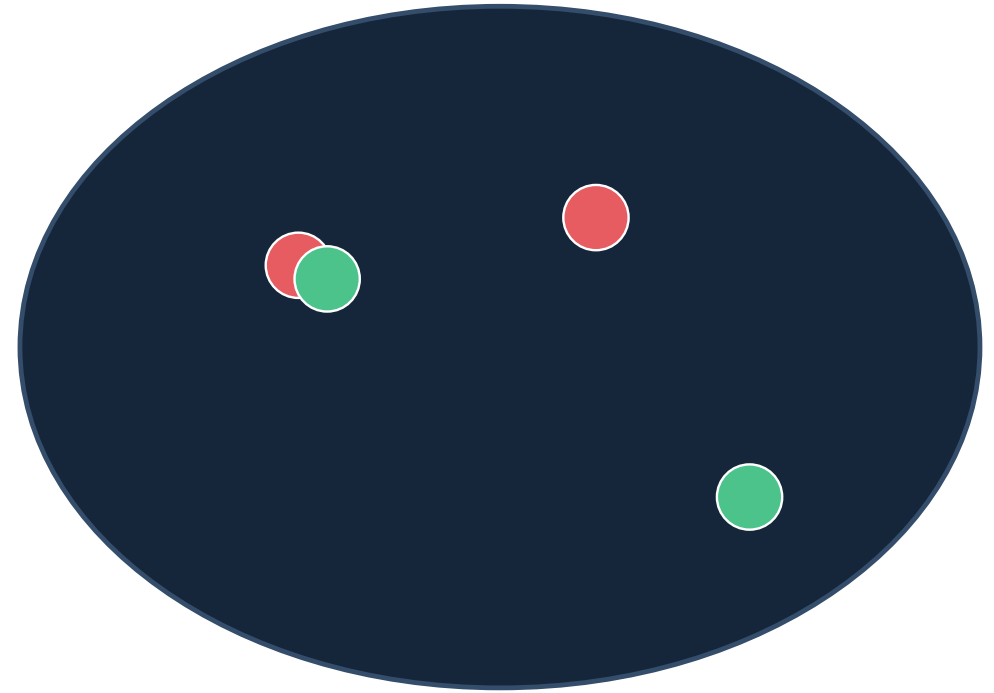
- Signal separation does not directly determine the partner chromosome
- It also does not sequence the breakpoint or prove fusion-transcript expression
- An orthogonal or partner-specific assay may be required



MYC signal separation differs from MYC gain

- Break-apart detects changes in spatial relationship between probe colors
- Amplification is a copy-number phenomenon involving increased signal abundance
- An assay may reveal both types of abnormalities; Classify the visual pattern correctly

One paired allele and one split allele



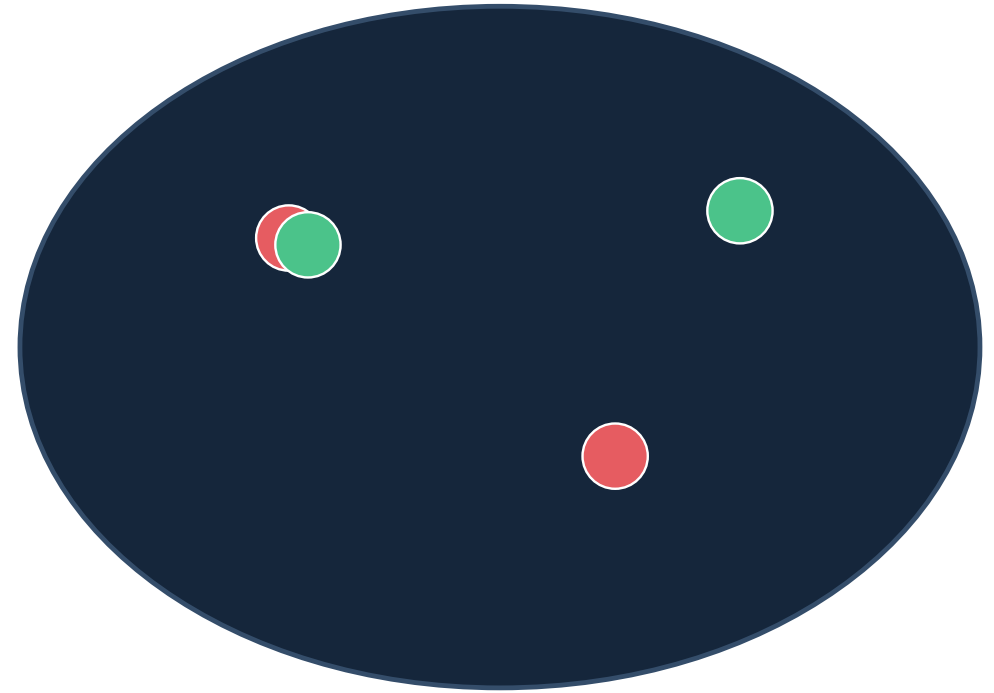
Schematic — illustrative signal pattern

Partner cannot be identified from split alone

Fusion-oriented probes test a partner hypothesis

- Two selected genomic loci are assigned distinct labels
- A relevant chromosomal rearrangement may juxtapose the labels
- Colocalization must be assessed against expected background and assay geometry

Juxtaposition of two named loci

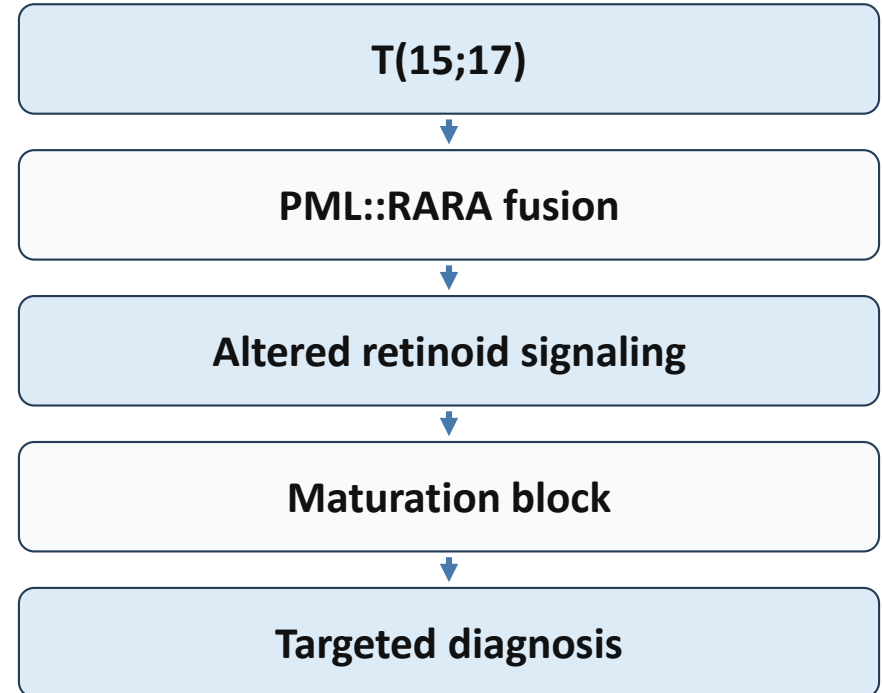


Schematic — illustrative signal pattern

Proximity is interpreted using a validated assay

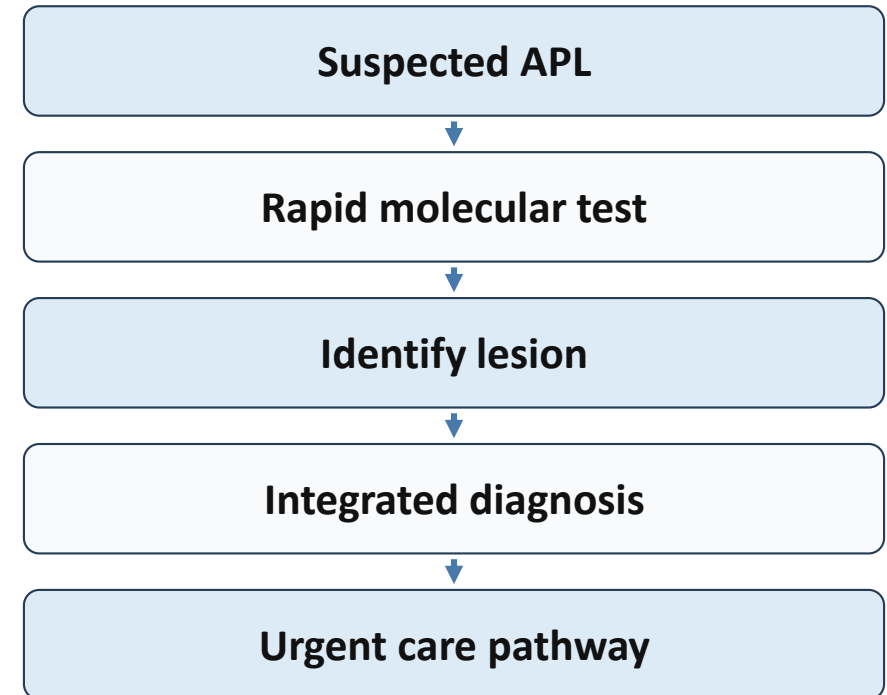
PML::RARA is an important disease-associated fusion

- Acute promyelocytic leukemia typically carries t(15;17), generating PML::RARA
- The altered retinoic-acid receptor biology contributes to impaired myeloid maturation
- Identification of this lesion has urgent clinical implications



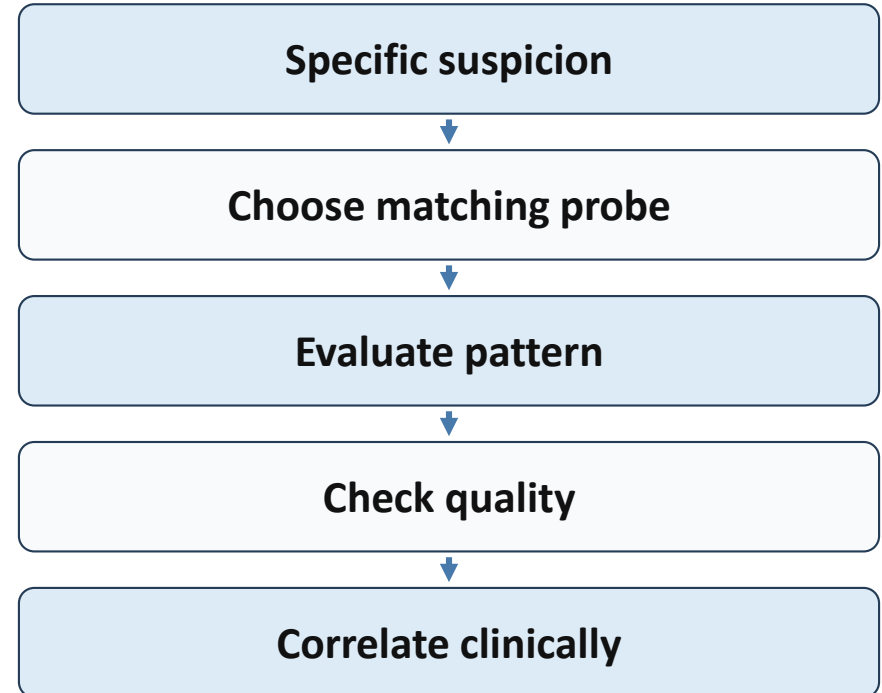
Suspected APL demands a rapid diagnostic pathway

- Suspected acute promyelocytic leukemia as a setting for rapid FISH
- Detection of the relevant retinoic-acid receptor rearrangement supports lesion-directed care
- Clinical urgency is not a justification to treat an unvalidated signal pattern as definitive



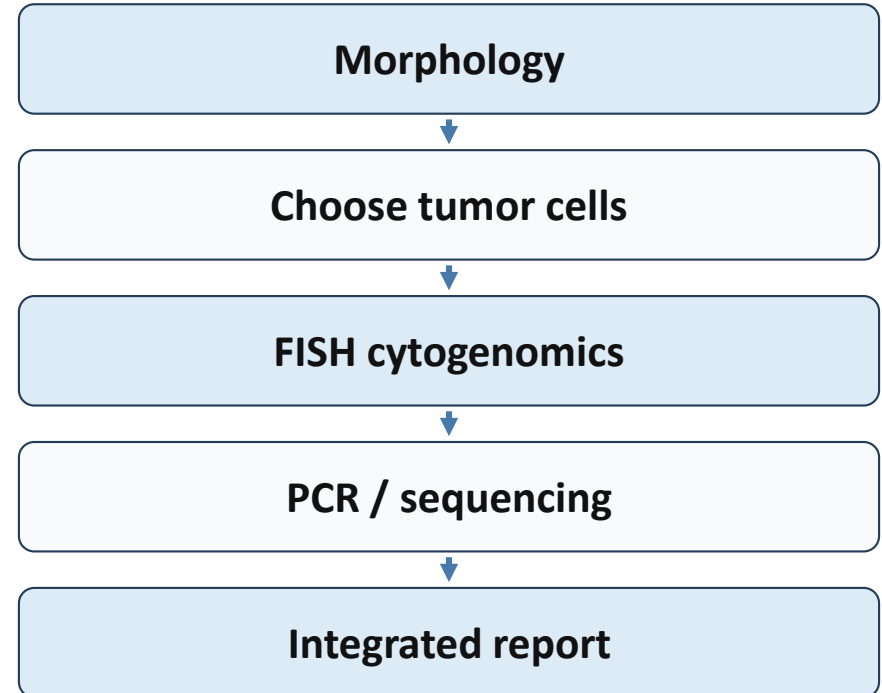
The clinical decision requires the correct target

- A generic structural abnormality is not equivalent to identifying PML::RARA
- Choose a probe configuration appropriate for the suspected leukemia
- Correlate the molecular result with morphology and the clinical presentation



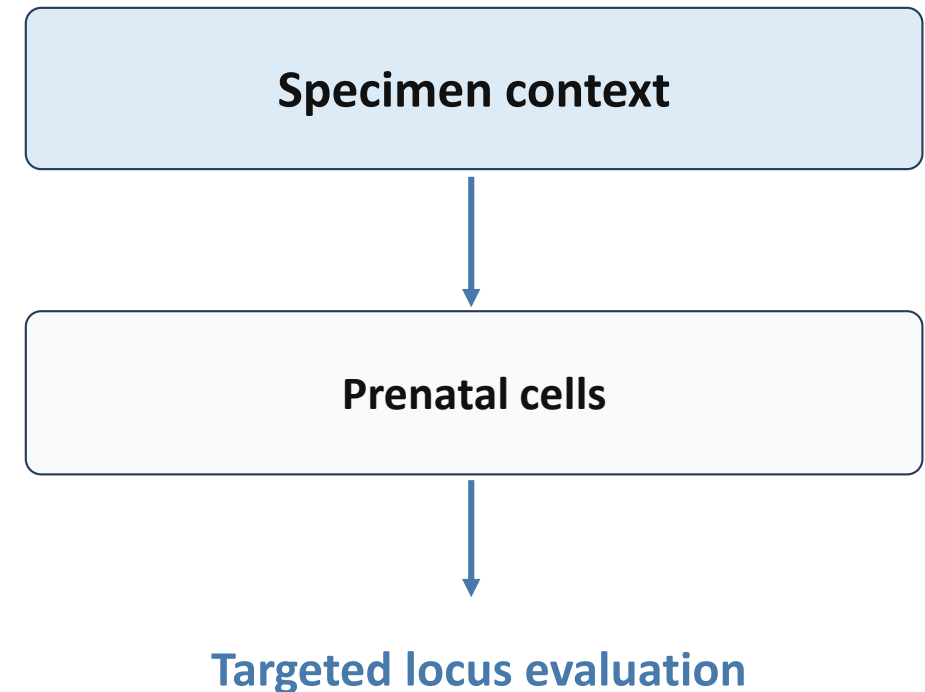
FISH complements other diagnostic modalities

- Morphologic examination selects the relevant cell population
- Cytogenetics can provide chromosome-wide structural context
- Sequencing or transcript assays may provide base-level or expression information



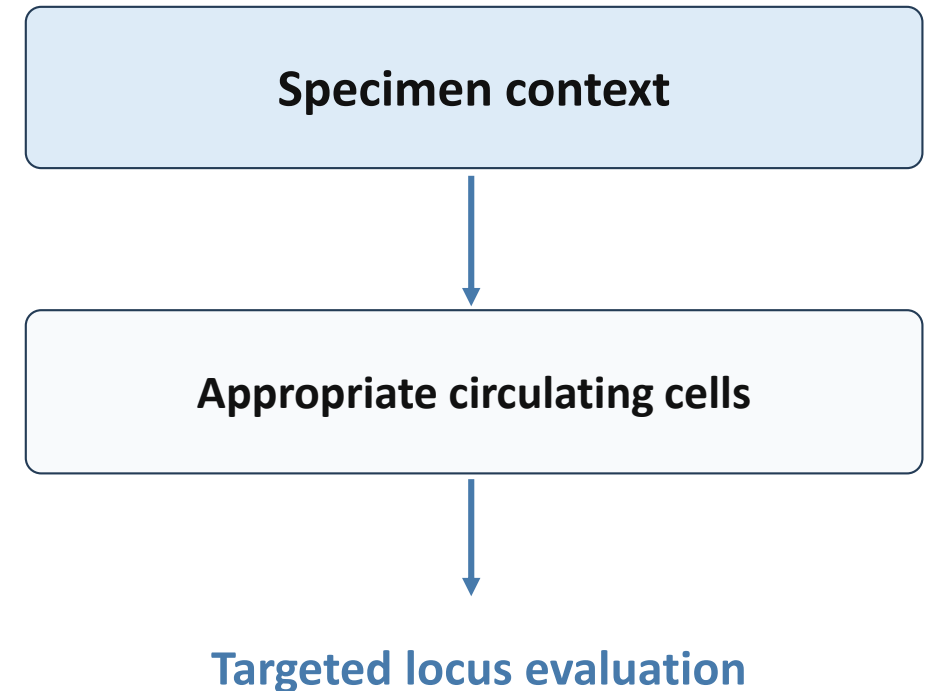
Prenatal material can support targeted FISH

- Interphase or metaphase preparations can assess selected chromosomal loci
- Targeted FISH can be useful when a specific aneuploidy or deletion is suspected
- Results must be interpreted within a validated prenatal-testing workflow



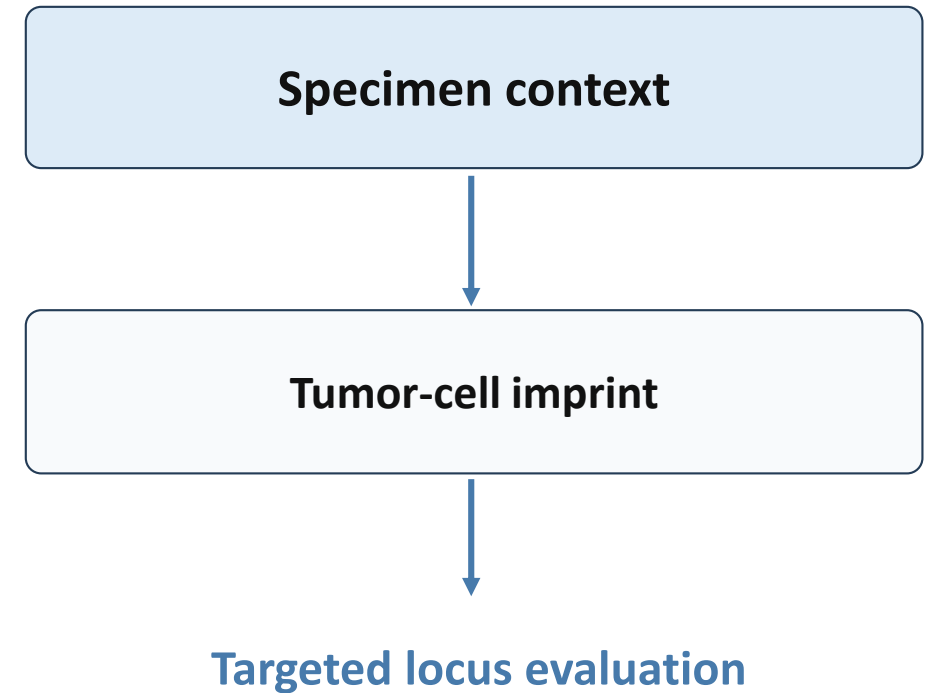
Peripheral blood is suitable in selected settings

- Circulating cells may provide material for constitutional cytogenetic assessment
- Hematologic malignancies may be investigated in blood when abnormal cells are present
- Specimen appropriateness depends on the clinical and biologic compartment



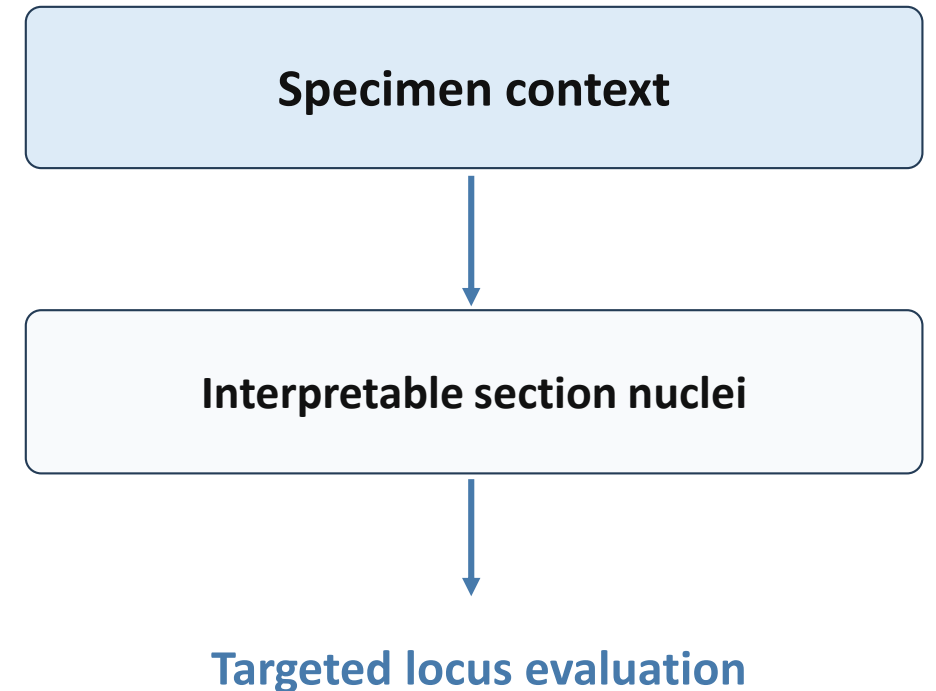
Touch preparations retain accessible tumor nuclei

- Fresh biopsy touch imprints can preserve cells for hybridization
- Direct nuclear evaluation can be tied to material sampled from a tumor
- Poor cellularity or wrong target-cell selection can compromise the result



Archival tissue expands diagnostic use

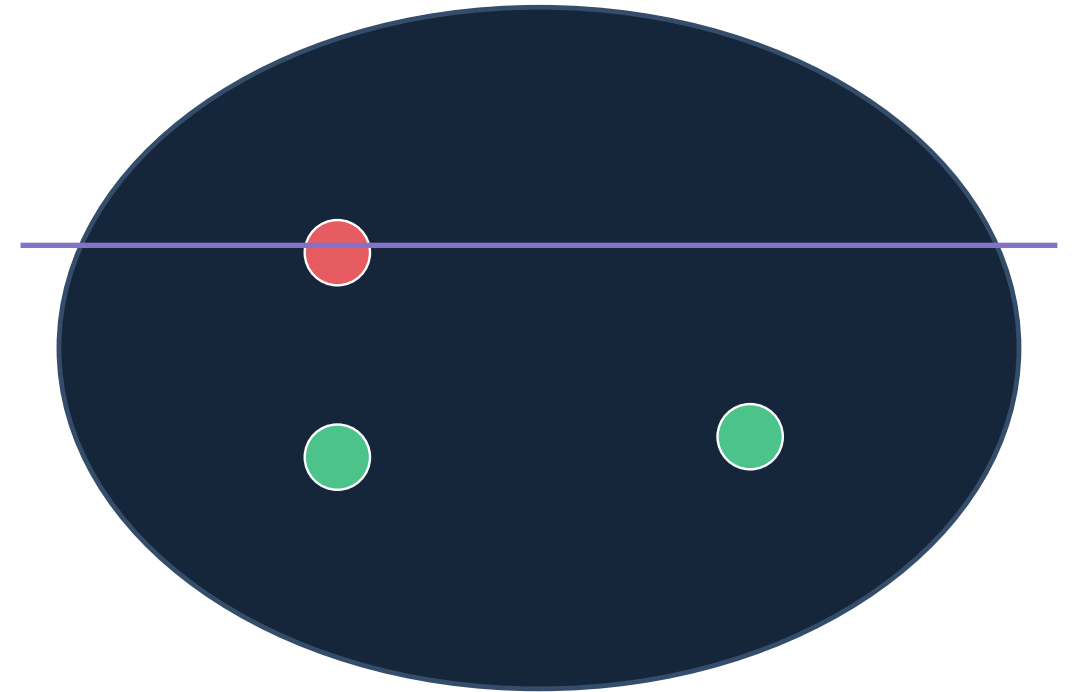
- FISH on fixed archival tissue sections
- Sectioned nuclei allow molecular assessment alongside histopathologic context
- Incomplete nuclear profiles can underestimate visible copy number



Sectioning may remove part of the nucleus

- A thin section may not contain every locus copy from a three-dimensional nucleus
- A missing signal can therefore be artifactual
- Scoring criteria and adequate control patterns reduce overinterpretation

Partial nucleus intersects a tissue section

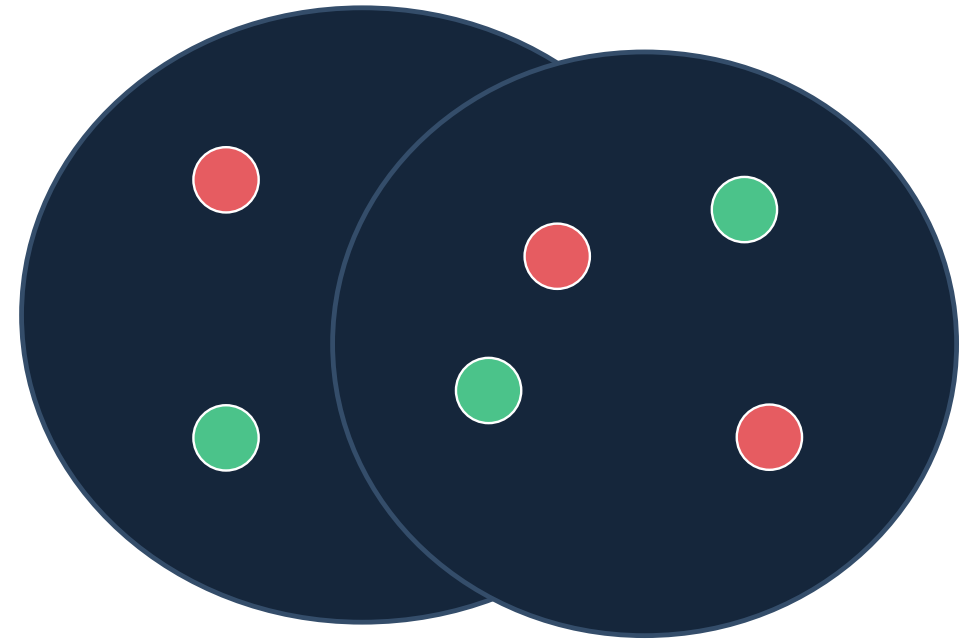


Schematic — illustrative signal pattern
Section plane (schematic)

Overlapping cells can simulate extra signals

- Superposed nuclei may appear to contain more signals than either cell truly carries
- Crowded regions also obscure nuclear boundaries
- Select well-preserved, separable, representative nuclei

Overlapping nuclei create false aggregates



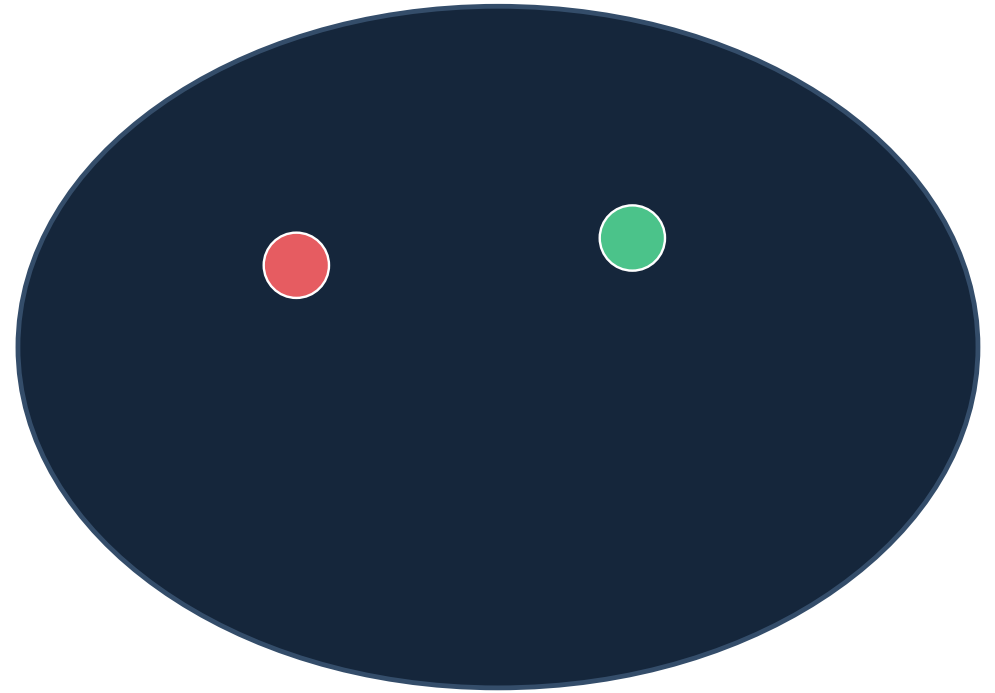
Schematic cell 1

Schematic cell 2

Hybridization failure can mimic true loss

- Inadequate DNA access or probe binding can reduce signals
- Weak or absent labeling must be evaluated with controls and quality criteria
- Failed technical runs are not interpretable negative molecular findings

Loss of probe signal need not mean deletion

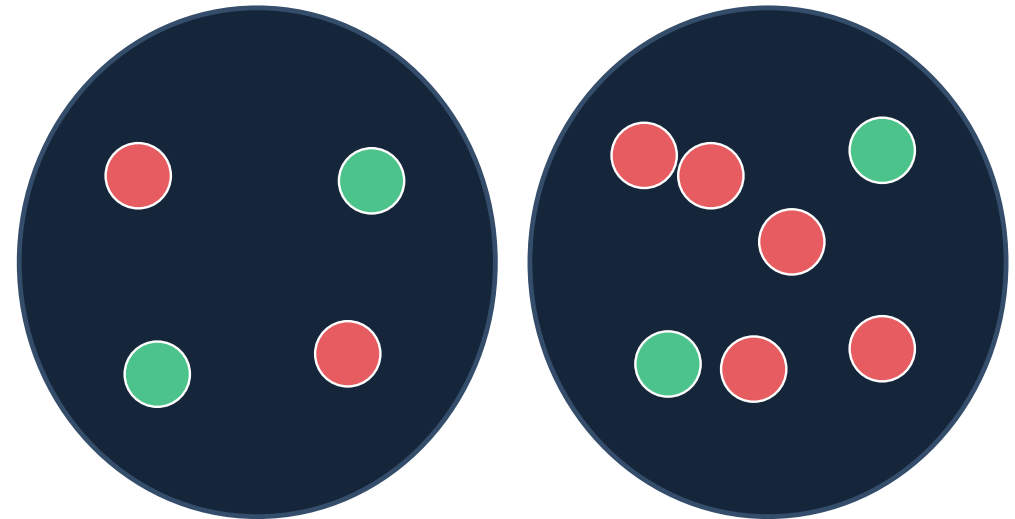


Schematic — illustrative signal pattern
Interpret only if controls and assay QC pass

Signal patterns may vary within a tumor

- Biologic heterogeneity and specimen sampling influence apparent fractions
- Neoplastic and non-neoplastic nuclei may coexist in the field
- A valid interpretation identifies the population actually evaluated

Intermingled tumor-cell populations



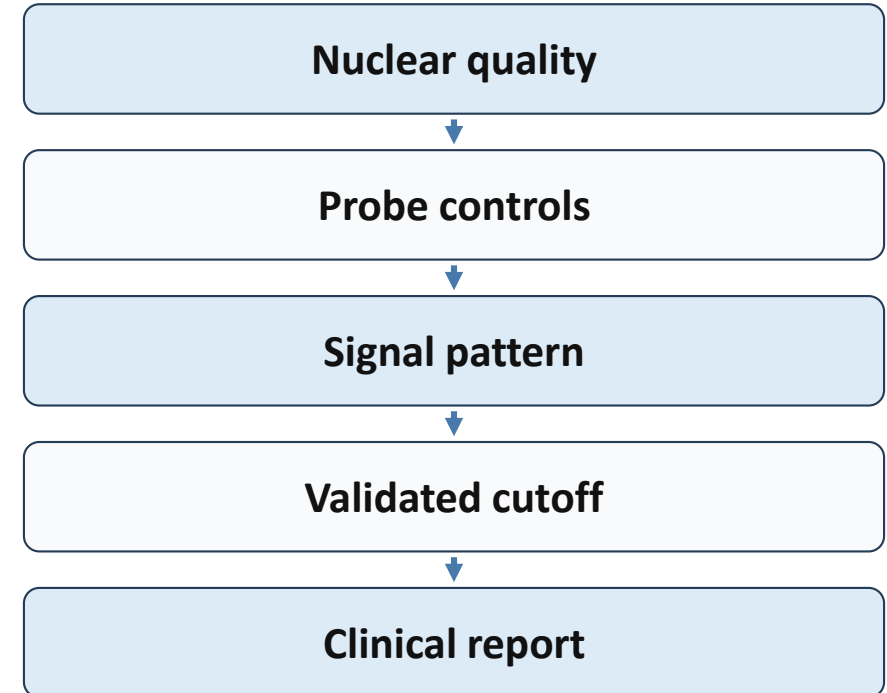
Lower-copy cell

Higher-copy cell

Cell identity and selection matter

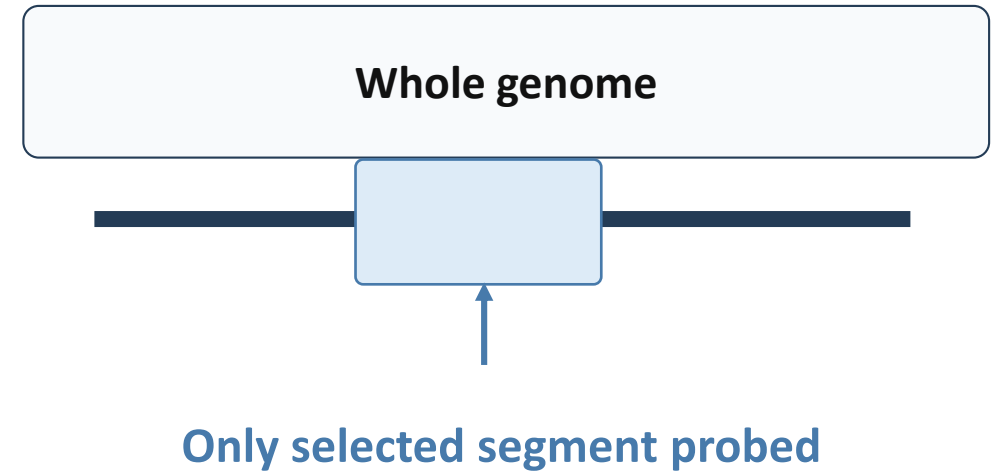
Laboratories need assay-specific scoring criteria

- Signals have finite optical size and spatial variability
- Cutoffs, minimum nuclei, and acceptance criteria depend on the validated probe/application
- Do not transfer an arbitrary numeric threshold between FISH assays



A normal targeted FISH assay has limits

- Unprobed loci and unrelated rearrangements remain outside its interrogation range
- Single-nucleotide substitutions cannot be characterized by routine locus-level FISH
- Some cryptic rearrangements may not produce detectable probe separation



PCR and FISH interrogate different features

Characteristic	FISH	PCR
Principal readout	Position / count of locus signals	Amplified target sequence
Resolution	Probe / optical context	Short defined nucleic acid interval
Cell-by-cell	Yes	Typically bulk extraction
Best question	Copy / rearrangement at locus	Defined sequence-level target

Editable comparison table

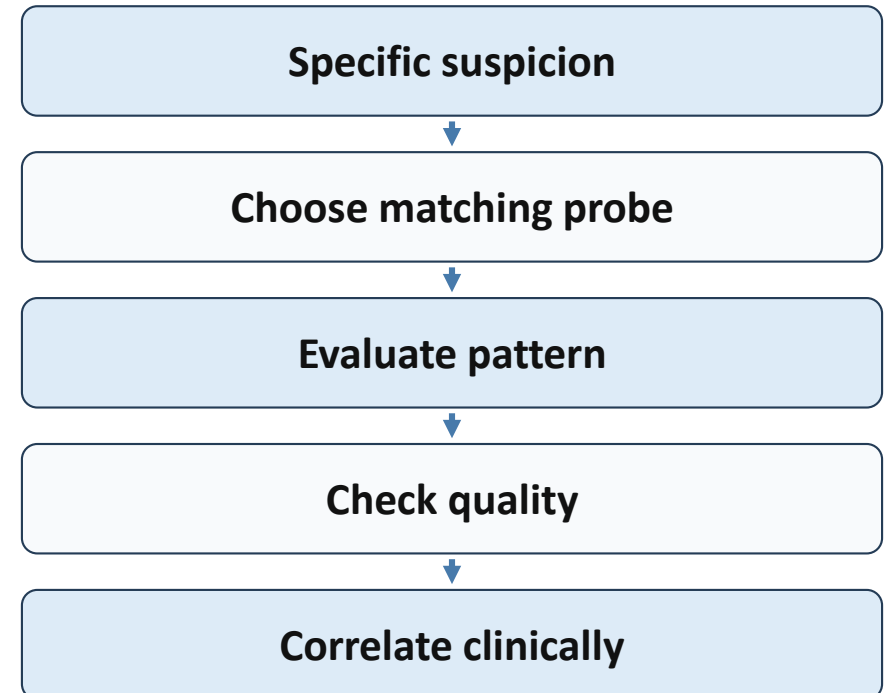
Arrays answer the genome-survey question

Characteristic	FISH	Array strategy
Coverage	Selected locus/loci	Many loci genome-wide
Readout	Spatial signals in cells	Aggregate copy-number data
Balanced event	Can detect with suitable probe	Not ordinarily by copy number alone
Best context	Specific alteration suspected	Locus not known

Editable comparison table

Selecting a test starts with the abnormality

- Suspected known locus deletion: Targeted locus/control FISH
- Partner-variable rearrangement: Gene-specific break-apart design
- Unexplained genome-wide copy-number changes: Consider an array-based strategy

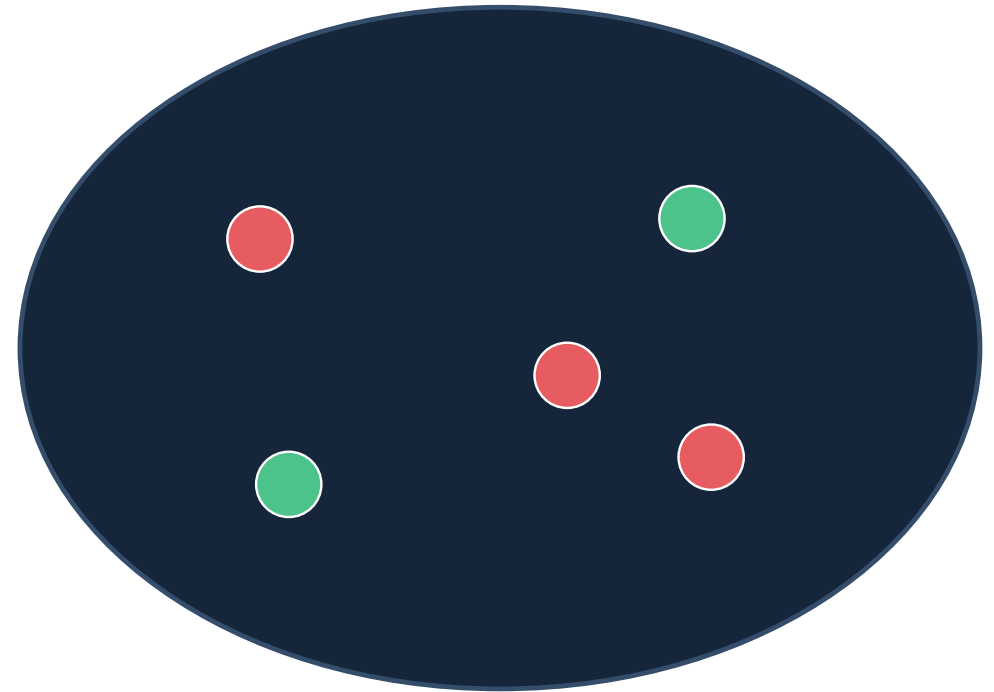


Case 1 — a possible chromosome-number abnormality

Clinical reasoning

- A child is being evaluated for a suspected chromosome-21 gain
- Illustrative nuclei show three chromosome-21 locus signals and two reference signals
- Interpret as a pattern consistent with trisomy 21; Confirm within the full diagnostic context

Three target / two reference signals



Schematic — illustrative signal pattern

● Target A

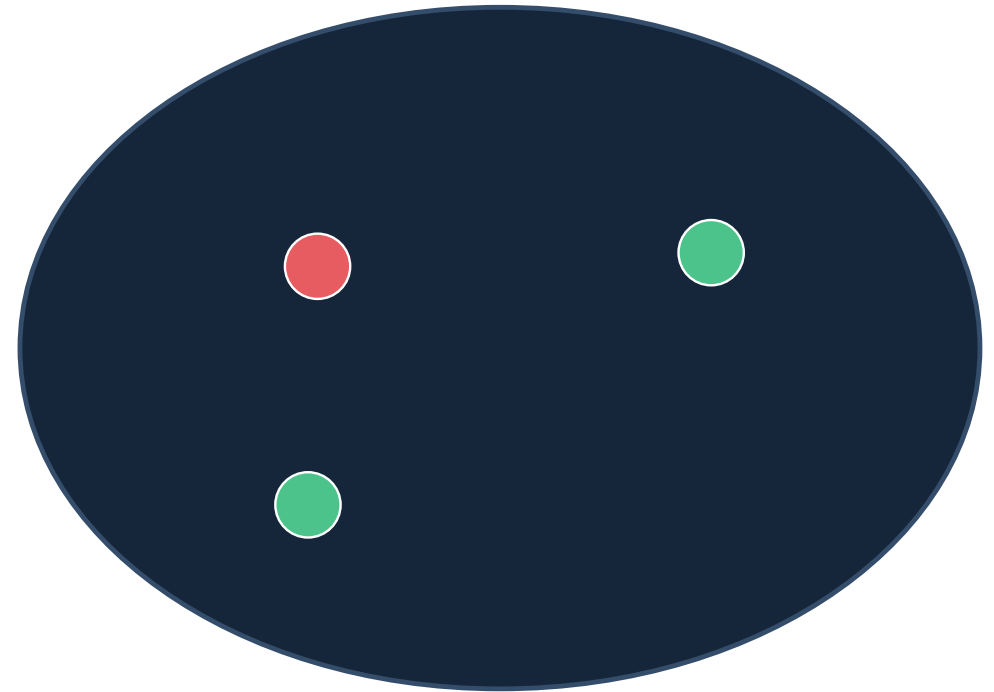
● Target B / control

Case 2 — an occult chromosome-22 deletion

Clinical reasoning

- A child has a phenotype prompting investigation of 22q11.2 loss
- One target 22q11.2 signal is present, whereas both control signals remain
- The pattern supports deletion of one interrogated 22q11.2 locus

22q11.2 deletion pattern



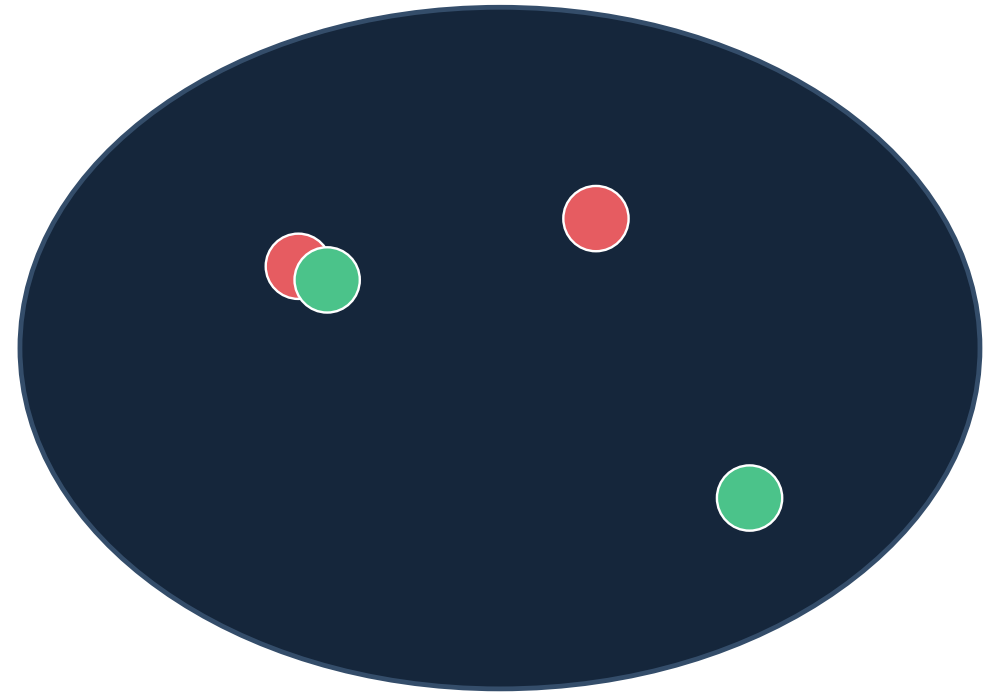
Schematic illustrative signal pattern
One 22q11.2 target **Two chr22 controls**

Case 3 — a rearrangement with unknown partner

Clinical reasoning

- Tumor nuclei show a reproducible separation of a MYC break-apart probe pair
- The arrangement indicates involvement of the MYC locus
- Do not assign a specific partner or transcript from break-apart signals alone

One paired allele and one split allele



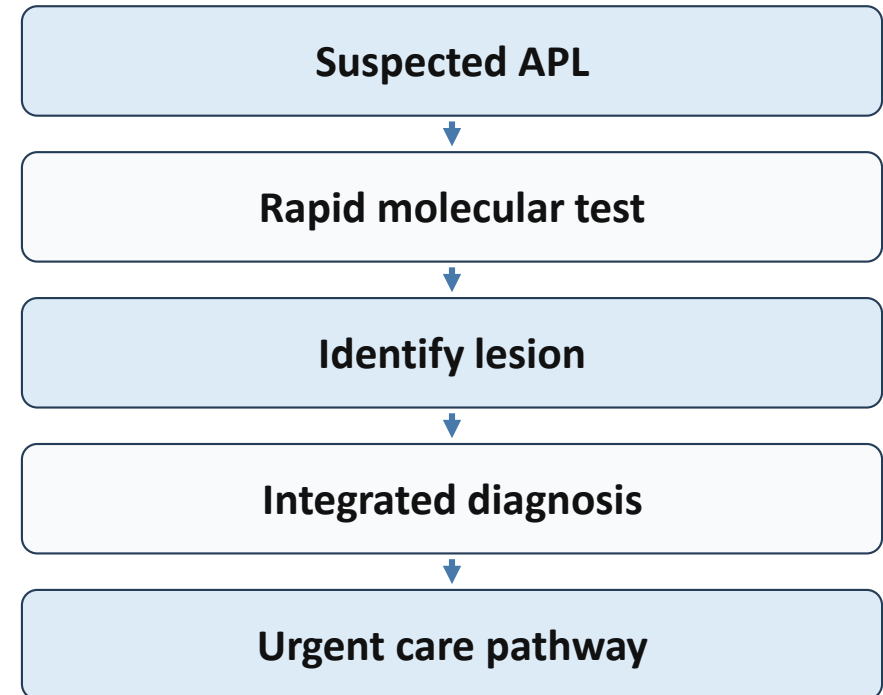
Schematic — illustrative signal pattern

Partner cannot be identified from split alone

Case 4 — rapid testing in suspected APL

Clinical reasoning

- Morphology raises concern for acute promyelocytic leukemia
- A rapid targeted genetic assay is requested to address the relevant rearrangement
- Interpret the assay alongside urgent specialist clinical and pathologic assessment



Core principles to retain

- Targeted hybridization: A probe must match the suspected genomic interval
- Pattern-based reading: Count, proximity, and internal controls all matter
- Clinical integration: Target selection, sample quality, and validated interpretation govern utility
- A negative result excludes only abnormalities detectable by the selected probe design

Principal reference

- Robbins, Cotran & Kumar: Pathologic Basis of Disease, 11th edition (2026)
- Chapter 5: Genes and Human Diseases — molecular genetic diagnosis and FISH
- Related illustrations and context: Figures 5.21, 5.23, and 23.19; APL molecular genetics
- The schematic signal patterns in this lecture are original teaching diagrams, not specimen images