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TECHNIQUE

A miniature living recording device

The ability to detect and store environmental changes in biological organisms has tremendous possibilities, for example, to record the presence of metabolites or regulatory changes. Sheth *et al.* now report the generation of modified bacteria that can record and store information about the presence of biological signals over time in DNA.

The Temporal Recording in Arrays by CRISPR Expansion (TRACE) system developed by Sheth *et al.* builds on the CRISPR–Cas adaptation process — a naturally occurring memory system in which foreign DNA is processed and incorporated in genomic CRISPR arrays in the form of spacers by the Cas1 and Cas2 proteins. New spacers are integrated in a unidirectional manner and, consequently, the CRISPR arrays store temporal information on the presence of foreign DNA. The TRACE system combines the CRISPR–Cas adaptation process with a trigger-induced copy number increase of a plasmid (that is, a trigger plasmid). Sequencing of the CRISPR arrays gives information on the temporal presence or absence of the trigger based on the presence and location of trigger plasmid-derived spacers versus reference spacers that are acquired at a background level from, for example, the genome.

The CRISPR–Cas adaptation system was engineered in *Escherichia coli* cells via an inducible recording plasmid that expresses Cas1 and Cas2. The trigger plasmid consists of the gene encoding the phage P1 lytic replication protein downstream of an inducible promoter. The input trigger induces expression of this protein, which in turn initiates plasmid replication. Exposure of the modified *E. coli* cells to the trigger leads to increased levels of trigger plasmid in the bacteria and trigger plasmid-derived spacers in CRISPR arrays. Robust recording requires trigger presence for 6 hours — the demonstrated temporal resolution of this system.

To assess whether TRACE can capture time-course information, bacteria were cultured in the presence or absence of the trigger for 4 days, resulting in 16 different signal exposure profiles and incorporation of up to five spacers. As the length of the CRISPR arrays determines the level of temporal information, a size enrichment protocol was implemented in the sequencing strategy to facilitate analysis of longer arrays. Using this strategy, the pattern of trigger spacer frequencies corresponded well to the respective temporal signal profiles. The authors also developed a model to accurately match the recorded array pattern to its corresponding signal profile. When information from arrays containing 2–4 spacers was used, all spacer profiles were successfully matched to the original signal exposure profile. Thus, temporal information can be recorded in and consequently reconstructed from CRISPR arrays.

As a proof of principle, Sheth *et al.* tested multiplex temporal recordings of three biologically relevant signals by generating three bacterial strains, each expressing a different trigger plasmid with a unique sensor and barcoded CRISPR array. Sequencing of the CRISPR arrays and demultiplexing using the barcodes enabled the identification of bacterial exposure profiles. When assessing 16 different temporal signal profiles, half of them were perfectly classified whereas the remaining incorrect predictions were close to the actual profiles. Thus, although technical improvements are needed, TRACE sets the stage for massive parallel recording of biological inputs.

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Nature Reviews Disease Primers

> “temporal information can be recorded in and consequently reconstructed from CRISPR arrays”

ORIGINAL ARTICLE Sheth, R. U. *et al.* Multiplex recording of cellular events over time on CRISPR biological tape. *Science* http://dx.doi.org/10.1126/science.aao0958 (2017)