Massively parallel biophysical analysis of a CRISPR-Cas complex on repurposed next generation sequencing chips

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Bacterial CRISPR-Cas systems recognize foreign DNA via complementary base pairing with an RNA-guided nucleoprotein complex. Although off-target DNA binding has been reported, a quantitative description of sequence-dependent DNA binding and nuclease activation remains elusive. Here, we describe a chip-hybridized affinity mapping platform (CHAMP) that repurposes modern next-generation sequencing chips to directly measure the interactions between proteins and ~107 unique DNA sequences. Using CHAMP, we interrogate DNA recognition by a Type I-E CRISPR/Cas (Cascade) complex and the Cas3 nuclease. These results show that Cascade is sensitive to an extended protospacer adjacent motif (PAM). Analysis of mutated target sequences reveals a surprising three-nucleotide periodicity in Cascade-DNA interactions. The identity of the PAM and the PAM-proximal nucleotides control Cas3 recruitment, providing an additional DNA-guided proofreading mechanism. These findings are used to develop a model for the biophysical constraints governing off-target DNA binding and provide a framework for high-throughput, quantitative analysis of protein-DNA interactions.