The human genome project, completed in 2003, gave scientists new tools to understand human evolution and medicine (genome.gov). With these new found tools, the next logical step in the genomic era is understanding whole genome regulation by determining the mechanisms of chromosomal positioning, nuclear arrangement and loci interactions. Overall nuclear organization is highly elaborate and structured, nonetheless, there is still great debate whether structure dictates function or vice versa (Mistelli 2007). Furthermore, differential chromosome positioning in the nucleus has been implicated in diseases such as progeria, muscular dystrophy, cancer and others (reviewed in Ferraiet et al. 2010). Current molecular biology tools are limited for following chromosome movement in living cells to fully understand how chromosome positioning and movement plays a role in disease (Chen et al. 2013). To address this problem, Chen and colleagues sought to further develop the CRISPR-Cas9 (clustered regularly-interspaced short palindromic repeats - CRISPR associated protein) system into an imaging tool to observe genomic element position and movement in living cells. A nuclease deficient Cas9 protein fused to enhanced green fluorescent protein (EGFP) was generated that complexes with a synthetic single guide RNA (sgRNA). Transfection of modified Cas9 along with synthetic sgRNA designed to target repetitive genomic sequences in telomeres and the MUC4 gene demonstrated the design was efficient at tagging and imaging unique repetitive locations in the genome. Further, transfection of the same modified Cas9 with sgRNA targeting non-repetitive regions of the MUC4 gene demonstrated their system was specific and robust enough to identify non-repetitive regions of the genome. Taking this new imaging tool a step further, they used the repetitive sequence sgRNA targeting MUC4 to follow MUC4 positioning through mitosis. They showed that the MUC4 gene segregates symmetrically through cell division. Furthermore, when compared to methods like fluorescence in situ hybridization (FISH), CRISPR imaging proved similar specificity and accuracy in detecting telomere and gene number and changes in telomere length with the added benefit that they could evaluate this information in living cells. This modified CRISPR-Cas9 imaging system was the first to identify both repetitive and non-repetitive genomic elements, detect gene number, and movement of genes through space and time. Further, their optimized design has the potential to reduce off-target binding and gene regulation in other CRISPR-Cas9 systems. This imaging platform is a novel tool for molecular biology allowing for the imaging and characterization of spatial relationships of different genomic elements, replication timing and genomic organization. This study was the first to show utility of CRISPR-Cas9 for imaging specific genomic loci. Future advances of this tool will provide for enhanced signal amplification of single molecules (Tanenbaum et al. 2014), multiplex labeling (Chen et al. 2013, Chen et al. 2016) and visualization of RNA localization (Chen et al. 2013, Nelles et al. 2015). CRISPR-Cas9 imaging of live cells will provide invaluable understanding of development and disease as well as utility in all fields of molecular biology.

References
Chen B., Gilbert L.A., Cimini B.A., Schnitzbauer J., Xhang W., Li G., Park J., Blackburn E.H.,


National Human Genome Research Institute <http://www.genome.gov/10001772>
