Complete alignment identification of CRISPR-Cas9 genomic off-targets using Edit-R CRISPR specificity tool and a comprehensive analysis of positional mismatch tolerance

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Abstract

The CRISPR-Cas9 system has the potential to advance basic and applied research. However, specificity of this electrifying CRISPR-Cas9 system is not yet completely understood. New findings on off-target effects show that genome-wide off-target effects exist and are being frequently detected. Recent work has demonstrated gene editing by CRISPR-Cas9 in both mammalian and bacteria systems, but existing design tools are unable to detect purine off-targets based on gapped alignments. We present the Dharmacon™ Edit-R CRISPR specificity tool, a simple, web-based tool that predicts functional activity of CRISPR-Cas9 off-target activity. The Edit-R CRISPR specificity tool combines the known target (20-bp) sequences with an additional 15-bp flanking region, thereby creating a 35-bp target region used in the alignment. The tool compares the target region to the genome and calculates the percentage of DNA mismatches. The Edit-R CRISPR specificity tool can predict for a range of CRISPR-Cas9 off-targeting and provides additional crRNA design rules for mammalian off-targeting.

A high-throughput functional assay for proteasome function

A high-throughput functional assay for proteasome function using a single-mismatched scaffold. The system is based on a single-mismatched scaffold that directs the degradation of the N-terminus of a Ubiquitin-GFP U2OS-Cas9 cell.

Gene targeting and relative off-targeting in Ubiquitin-GFP U2OS-Cas9 cells

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Conclusions

- The Dharmacon™ Edit-R CRISPR specificity tool is both fast and exhaustive at predicting crRNA genomic off-target sites with up to three mismatches.
- Single mismatches are fairly well-tolerated, especially in the 5'-PAM distal-end of the crRNA.
- crRNAs with mismatches in the seed region, position 11-20 of the crRNA, did not exhibit significant activity.
- Consecutive two-base mismatches are generally not active.
- Two-base mismatches exhibiting function tend to be nonconservative (in the non-seed region) and most often involve one mismatch at the most distal end from the PAM.