

Comprehensive NGS versus qPCR and Sanger sequencing

In contrast to qPCR and Sanger sequencing, next-generation sequencing (NGS) can simultaneously identify variants across thousands of target regions down to single-base resolution in a single experiment. Explore the benefits and limitations of each method to understand which one meets your needs.¹⁻³

qPCR

qPCR analyzes the amount of a specific sequence in a sample by a cycle of amplification and quantification.

✓ Benefits

- Familiar workflow
- Lab equipment is readily available

✗ Challenges

- Restricted to a predefined set of variants
- Minimal discovery power
- Limited throughput and variant resolution

Variant present

Sequence information for short fragments (300–1000 bp)

Sanger sequencing

Sanger sequencing, also known as sequencing by capillary electrophoresis (CE), interrogates a sequence of nucleotides in one DNA fragment at a time.

✓ Benefits

- Familiar workflow
- Cost effective when sequencing 1–20 targets

✗ Challenges

- Low sensitivity, throughput, and discovery power
- Only cost effective for < 20 targets

NGS

NGS interrogates sequences of nucleotides for millions of DNA fragments simultaneously, enabling screening of hundreds to thousands of variants.

✓ Benefits

- High sequencing depth enables high sensitivity
- Exceptional discovery power
- Single-base variant resolution
- High-throughput workflows and large data sets
- Sensitive detection of gene expression changes

✗ Challenges

- Possibly inefficient for simple detection of a low number of targets

Sequence information for small gene panels to entire genomes

Which to choose—and when?

Sanger sequencing and qPCR are good choices if you need to interrogate a small region of DNA on a limited number of samples.

Otherwise, NGS is more likely to suit your needs. For variant screening studies with a high number of samples, NGS is the most efficient and cost-effective approach for sequencing tens to thousands of targets compared to conventional methods.

Learn more about targeted NGS:
illumina.com/ngs-explained

References

1. Illumina. High-impact discovery through gene expression and regulation research. illumina.com/content/dam/illumina-marketing/documents/gated/gene-expression-profiling-e-book-web.pdf. Accessed February 7, 2024.
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3. Illumina. Differences between NGS and Sanger Sequencing. illumina.com/science/technology/next-generation-sequencing/ngs-vs-sanger-sequencing.html. Accessed February 7, 2024.