NEW CRISPR TOOLS FOR STUDYING TRANSCRIPTION

Both CRISPRi and CRISPRa rely on a mutated version of the Cas9 endonuclease (dCas9) that cannot cut the desired gene at the usual sites.

❶ dCas9 can still form a complex with a single-guide RNA, which indicates where in the genome the complex should bind. Covalently attaching a 50-amino-acid KRAB domain from a zinc-finger protein to dCas9 provides better transcription blockage of both protein-coding and RNA genes.

❷ Attachments to dCas9 can also allow for the activation of gene expression. A SunTag array—a string of peptide epitopes that recruits transcription activators—or a tandem array of activators fused to dCas9 (VP64, p65, Rta) enhance the amount of gene expression.

❸ Adding particular sequences of RNA to the single-guide RNA allows it to attract transcription activators. This method also fuses a transcription activator to dCas9 (VP64).