

Targeted next-generation sequencing versus qPCR and Sanger sequencing

Technologies used to interrogate DNA and RNA have come a long way. From Sanger sequencing to quantitative PCR (qPCR) to next-generation sequencing (NGS), explore the benefits and the limitations of each to understand which method you should choose.

qPCR

qPCR allows for the analysis of particular variants at specific locations.

✓ Benefits

- High sensitivity
- Quick and simple workflow
- Capital equipment already found in most labs

✗ Limitations

- Only examines a small set of variants
- Virtually no discovery power¹⁻⁴
- Low variant resolution¹⁻⁴
- Low scalability⁵

Variant present

Sanger sequencing

Sanger sequencing, also known as sequencing by capillary electrophoresis, interrogates a gene of interest.

✓ Benefits

- Cost effective for small stretches of DNA
- Quick and simple workflow

✗ Limitations

- Low sensitivity (down to 20%)^{6,7}
- Low discovery power¹⁻⁴
- Not cost effective for large stretches of DNA⁵
- Low scalability³⁻⁵

Targeted NGS

Targeted NGS simultaneously screens several hundred to thousands of genes.

✓ Benefits

- Expanded discovery power through comprehensive genomic coverage
- Higher analytical sensitivity^{6,7}
- Greater resolution of genomic variants¹⁻⁴
- More data from smaller DNA amounts⁵
- Higher throughput with sample multiplexing⁵

✗ Limitations

- May be less cost effective when interrogating a low number of samples
- Requires a dedicated data-handling workflow

References

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Which to choose—and when?

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

Otherwise, targeted NGS is more likely to suit your needs. It allows you to screen more samples and detect multiple variant types across targeted areas of the genome, which would be a costly and time-consuming effort with the Sanger and qPCR methods.

To learn more about targeted NGS, visit:
www.illumina.com/ngs-explained