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BREAKTAG - NOVEL METHOD FOR DETECTION OF DS-BREAKS AND ANALYSIS OF CRISPR-INDUCED ON- AND OFF-TARGET EFFECTS

Keywords: genome editing, CRISPR/Cas, Cas9, off-target, DSB, gRNA, tagmentation, cell engineering, T cell, cell therapy

INVENTION NOVELTY

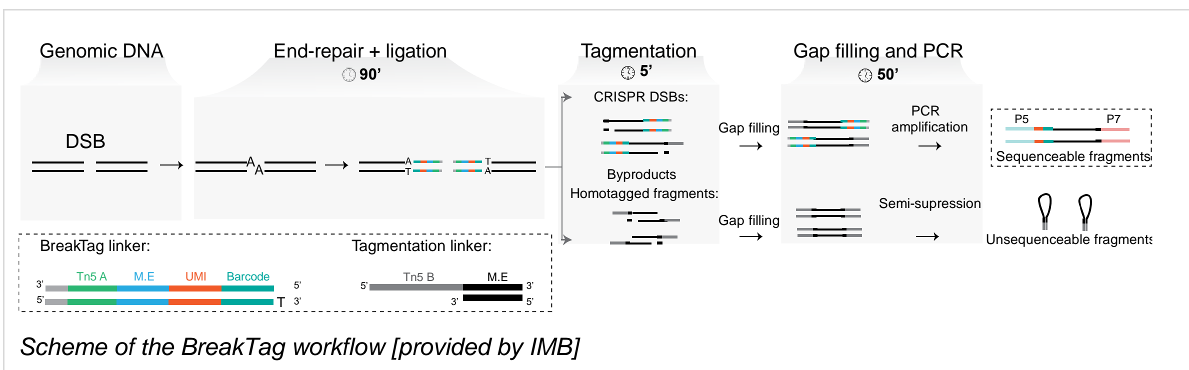
BreakTag is a high throughput method for detecting the locations of on- and off-target DNA double-strand breaks (DSBs) induced by genome editing nucleases *in vitro* or *in vivo* via preparation of a DNA library for next-generation sequencing, allowing flexible preclinical testing for optimization of nuclease cleavage and guide RNAs design for higher specificity.

VALUE PROPOSITION

BreakTag relies on direct labelling of DNA DSB ends. It is preferably used for discovering on- and/or off-targets of genome-editing nucleases, in particular CRISPR nucleases such as Cas9 and Cas12 *in vitro* or in cells. In a core step, it uses a PCR suppression step to enrich for DSBs via tagmentation. The BreakTag method can also be adapted to test off-target sites for base editors by introducing artificial DSBs at the nicked site using the repair enzyme endonuclease V. Compared to other known methods for analyzing off-target effects, BreakTag's enrichment protocol is simplified and robust, and does not require steps such as cell transfection, circularization of the genome, *in vitro* transcription, nested PCR or whole genome sequencing. These advantages in combination with the short protocol time of less than 6 hours would allow this methodology to be easily implemented into research or clinically routine as for instance, to screen for gRNAs with minimal off-targets.

TECHNOLOGY DESCRIPTION

BreakTag is a 4-step protocol for profiling CRISPR off-targets in a cell-free or cell-based mode. The protocol uses genomic DNA, either extracted from living cells or *in vitro* digested with ribonucleoprotein complexes after CRISPR delivery. Free DSB-ends are labeled with a BreakTag linker, followed by a second linker which is rapidly added via a tagmentation step. DNA fragments that are labeled with both linkers are selectively amplified during a PCR step, whereas by-products (such as non-DSBs) form intramolecular hairpins are not enriched, resulting in ready-to-sequence libraries.



Scheme of the BreakTag workflow [provided by IMB]

COMMERCIAL OPPORTUNITY

The technology is available for licensing or further co-development.

DEVELOPMENT STATUS

BreakTag was implemented *in vitro* testing several nucleases (Cas9 variants and Cas12) and the cytosine base editor BE3. Using cell-free BreakTag, the off-target landscape for 46 single-guide RNAs (sgRNA) from clinically relevant CAR-T genes was analyzed.

PATENT SITUATION

A priority establishing European patent application was field in 2022 (EP22189451.2).

FURTHER READING

Manuscript under review.

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