A114 · TANGO: High-Throughput, Highly Sensitive Measurements of dCas9 Binding to On- and Off-Target Sequences

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CRISPR-based epigenome editing technologies, including CRISPR interference (CRISPRi) and activation (CRISPRa), enable programmable gene regulation and double-strand break-free genetic medicines. However, dead Cas9 (dCas9) targeting efficiency remains a bottleneck — in some studies, up to 80% of guide RNAs (gRNAs) directed against distal regulatory elements fail to modulate gene expression in vivo, while off-target binding remains prevalent. Current computational algorithms and low-throughput assays struggle to accurately capture these inefficiencies or discover promiscuous off-targets. To address this critical gap, we developed TANGO (Targeted Array-based Nucleic acid-Guided Occupancy), a sensitive, high-throughput in vitro platform that quantitatively measures dCas9 ribonucleoprotein (RNP) binding across tens of thousands of pre-designed DNA sequences. By isolating intrinsic RNP:DNA recognition from complex cellular chromatin dynamics, TANGO generates high-resolution profiles capturing on-target affinities, PAM dependencies, and position-specific mismatch tolerances with superior sensitivity over existing high-throughput assays. Array-measured binding intensities and PAM-proximal mismatch tolerances mechanistically explain and strongly correlate with genome-wide dCas9 binding (ChIP-seq) and cellular CRISPRi activity. TANGO reliably distinguishes working from non-working gRNAs within tight genomic loci sharing comparable epigenetic environments. Furthermore, the platform implicates novel mechanistic drivers of gRNA promiscuity, such as internal NGG motifs within the protospacer seed sequence that may be utilized as alternative PAMs. By shifting gRNA evaluation from target cleavage to binding affinity, TANGO elucidates the intrinsic sequence determinants of CRISPR specificity. These datasets provide informative inputs for machine learning, enabling the development of foundational rulesets for the prediction of on- and off-target binding across genomes, thus supporting the de novo design of potent, de-risked, high-fidelity gRNAs for safe clinical epigenome editing.

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