Massively parallel lineage tracing using CRISPR/Cas9 induced genetic scars

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Introduction

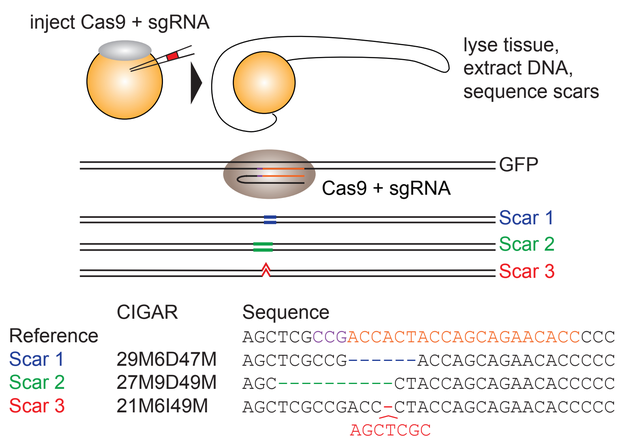
Analysis of the lineage history of cells can reveal the developmental origin of cell populations. Direct observation of all cell divisions is generally only possible at the earliest developmental stages. Pioneering studies using somatic mutations[1], transposon locations[2], or viral inserts[3,4] as DNA barcodes have yielded important insights into developmental lineage decisions. However, they are typically limited to tracing the lineage of a small subset of cells within the organism. In our method, scartrace, we use CRISPR/Cas9 genome editing to barcode a majority of all cells with random indels.

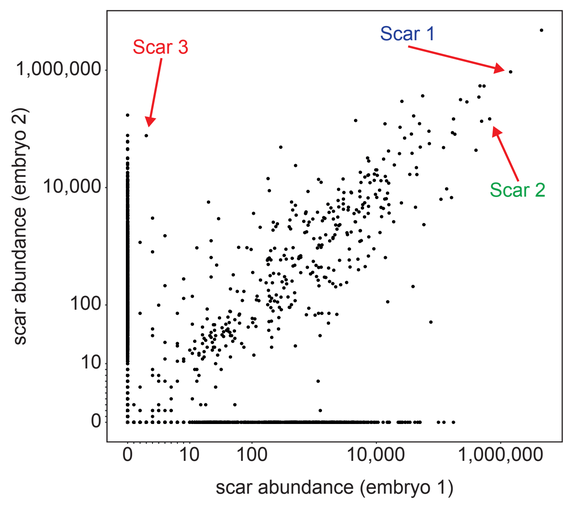
Results

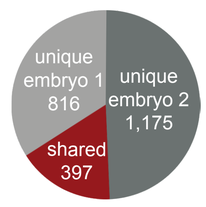
Scartrace is a simple approach for lineage analysis of whole organisms. Cell labeling and detection of barcodes is performed in a high-throughput manner. Using a fish line that is present in most zebrafish facilities, and easily available reagents, we created over thousand different barcodes in a single zebrafish. The majority of cells have up to four barcodes that can be used to construct lineages. In the caudal fin, we observed that development and regeneration use the same cell lineages. We expect scartrace and related methods[5] to significantly advance the understanding of processes in development and regeneration.

Cas9 generates large diversity of single-cell barcodes

Injection of Cas9 and sgRNA into the zygote marks cells with genetic scars. Scars are classified by the CIGAR code, which describes length and position of indels.



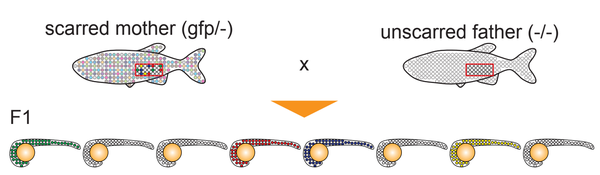




Correlation of scar abundances between two 24 hpf embryos injected with sgRNA and Cas9 mRNA.

Clonality of the germ cell pool

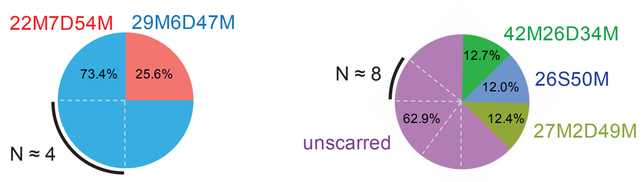
An adult female with scars was crossed with a wildtype male. We sequenced the scars of the F1 generation.



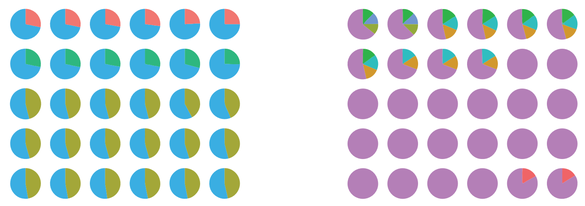
Cas9 protein injected mother

Cas9 mRNA injected mother

Representative examples of scar frequencies for two individual embryos.

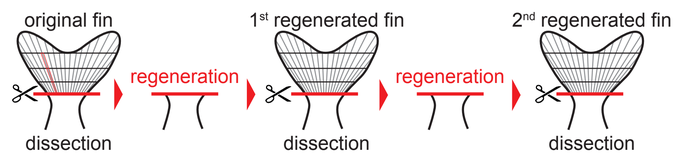


Representative subset of embryos.

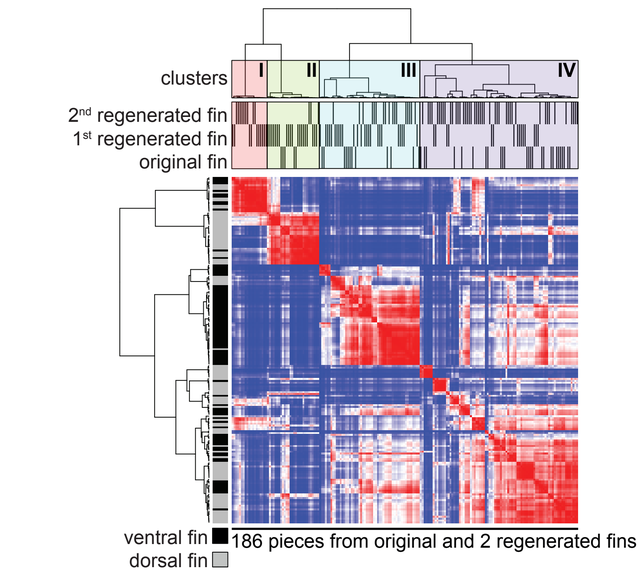


Lineage trees for the F1 clones derived from a protein-injected and an mRNA-injected parent.

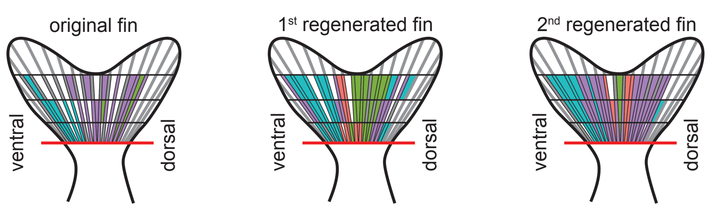
Clonality of the regenerating caudal fin



The caudal fin of an adult zebrafish with Cas9 mRNA-induced scars was dissected.



Clustering of fin pieces by similarity of scar profiles, normalized by global scar probability.



Spatial profile of scar clusters. Color code as above.

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Junker J.P., B. Spanjaard *et al.* Massively parallel clonal analysis using CRISPR/Cas9 induced genetic scars. *Biorxiv*, doi: 10.1101/056499