UTILIZING CRISPR/CAS9 TO IDENTIFY CHROMOSOMAL LOCI

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Current methods for imaging transgene integration sites use DNA-FISH, which is a time- and labor-intensive process. Every genomic target requires a costly probe specific to the locus of interest. Through CasFISH, the only customized reagent needed is a target-specific crRNA, while a singular tracrRNA, dCas9, and antibody set can be applied to any newly synthesized crRNA, significantly reducing cost and effort when targeting multiple sites. The CRISPR/Cas9 system provides highly specific targeting of genomic DNA by means of a Cas9 nuclease complexed with guide-RNA which binds a 20 base-pair target sequence within the genome. Introducing two point-mutations to the catalytic region of Cas9 results in a nuclease-inactive enzyme referred to as dCas9. This catalytically “dead” system can be used by leveraging the intact DNA-interrogative properties to direct proteins to areas of interest within the genome. Our group has applied this approach to image telomere-specific repeats on chromosomes in metaphase spreads via immunofluorescence within our host cell line, CHOZN GS-/-. Utilizing the CasFISH method has been successful for us and others in targeting large, repetitive regions of interest, but to our knowledge, there has been no success in targeting smaller, non-repetitive regions using CasFISH alone. Our current work seeks to solve this problem by combining CasFISH with other existing technologies, such as DuoLink PLA (proximity ligation assay) or quantum dots, which are artificial semiconductor nanoparticles. Here, we detail our efforts in exploring these technologies, which promise to reduce the time and effort spent in visualizing transgene integration sites and other genomic loci of interest.