

Clinical Applications of Next-Generation DNA Sequencing

From sequencing strategy to clinicopathologic decisions

Dr Abu Suraih Sakhri

MBBS, MD (Pathology)

www.abusuraihsakhri.com

Learning objectives

- Explain why massively parallel sequencing accommodates heterogeneous clinical DNA.
- Trace the analytical sequence from specimen to clinically interpreted variants.
- Select among targeted panels, whole-exome sequencing, and whole-genome sequencing.
- Apply sequencing results to inherited disease and cancer evaluation.

Clinical sequencing begins with a question

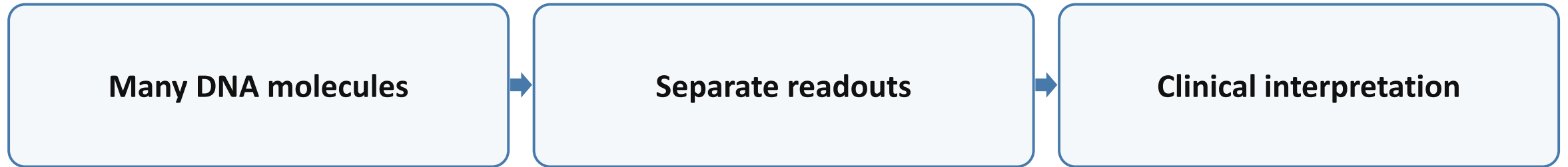
Constitutional disease

Tumor profiling

Recurrence / resistance

- Inherited disease: Which constitutional change may explain a phenotype?
- Cancer: Which somatic alterations have treatment or prognostic relevance?
- Recurrence: Have new alterations emerged that could explain drug resistance?
- Test scope must be chosen to fit the specific diagnostic question.

Why next-generation sequencing became clinically useful



- Massively parallel read generation permits broad or deep analysis.
- Large-scale sequencing is feasible without separately sequencing every region by Sanger methods.
- The analysis can interrogate DNA mixtures containing different molecules.

Sanger and NGS handle mixtures differently

Sanger: Composite signal from template material

NGS: Sequence reads from separated molecules

- Sanger sequencing generally requires a homogeneous DNA template for interpretable output.
- Heterogeneous mixtures may obscure the sequence represented in a Sanger trace.
- NGS reads DNA molecules separately, enabling analysis of heterogeneous samples.

Molecular heterogeneity matters in oncology

Schematic alignment of reads (not patient data)

Reference

C G T A C G T C G A T C G T A C

C G T A C G T C G A T C G T A C

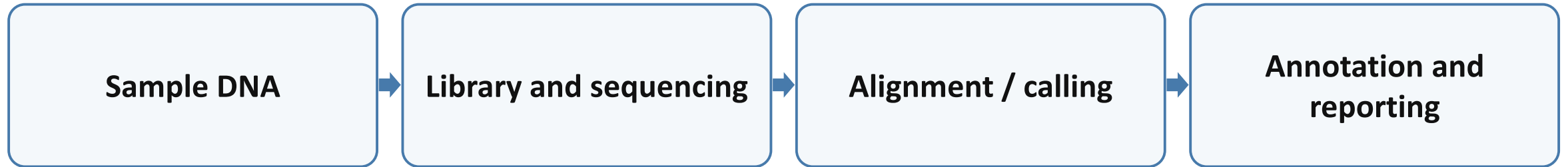
C G T A C G T C T A T C G T A C

C G T A C G T C G A T C G T A C

C G T A C G T C G A T C G T A C

- A tumor sample contains DNA molecules that need not share identical sequences.
- Parallel reads preserve information about different alleles within the sample.
- This makes tumor mutation profiling feasible despite sequence heterogeneity.

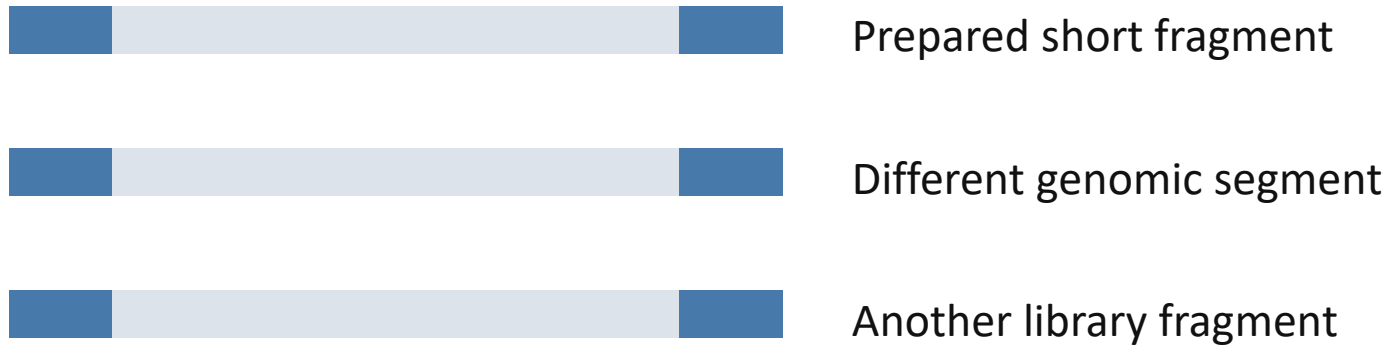
The clinical NGS workflow



- Laboratory preparation and sequencing produce reads.
- Computational analysis locates differences from a reference genome.
- Clinical review converts annotated variants into a medically interpretable report.

Library preparation precedes sequencing

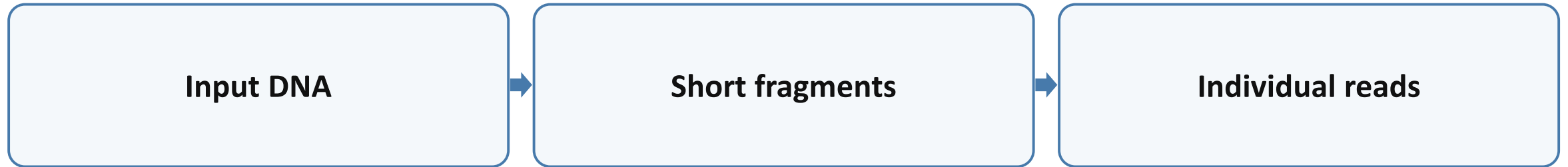
Source DNA is reorganized into a sequenceable library



Blue ends: Schematic universal adaptors; Grey center: Sample-derived DNA

- The source DNA is prepared as a collection of short DNA fragments.
- Regions of interest may be enriched before the sequencing run.
- The selected library defines what is available for downstream analysis.

Fragmentation makes parallel reading possible



- An NGS library consists of shorter sequences derived from input DNA.
- In the illustrated short-read approach, fragments are prepared for immobilization and sequencing.
- Each fragment subsequently yields a corresponding sequence read.

Capture enrichment focuses the library

Sequence complementarity selects DNA regions of interest



Original schematic — hybrid capture; Not a laboratory assay image

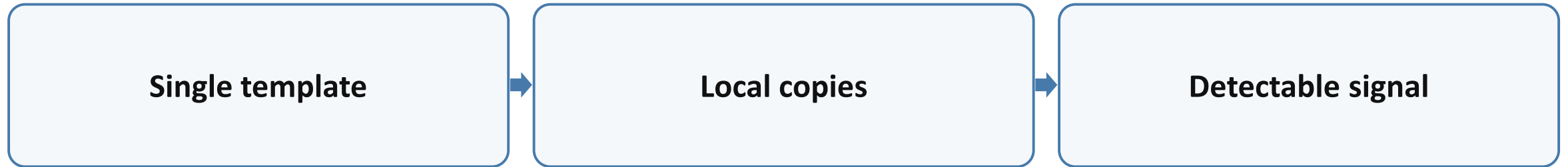
- Hybridization probes recognize sequences complementary to selected genomic regions.
- Target enrichment directs analytical capacity toward regions of interest.
- Target design is therefore central to the clinical assay's scope.

Spatial separation is the first NGS principle



- Input molecules become physically separated at distinct locations.
- Platform details vary; Physical separation permits distinct sequence signals.
- The essential concept is that different templates are read separately.

Local amplification increases signal



- Separated templates undergo localized amplification in the source's illustrated approach.
- Copies at each location improve signal detection.
- The amplified cluster remains associated with its starting DNA molecule.

Parallel sequencing produces many reads

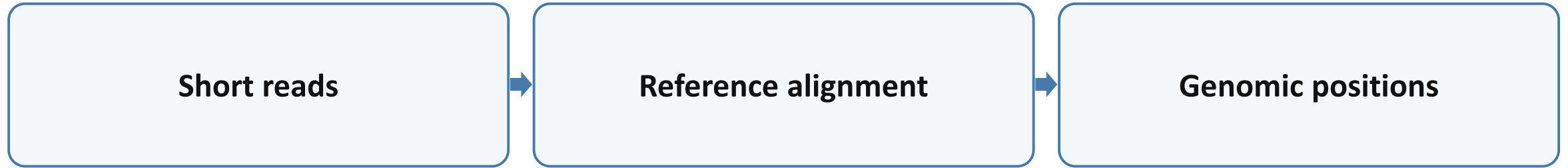
Parallel reactions

Sequence reads

Large read collection

- Many spatially separated amplified templates are sequenced simultaneously.
- Each original template gives rise to a read.
- The source describes typical reads as short, generally under 500 bp.

Reads require genomic context



- Raw read sequences are not yet equivalent to a diagnosis.
- Alignment maps reads to a reference genome.
- Mapped reads can then be evaluated for sequence differences at genomic positions.

Alignment links reads to a reference genome

Schematic alignment of reads (not patient data)

Reference

C G T A C G T C G A T C G T A C

C G T A C G T C G A T C G T A C

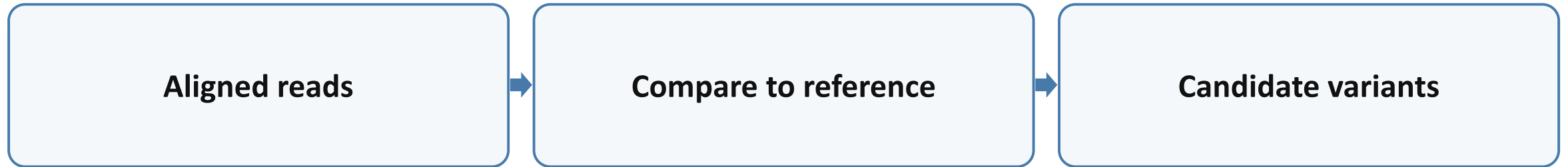
C G T A C G T C T A T C G T A C

C G T A C G T C G A T C G T A C

C G T A C G T C G A T C G T A C

- A reference genome supplies a common coordinate framework.
- Sequenced fragments are mapped to matching reference intervals.
- Alignment is a prerequisite for systematic comparison with reference sequence.

Variant calling identifies sequence differences



- The pipeline compares aligned sample reads with a reference sequence.
- Differences can then be detected in the intervals covered by the assay.
- Calling depends on adequate sequencing coverage and downstream review.

Depth and breadth answer different questions

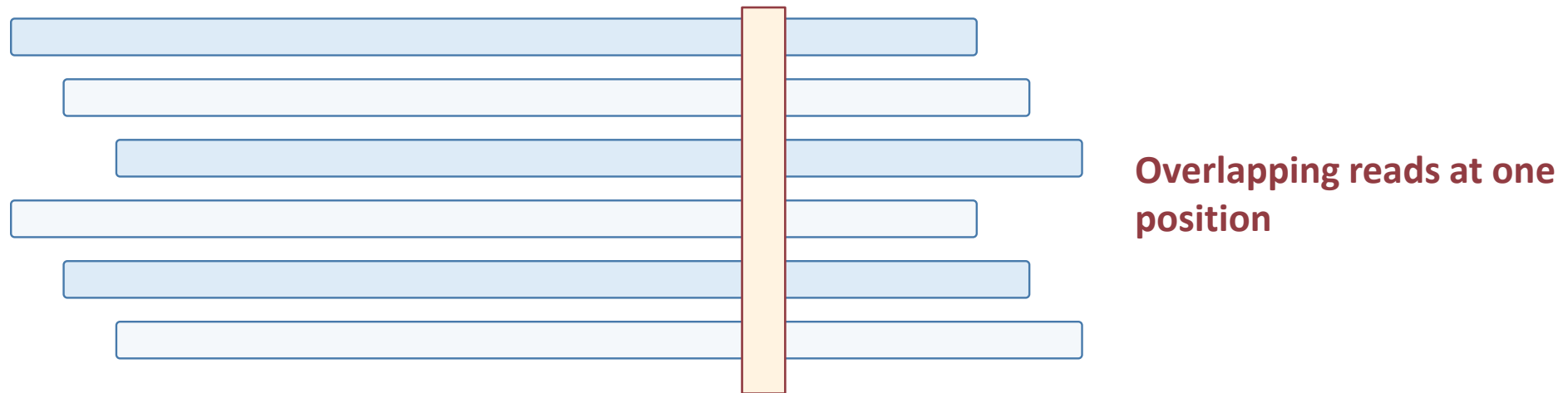
Breadth: Number or extent of target regions

Depth: Repeated observation at a position

- Breadth describes how much genomic territory is interrogated.
- Depth describes the number of reads covering a genomic position.
- Greater depth can improve detection of a low-frequency variant in mixed DNA.

Sequencing depth supports rare-allele detection

Conceptual illustration: Greater overlap increases evidence at a locus



- Low-frequency alleles may be represented by relatively few template molecules.
- More reads at the relevant position increase the opportunity to observe these alleles.
- A target outside the assay or without adequate coverage cannot be confidently assessed.

Variant allele frequency represents a mixture

Schematic alignment of reads (not patient data)

Reference

C G T A C G T C G A T C G T A C

C G T A C G T C G A T C G T A C

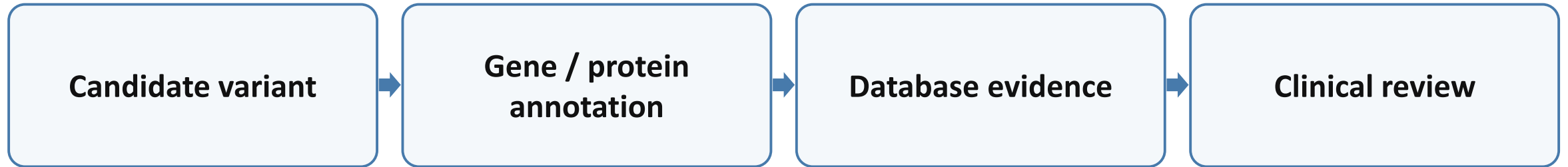
C G T A C G T C T A T C G T A C

C G T A C G T C G A T C G T A C

C G T A C G T C G A T C G T A C

- Variant allele frequency (VAF) refers to the fraction of observations supporting a variant.
- The source links higher read depth to detection of variants at low VAF.
- VAF should be interpreted in its specimen and assay context—not as a diagnosis on its own.

Variant annotation adds biological meaning

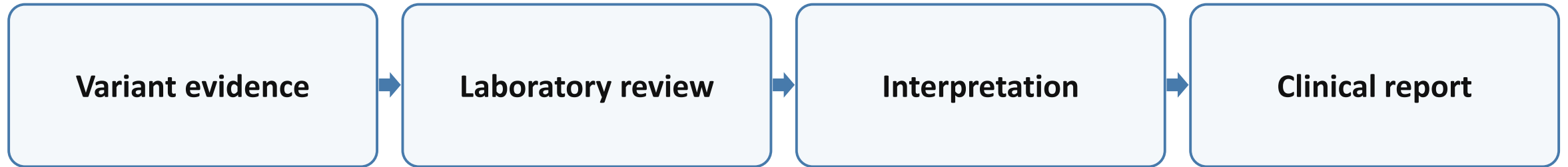


- Annotation identifies the affected gene and the sequence or coding change.
- Predicted protein effects and reference databases provide interpretation context.
- Clinical relevance is assessed rather than inferred from a sequence change alone.

Variant interpretation is not automatic

- A variant must be interpreted with the patient's disease or tumor context.
- Databases include common and disease-associated variants.
- A clinical laboratory reviews annotation before final reporting.

The report connects DNA to a clinical decision



- Reports describe biologic and clinical significance when it can be established.
- Multiple pathogenic changes may need explanation in cancer specimens.
- The goal is an intelligible result, not merely a list of sequence differences.

Mutational patterns provide additional information

Individual variants

Pattern recognition

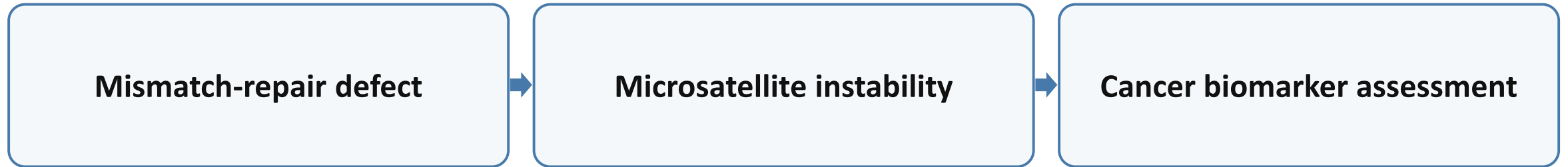
Biologic associations

- Analysis need not stop after counting individual mutations.
- Mutation patterns may suggest certain exposures or defects in DNA repair.
- The source gives ultraviolet light, smoking, and DNA repair defects as examples.

Tumor mutational burden can be inferred from NGS

- Tumor mutational burden summarizes the burden of detected tumor mutations.
- The chapter links high TMB in some tumors to a tendency to respond to checkpoint blockade.
- TMB is a context-dependent biomarker; A generic NGS result does not itself guarantee response.

Microsatellite instability is clinically relevant



- Acquired mismatch-repair defects can produce microsatellite instability (MSI).
- The source associates MSI with responsiveness to immune checkpoint inhibitors in some cancers.
- NGS bioinformatics may assess MSI as part of cancer profiling.

Three principal assay strategies

Targeted sequencing

Whole exome

Whole genome

- Targeted panels: Curated genes or exons with substantial sequencing depth.
- WES: Capture most protein-coding exons across the genome.
- WGS: Sequence across the genome, including noncoding regions.

Targeted sequencing restricts the interrogated space

Gene panel



Whole exome



Whole genome



Conceptual coverage of selected regions; Blocks are not genomic-scale measurements

- A panel focuses on selected exons and genes relevant to the indication.
- Concentrating the analysis supports greater depth and practical reporting.
- The scope depends on expert selection of panel targets.

Clinical panels are designed around a phenotype



- A targeted assay is appropriate when candidate genes are already established.
- An inherited disorder may justify one gene or a panel of disease-related genes.
- Tumor testing requires genes relevant to the oncologic management question.

Hybrid capture uses complementary probes

Sequence complementarity selects DNA regions of interest

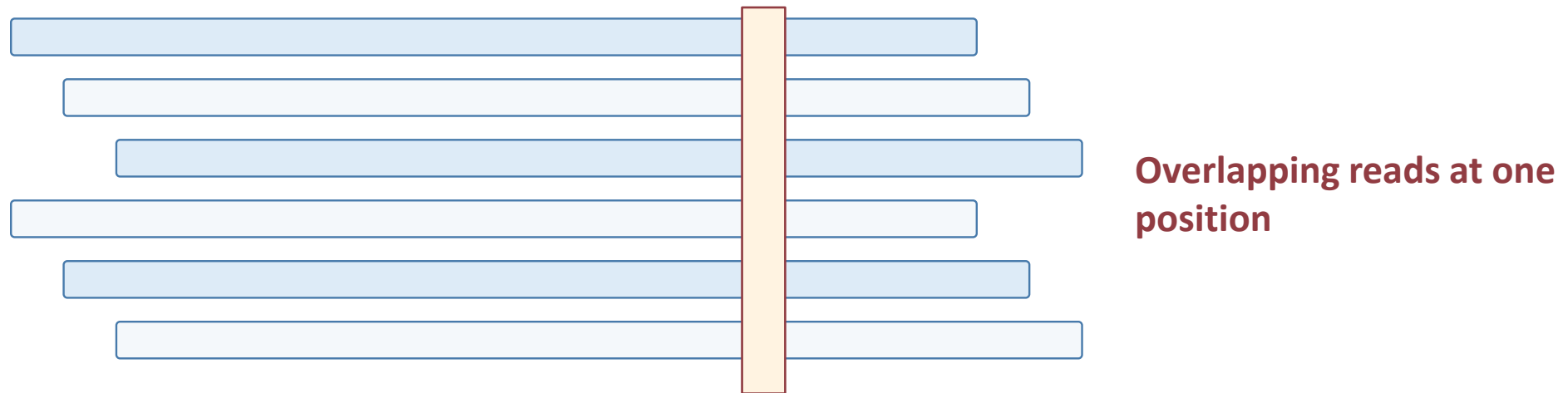


Original schematic — hybrid capture; Not a laboratory assay image

- The source describes synthesized biotinylated probes used for hybrid capture.
- Probes hybridize to complementary DNA segments in the prepared library.
- Enriched fragments are then prioritized for sequencing.

Why targeted panels offer sequencing depth

Conceptual illustration: Greater overlap increases evidence at a locus



- Sequencing effort is concentrated on a defined set of regions.
- The chapter emphasizes maximal depth alongside reduced expense and interpretation burden.
- This depth advantage is particularly useful when the specimen contains variant mixtures.

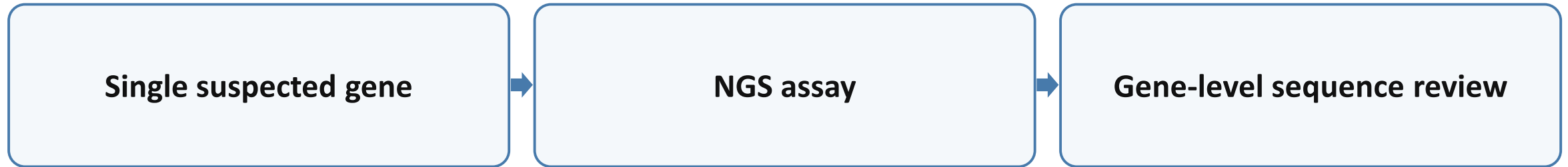
Targeted panels limit interpretation workload

Focused targets: Manageable candidate set

Untargeted territory: Outside panel scope

- Fewer examined regions usually mean fewer variants requiring clinical review.
- Focused assays reduce time and expense associated with interpretation.
- The trade-off is that variation outside panel targets is not comprehensively assessed.

Single-gene testing also uses NGS



- NGS is not limited to very large gene panels.
- The chapter describes migration from multiple Sanger amplicons to NGS-based analysis.
- Comprehensive CFTR sequencing is given as an illustrative single-gene application.

An inherited-gene panel can fit a defined syndrome

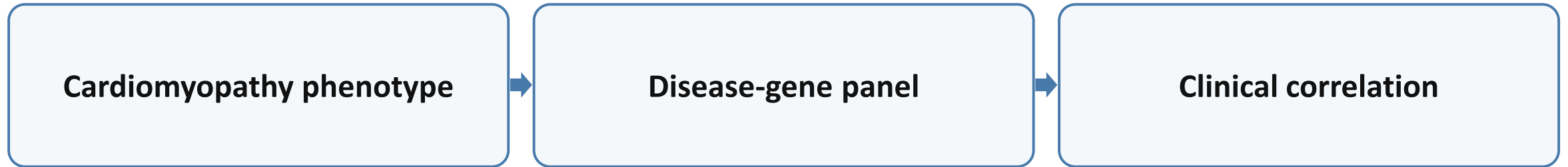
Clinical phenotype

Candidate gene set

**Phenotype-linked
interpretation**

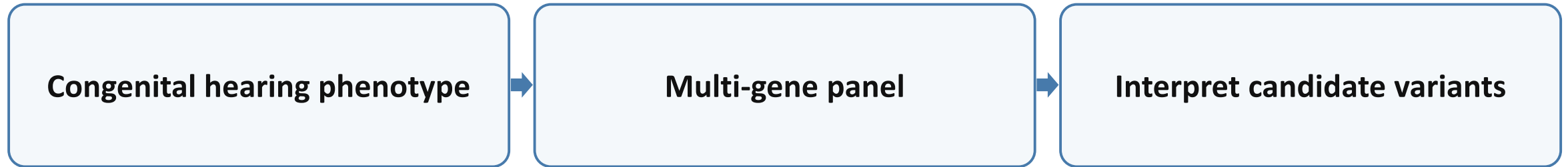
- When multiple genes can explain a similar phenotype, a multi-gene panel is attractive.
- The textbook specifically mentions cardiomyopathy and congenital deafness.
- Clinical phenotype guides the panel choice and interpretation.

Cardiomyopathy: A panel-based diagnostic model



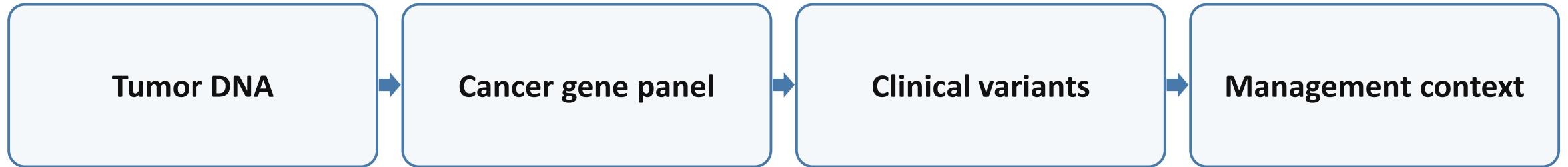
- The clinical entry point is a phenotype consistent with heritable cardiomyopathy.
- A panel interrogates established candidate genes rather than the entire genome.
- Detected variants need correlation with disease features and family context.

Congenital deafness: Genetic heterogeneity



- A single clinical presentation may prompt evaluation of many possible genes.
- Panel testing interrogates multiple selected genes in one sequencing strategy.
- A technically positive change still requires clinically meaningful interpretation.

Cancer panels profile somatic alterations



- Each tumor may harbor its own combination of acquired mutations.
- NGS panels seek tumor changes with therapeutic or prognostic significance.
- A broad readout can inform more individualized oncology decisions.

Tumor-specific profiles are not interchangeable

Tumor A: One combination of alterations

Tumor B: A different combination

- Two tumors of the same broad category need not have the same set of somatic mutations.
- Profiling seeks the alterations actually present in the specimen.
- This molecular information complements, rather than replaces, the clinicopathologic diagnosis.

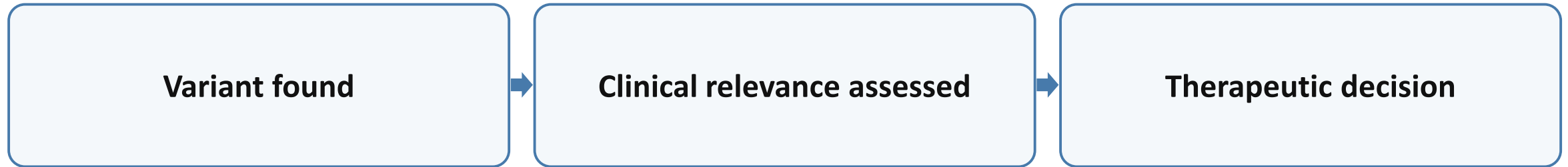
Separate predictive from prognostic information

Predictive: Expected benefit from an intervention

Prognostic: Association with disease course

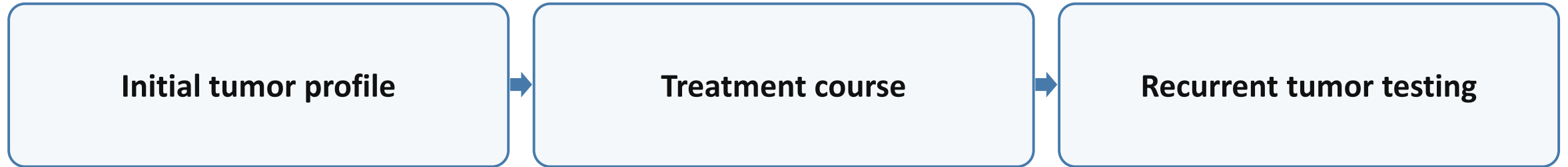
- A treatment-relevant alteration can guide consideration of a targeted therapy.
- A prognostically relevant alteration informs anticipated disease behavior.
- The same molecular report may contain findings with different clinical roles.

Mutation detection does not equal drug selection



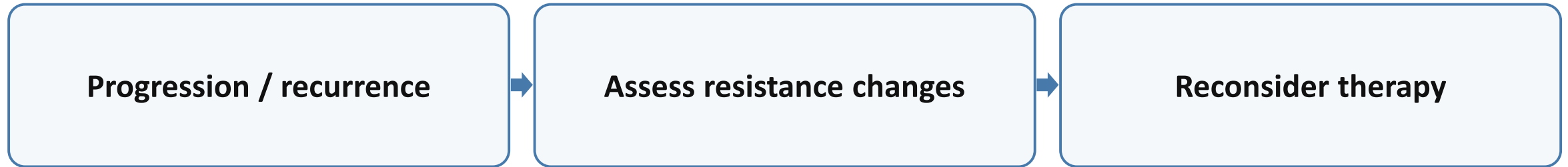
- Panels aim to detect as many treatable or prognostic mutations as possible.
- The presence of a variant requires disease-specific clinical evaluation.
- Sequencing informs a decision; It does not provide a complete treatment plan.

Recurrence is a reason to re-evaluate the genome



- Tumor molecular features may differ after disease progression or therapy.
- The chapter notes increasing use of repeat sequencing at recurrence.
- The objective is to understand mechanisms underlying acquired resistance.

Molecular resistance can alter second-line choices



- Newly identified resistance-associated changes may explain therapeutic failure.
- Repeat testing can identify information relevant to subsequent treatment.
- Results require integration with prior molecular findings and clinical status.

A negative targeted panel has limited scope

Assayed genes: Evidence available

Genes outside panel: Not comprehensively evaluated

- No detected panel variant means none was reported within the assay's investigated targets.
- The result cannot exclude disease-related variants in regions the panel did not capture.
- If clinical suspicion persists, the question may warrant a broader sequencing approach.

Whole-exome sequencing expands the target set

Gene panel



Whole exome



Whole genome

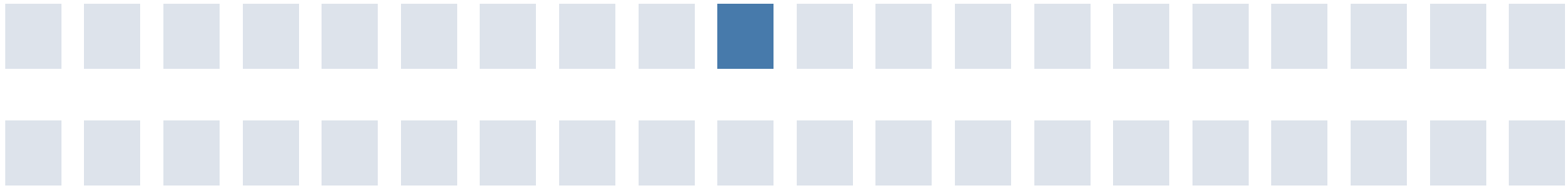


Conceptual coverage of selected regions; Blocks are not genomic-scale measurements

- WES uses extensive capture to enrich protein-coding exons before sequencing.
- It is broader than a phenotype-selected multi-gene panel.
- WES remains a form of targeted sequencing—not whole-genome sequencing.

The exome occupies a small genomic fraction

Coding exons are a small part of the whole genome

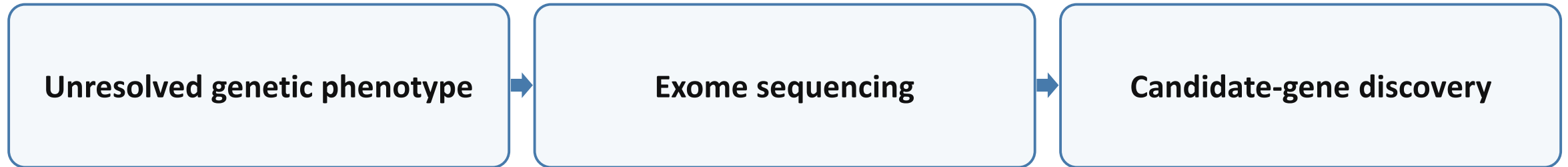


Coding exons $\approx 1.5\%$ of human genomic sequence (source estimate)

The blocks are schematic—not a proportional depiction of all exons.

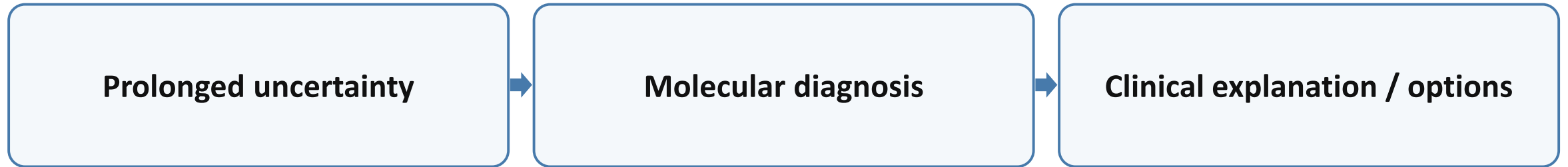
- The chapter describes protein-coding exons as roughly 1.5% of the genome.
- Exome capture focuses analytical resources on these coding regions.
- A large amount of noncoding genomic sequence lies outside the WES target.

WES is relevant when the diagnosis is uncertain



- A rare inherited disease may lack a clear single candidate gene.
- A broader analysis can evaluate coding variants beyond a narrow panel.
- The chapter emphasizes diagnoses achieved after prolonged unsuccessful evaluations.

Ending a diagnostic odyssey has clinical value



- Identifying a molecular basis can convert an unexplained syndrome into a defined diagnosis.
- The chapter describes benefits for children with rare orphan diseases.
- For some diagnosed conditions, finding the molecular cause can open therapeutic options.

WES also supports cancer gene discovery

Routine panels: Decision-focused tumor profiling

Exome research: Broader gene discovery

- In oncology, WES has been used mainly for research applications discussion.
- Its breadth facilitates study of genes that are infrequently mutated.
- This is distinct from the routine targeted-panel goal of actionable tumor profiling.

The exome still misses important territory

Coding exons: Principal WES targets

Noncoding genome: Largely outside target

- An exome assay preferentially interrogates protein-coding exons.
- Potentially relevant noncoding changes are not comprehensively covered.
- Persisting clinical suspicion after a non-diagnostic exome can justify considering WGS.

Whole-genome sequencing is the broadest DNA scope

Gene panel



Whole exome



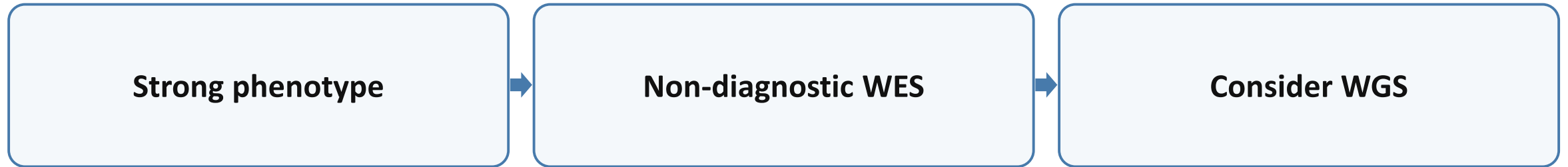
Whole genome



Conceptual coverage of selected regions; Blocks are not genomic-scale measurements

- WGS examines coding and noncoding genomic regions.
- It is the most comprehensive sequencing strategy discussed in this chapter.
- Greater breadth increases analysis and interpretation demands.

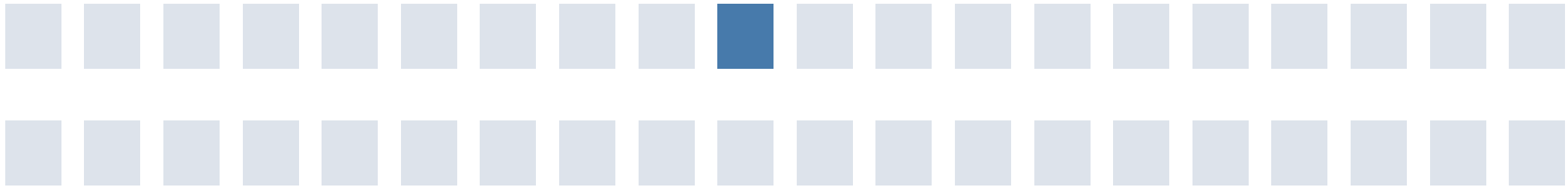
After a negative exome, WGS may be useful



- The chapter places most medical-genetics WGS indications after unsuccessful WES.
- The clinical prerequisite is persistently high suspicion of genetic disease.
- Broadening assay territory may reveal changes missed by exon-focused testing.

WGS includes noncoding sequence

Coding exons are a small part of the whole genome

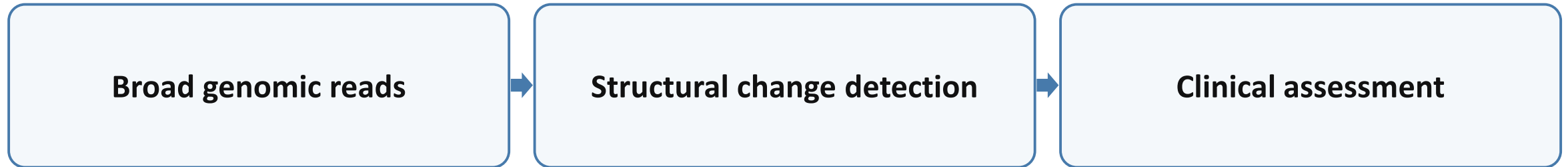


Coding exons $\approx$ 1.5% of human genomic sequence (source estimate)

The blocks are schematic—not a proportional depiction of all exons.

- A broader genomic assay can evaluate variation outside protein-coding exons.
- This matters when a clinically important alteration may not lie in an exonic sequence.
- Comprehensive coverage does not automatically confer comprehensive interpretation.

WGS can detect structural rearrangements



- The chapter gives insertions, deletions, and translocations as examples.
- At sufficient depth, WGS can characterize novel structural genomic changes.
- Such alterations may be relevant to cancer biology or clinical management.

Cancer WGS may reveal noncoding alterations

Noncoding variants

Structural alterations

Clinical relevance review

- A tumor genome includes potentially important changes outside conventional panel regions.
- Broad analysis may find noncoding variants and novel structural rearrangements.
- The value of a finding still depends on established biologic and clinical meaning.

WGS is not yet a universal routine replacement

Cost

Turnaround

Informatics burden

- The chapter identifies high costs as a barrier.
- Longer turnaround and substantial informatics demands also hinder routine implementation.
- A broader assay is useful only when it helps answer the clinical question.

Breadth must be balanced against practical depth

Gene panel



Whole exome



Whole genome



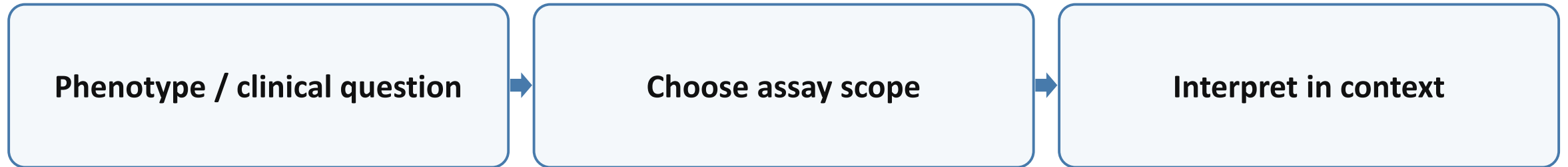
Conceptual coverage of selected regions; Blocks are not genomic-scale measurements

- Targeted testing concentrates resources on selected regions.
- WES greatly expands the set of exons considered.
- WGS provides broad genomic scope but increases the reporting and analysis burden.

Targeted panel, WES, and WGS compared

Approach	Principal target	Typical purpose	Key trade-off
Gene panel	Selected genes / exons	Focused clinical diagnostics	Limited to chosen targets
WES	Protein-coding exons	Broad coding-variant search	Noncoding DNA largely excluded
WGS	Coding + noncoding genome	Most comprehensive DNA analysis	Cost, time, informatics

Match test breadth to the clinical indication



- Known disease-related gene set → targeted panel often provides efficient analysis.
- Rare unexplained inherited disease → WES can expand the coding search.
- High suspicion after unrevealing WES → WGS may examine broader genomic territory.

Constitutional and somatic sequencing differ

Constitutional: Explain a hereditary phenotype

Somatic: Characterize a tumor's alterations

- Constitutional disease evaluation asks about inherited or constitutional variation.
- Tumor profiling seeks acquired somatic changes relevant to an individual cancer.
- Clinical interpretation and reporting priorities follow the indication.

Illustrative case: Congenital deafness

Clinical reasoning exercise — hypothetical scenario

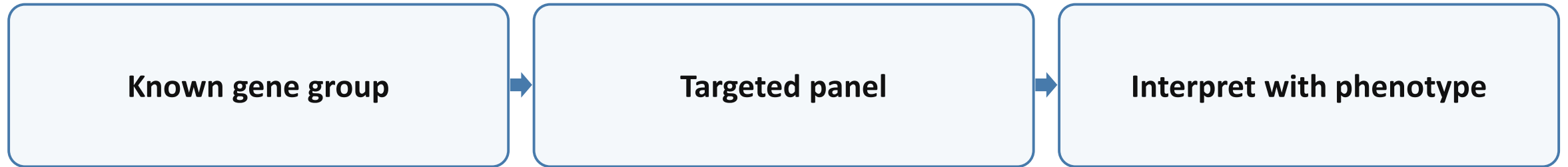
- A child has congenital deafness; A molecular diagnosis is sought.
- The clinical question implicates a defined group of genes rather than a single proven gene.
- Which sequencing breadth best matches the initial question?

Clinical phenotype

Panel / WES / WGS?

Justify choice

Case discussion: Choose a hearing-loss panel



- Targeted sequencing can cover a curated gene set relevant to congenital deafness.
- Concentrated depth and focused interpretation are advantages.
- If the panel is unrevealing, the residual suspicion and assay scope govern next steps.

Illustrative case: Tumor recurrence

Clinical reasoning exercise — hypothetical scenario

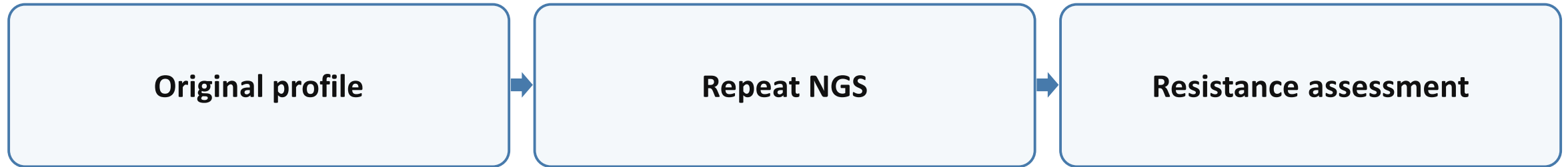
- A patient's malignancy recurs after treatment.
- An earlier molecular profile is available.
- What additional question can repeat NGS testing address?

Prior profile

Recurrent tumor

Resistance mechanism?

Case discussion: Repeat profiling at relapse



- Compare the recurrent tumor's detected changes with the earlier profile.
- Look for molecular evidence that could explain acquired resistance.
- Review whether newly identified alterations are clinically relevant to later treatment.

Illustrative case: Exome-negative rare disease

Clinical reasoning exercise — hypothetical scenario

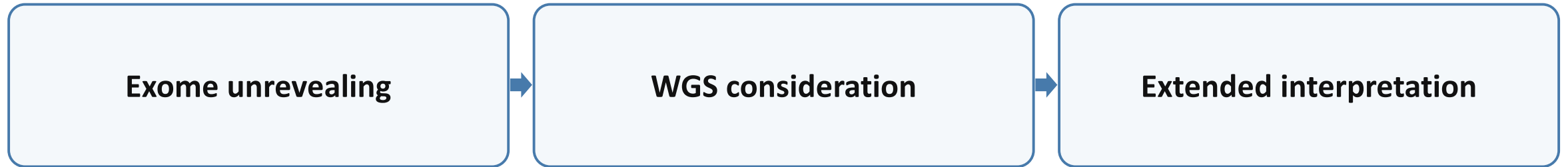
- A patient has a convincing genetic syndrome without an established molecular diagnosis.
- Whole-exome testing has not identified a cause.
- Should the genomic search end, or could it be expanded?

Strong clinical suspicion

Negative WES

What next?

Case discussion: Broaden beyond coding exons



- WGS consideration when WES is unrevealing and suspicion remains high.
- WGS offers noncoding coverage and broader structural alteration assessment.
- Even a more comprehensive assay requires careful interpretation.

Interpret a negative result in assay context

- A result can only be evaluated in relation to regions actually interrogated.
- Sequencing depth and analytical methods influence what can be detected.
- An unrevealing result does not negate a strong phenotype or clinical observation.

Genomic results require clinicopathologic integration



- Sequencing detects and characterizes molecular variation.
- The clinician and laboratory determine whether the finding explains the presenting disease.
- Cancer assays supplement morphology, diagnosis, treatment history, and other clinical facts.

What NGS can and cannot establish

NGS can supply	NGS alone does not establish
Sequence differences within assay scope	That every difference is pathogenic
Read-depth and allele information	That every target is adequately covered
Cancer molecular profiling	That a particular therapy will succeed
Expanded genomic searches	That a negative result excludes disease

Final clinical synthesis

Assay scope

Sequence evidence

Clinical significance

- Choose the narrowest assay that adequately answers the clinical question.
- Interpret depth, breadth, and target limitations together.
- For constitutional disease: Integrate genotype with phenotype.
- For cancer: Relate molecular findings to prognosis, therapeutic relevance, and recurrence.

Principal reference

- Robbins, Cotran & Kumar: Pathologic Basis of Disease, 11th edition (2026).
- Relevant textbook sections: molecular genetic diagnosis and next-generation sequencing.
- Clinical applications: targeted panels, whole-exome and whole-genome sequencing.
- NGS principles and bioinformatics are included as foundational context.