Docket #: S18-467

BARCODED CLONAL TRACKING OF GENE TARGETING IN CELLS

Researchers at Stanford and the Chan Zuckerberg Biohub have developed a methodology to monitor cell expansion and differentiation following targeted genomic modification.

Targeted DNA correction of disease-causing mutations offers promise in the treatment of genetic diseases. Following targeted genomic modification, it is often desirable to follow progeny cells, especially in situations where possible secondary mutations can occur in the genome (e.g., due to off-target effects) such that progeny cells act differently. However, because of the precise nucleotide-resolution nature of gene correction, it is not possible to track the output of any specific gene modified cell or understand clonal dynamics with current approaches.

Stage of Research
The inventors have developed TRACE-Seq (Tracking Recombination Alleles in Clonal Engraftment using Sequencing), a methodology that allows for both targeted genome modification and for the monitoring and tracking of different cells having the same targeted modification. TRACE-Seq utilizes barcoded donor vector libraries, carrying either in-frame silent mutations or semi-randomized nucleotide sequences outside the coding region, such that independent, and otherwise identical, targeted modifications can be monitored by the presence of different barcode sequences. The inventors demonstrate the impact of TRACE-Seq for translational and basic research through the targeted barcoding of the hemoglobin allele (HBB) and cell tracking within a heterogenous hematopoietic cell population.

Applications

- Methods for targeted homologous recombination-based genome modification and monitoring the development of a cell population, in vitro or in vivo

- Targeted DNA correction of disease-specific mutations in hematopoietic stem and progenitor cells (HSPCs) and tracking of in vivo contributions of gene targeted HSPCs

Advantages

- Any suitable DNA nuclease can be used (e.g., CRISPR/Cas, zinc finger nuclease, TALEN, meganuclease, etc.)
- The coding sequence can be any coding sequence desired (
- Donor template can be introduced into the target cell in any way desired (e.g., electroporation, etc.)
- Donor vectors contain synonymous mutations to introduce sequence diversity without modifying the amino acids produced by the target cells

Publications

- Sharma R, Dever DP, Lee CM, Azizi A, Pan Y, Camarena J, Köhnke T, Bao G, Porteus MH, Majeti R. The TRACE-Seq method tracks recombination alleles and identifies clonal reconstitution dynamics of gene targeted human hematopoietic stem cells. Nat Commun. 2021. Doi: 10.1038/s41467-020-20792-y
- PCT publication WO2020210225

Patents

- Published Application: WO2020210225

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