Nature Reviews Genetics | Published online 22 Jan 2018; doi:10.1038/nrg.2018.2

TECHNIQUE

SNP-CLINGing onto your post in the genome

Previous methods for understanding nuclear organization have been limited to a momentary snapshot, unable to capture the dynamics of a living genome. In particular, the positioning of alleles in real time *in vivo* has been impossible. Maass *et al*. address this limitation with single nucleotide polymorphism CRISPR live-cell imaging (SNP-CLING).

SNP-CLING uses catalytic dead Cas9 (dCas9) and several single guide RNAs specific to each allele of a target locus. The guide RNAs were modified with RNA-aptamer motifs that bind one of the RNA-binding proteins MS2, PP7 or PUM1 fused to different fluorescent reporters. *Streptococcus pyogenes* dCas9 requires a protospacer-adjacent motif (PAM) next to its target sequence; the ability of dCas9 to distinguish SNPs within the PAM motif was exploited to resolve specific alleles. Live-cell imaging of mouse embryonic fibroblasts and stem cells derived from a hybrid cross in which the PAM sequence in each allele varies by a SNP was able to distinguish maternal from paternal alleles at different loci. "SNP-CLING offers a variety of possibilities to study molecular biology and heterozygous disease states by distinguishing between parental alleles," explains Philipp Maass (Harvard University), lead author of the study.

Targeting *XIST* and *TSIX*, which are only 69 kb apart on the linear genome, in human retinal pigment epithelial (RPE-1) cells, the group could observe separate signals at a distance of 163 nm, indicating high spatial resolution.

To determine the 3D dynamics of alleles using SNP-CLING, the authors selected genes that are involved in heterozygous structural aberrations that cause brachydactyly — a condition characterized by disproportionately short fingers and toes — and that are located on chromosomes with distinct features. The team were thus able to probe interallelic differences in a range of genomic contexts, comparing, for example, *Hdac4* on a large chromosome versus a random locus on a small chromosome, as well as *Sox9* on a gene-dense chromosome and the long non-coding RNA (lncRNA) *Cistr-act* locus on a gene-poor one. Whereas chromosome size did not have an effect, alleles on gene-dense chromosomes were further apart than alleles in gene-poor regions. These positions relative to each other were stable in living cells.

Using the nucleoli and the nuclear periphery as landmarks, the group observed that alleles of a locus are stably positioned in the genome, and the loci themselves have unique positions. Turning to the gene encoding the lncRNA *Firre*, the authors confirmed that both alleles are positioned close to the nucleoli regardless of genetic background or its interaction with *Ypel4* through colocalization.

As SNP-CLING is functional in live cells, the team was able to use time-lapse imaging to track the allelic positioning of *Firre* over time. They observed that the alleles maintained their position relative to the nucleoli for close to 4 hours. This positioning drastically fluctuated during apoptosis.

The specificity of SNP-CLING to distinguish and visualize alleles in living cells moves us beyond the static snapshot provided by techniques such as fluorescent *in situ* hybridization and chromosome conformation capture-related techniques. "Many human diseases affect only one allele," concludes senior author John Rinn (University of Colorado). "SNP-CLING can target and track the positioning of disease alleles versus normal alleles in real time to yield insights into how genetic aberrations affect the dynamic architecture of the genome."

Ross Cloney, Senior Editor,
Nature Communications

> "SNP-CLING to distinguish and visualize alleles in living cells"

ORIGINAL ARTICLE Maass, P. G. *et al.* Spatiotemporal allele organization by allele-specific CRISPR live-cell imaging (SNP-CLING). *Nat. Struct. Mol. Biol.* https://doi.org/10.1038/s41594-017-0015-3 (2018)

SolStock/iStock/Getty