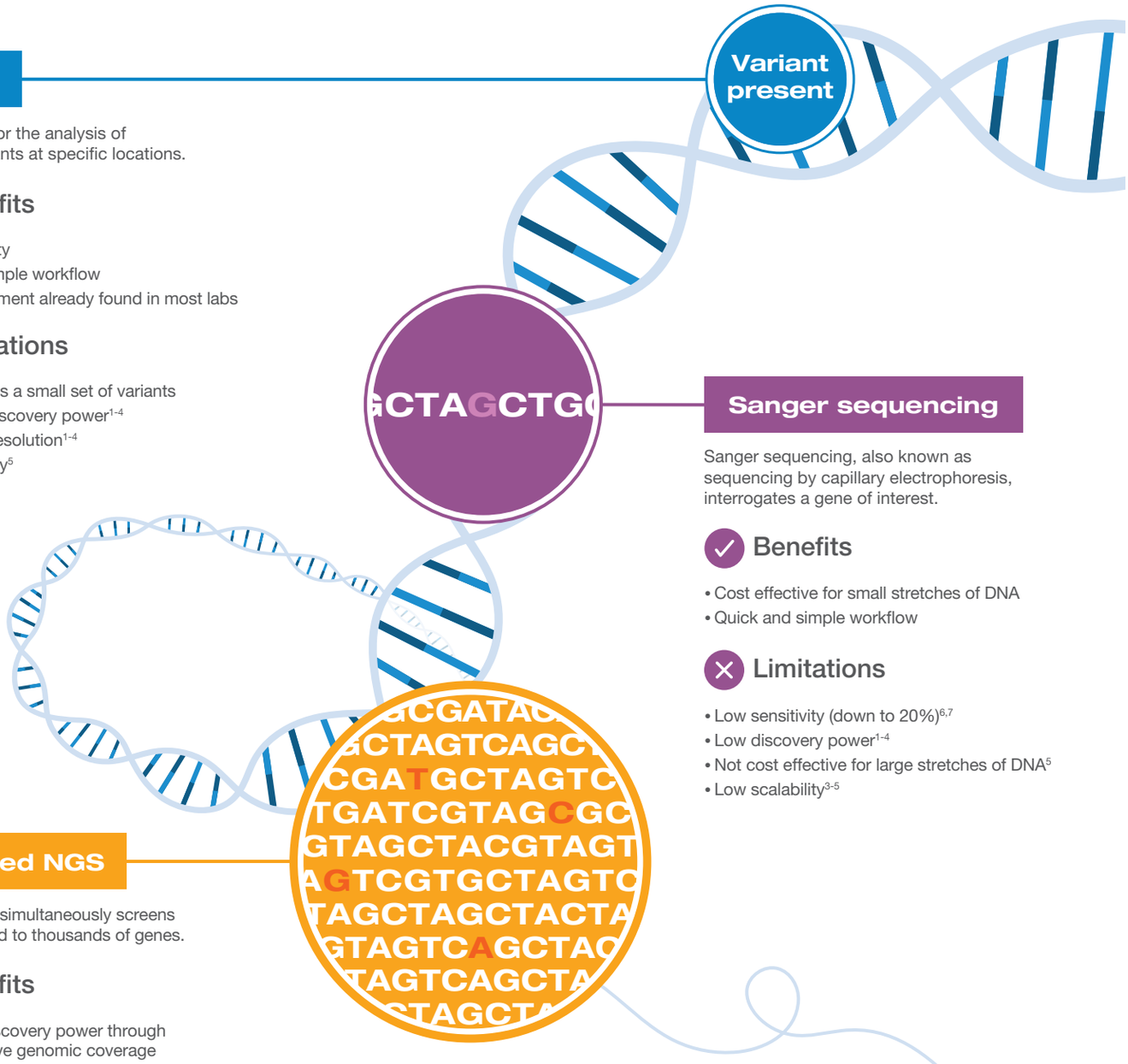
Targeted next-generation sequencing versus qPCR and Sanger sequencing

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 power[1-4]
- Low variant resolution[1-4]
- Low scalability[5]

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 power[1-4]
- Not cost effective for large stretches of DNA[5]
- Low scalability[3-5]

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 variants[1-4]
- More data from smaller DNA amounts[5]
- Higher throughput with sample multiplexing[5]

Limitations

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

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**

References

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