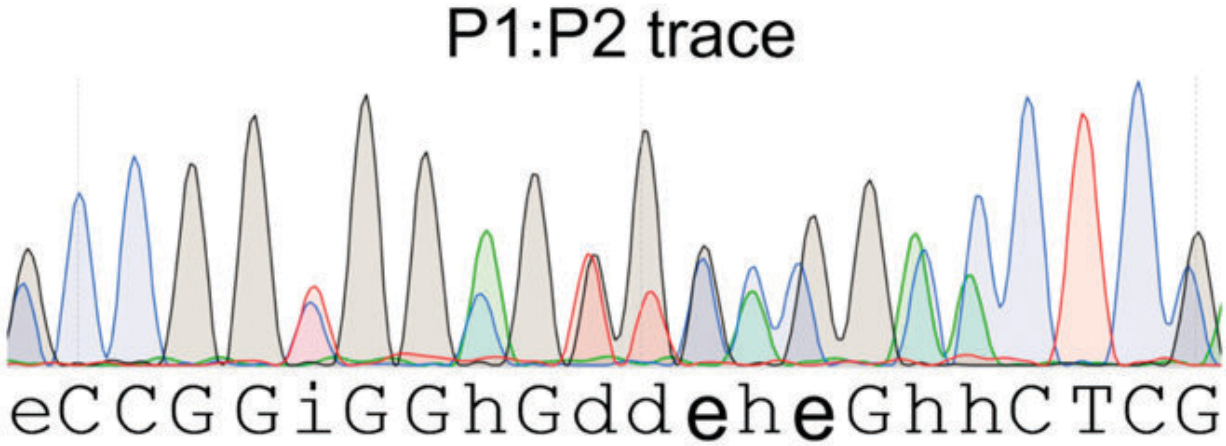
Quantifying genetic variations in bacterial cultures the qSanger way

March 6 2023

$$\begin{bmatrix} & G & A & T & C \\ G & G & b & d & e \\ A & b & A & f & h \\ T & d & f & T & i \\ C & e & h & i & C \end{bmatrix}$$



The P1:P2 trace is annotated using an extended alphabet that assigns a new letter at time points where 2 peaks are present. Top: Pairwise matrix showcasing how the extended alphabet assigns a letter to each nucleotide pair possible. Bottom: Example of electropherogram data analyzed by qSanger where new letters are placed at positions with 2 nucleotides. Credit: *BioDesign Research* (2023). DOI:

10.34133/bdr.0007

Genetic variations, such as mutations, recombinations, or transpositions occur naturally in cultured microorganisms and are often considered nonneutral mutations.

Neutral mutations are neither beneficial nor harmful to an organism and only affect a small proportion of the total population. On the other hand, nonneutral mutations can affect a larger proportion of the population by potentially changing the gene pool, depending upon the advantages or disadvantages provided by the genetic variant. These mutations can be quantified genotypically using different sequencing methods.

Quantitative polymerase chain reaction (qPCR) is considered an effective sequencing method to measure individual genetic variants. However, it can be expensive and time-consuming. In contrast, Sanger sequencing is fast and cost-effective, but its accuracy falls short in quantifying mutations in plasmid mixtures derived from a heterogeneous population.

Since neutral mutations can become dominant due to changing environmental conditions, resulting in transitory selection or counterselection, it is important to accurately assess ratios of mutated and wild-type DNA sequences. Moreover, such DNA quantification could improve the early diagnosis of diseases and effective drug development. Consequently, a team of researchers from Europe have devised a novel method—qSanger, to quantify genetic variants and identify neutral/nonneutral mutations.

"The qSanger methodology proposed in our study uses data from Sanger sequencing to measure the ratio of genetic variations in a bacterial

culture," says Prof. Alfonso Jaramillo, who is affiliated with the University of Warwick and Keele University and is the corresponding author of the study.

The study was published in *BioDesign Research*.

First, the team used Top 10 competent cells of Escherichia coli (E. coli), which were aerobically cultured in the presence of antibiotics. The bacterial colonies were co-transformed with two plasmids—P1 and P2. Next, they used Sanger sequencing to generate an electropherogram with multiple traces for the bacterial colonies co-transformed with P1 and P2. The two plasmids were also individually sequenced.

Thereafter, traces from the P1 and P2 sequences were aligned with the mixed P1:P2 traces. Finally, they applied the qSanger method to quantify the plasmid DNA, using amplitude ratios of the aligned electropherogram peaks from mixed Sanger sequencing reads.

To easily measure the plasmid ratios, the team used distinct fluorescent markers with two plasmid constructs—mCherry (red) in P1 plasmid and enhanced green fluorescent protein (EGFP) in P2 plasmid. These fluorescence-expressing plasmids also helped validate the qSanger method both, in-vitro and in co-transformed bacteria using standard DNA quantification methods.

First, they validated the qSanger method by measuring the plasmid DNA ratio in different mixtures of P1- and P2-only cells. Next, the team evaluated P1:P2 ratios in co-transformed E. coli cells using qPCR and fluorescence quantifications. In both cases, the P1:P2 ratio computed with the qSanger method correlated with the other plasmid DNA ratios.

These results demonstrate the accuracy of the qSanger methodology in quantifying genetic variants in cells from mixed Sanger sequences,

proving that its efficacy is comparable to other DNA quantification approaches. However, what makes this methodology stand out against technologies like qPCR and digital droplet PCR are its ease of use and significantly reduced costs and labor.

Discussing the potential applications of this novel approach, Prof. Jaramillo says, "Our methodology could be used to analyze mutant/nonmutant DNA ratios in cell populations after different implementations of gene editing, including base editing, prime editing, and promoter engineering by multiplex automated genome engineering. Furthermore, it could be used in applications requiring quantification of multiple DNA or RNA sequences in the same mixture."

Given the growing applications of DNA sequencing in different fields, the qSanger methodology might just push the envelope for accurate, time-saving and cost-effective DNA assessments.

More information: Satya Prakash et al, qSanger: Quantification of Genetic Variants in Bacterial Cultures by Sanger Sequencing, *BioDesign Research* (2023). DOI: 10.34133/bdr.0007

Provided by Nanjing Agricultural University The Academy of Science