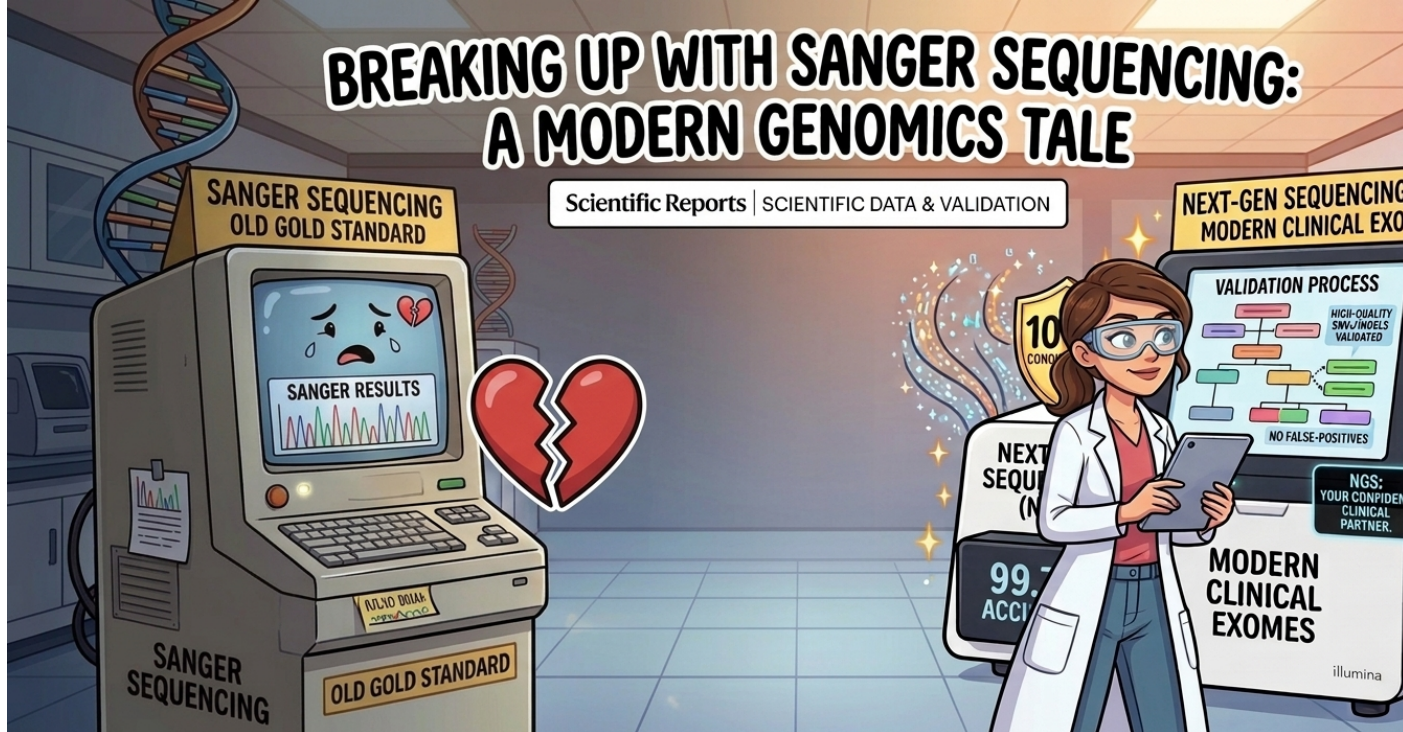




I'm sorry, Sanger, it's not me, it's you.

By Adrian Hill (HLLADR005)

Imagine you are sequencing a patient's DNA to find a specific disease-causing variant (mutation), but each time you do so, you have to spend more time and money confirming your findings. Just like that friend we always turn to for reassurance to make sure we're doing the right thing, Sanger sequencing has long played that role in validating Next-Generation Sequencing (NGS) results. However, as the accuracy of NGS has improved, Sanger's reassurance becomes increasingly redundant. Along with the added time and cost of confirming every finding, it is leading researchers to question whether Sanger Sequencing is still necessary as a validation step.

To investigate this, a recent study used the largest sample set reported to date using Illumina chemistry (an NGS platform), including 825 clinical exome samples.

Putting the technology to the test

All patients' clinical exome panels were sequenced using two different technologies. The sequencing data then underwent bioinformatic analysis, including variant calling to detect genetic variants. The researchers analysed different types of genetic changes, including single-letter DNA changes (SNVs), small insertions and deletions (indels), and larger missing or duplicated DNA segments (CNVs).

Quality metrics were then applied to the identified variants. Low-quality variants and misleading samples were excluded from further analysis. SNVs and indels were confirmed using Sanger sequencing, while CNVs were validated using MLPA or CGH arrays.

What they uncovered...

The results confirmed the researchers' suspicions. Among the high-quality variants assessed (866 SNVs and 213 indels), 100% concordance was observed within the predefined quality thresholds, with no false-positive SNVs or indels detected.

Ironically, the reassuring friend was occasionally the one making mistakes. While the NGS results remained correct, three discrepancies were identified during Sanger sequencing. The discrepancies were most likely caused by preferential. The study documented a total of ten Sanger sequencing issues during SNV and indel confirmation.

When reviewing the existing literature, the researchers found that several other studies also reported a 100% concordance for high-quality NGS variants. Together, these findings add further weight to the argument that routine Sanger confirmation may no longer be necessary.

Under specific circumstances, the researchers noted that the chances of detecting a false-positive result from NGS seemed lower than the likelihood of a false-negative result from Sanger sequencing. It is at this point that you realise you have gained the confidence to not seek reassurance from that friend.

Is it really over for Sanger?

Just because you no longer need that friend for reassurance does not mean you will cut them out of your life. While NGS is a robust technique for detecting SNVs and indels, it may eliminate the need for routine confirmatory testing of high-quality variants. However, laboratories should consider their own sequencing platforms and establish appropriate quality thresholds before deciding whether to remove confirmation studies.

Rather than signalling the end of Sanger sequencing, these findings suggest a shift in its role – from routine confirmation of every variant to targeted internal quality assurance where it is needed to prevent human error and obtain reliable results.

