

Modeling Genomic Recombination Potentials Regulated by Synthetic Donor DNA and Triplex-forming Molecules

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Short Abstract — Endogenous genome targeting and editing in an efficient and specific manner are technological challenges, particularly in development and translational settings, with significant foreseeable impacts. To address these challenges, quantitative modeling genomic recombination potentials of synthetic donor DNA and triplex-forming molecules from sequence content and structural conformation perspectives are developed. These designed sequence-specific and structure-resaping molecules are explored for their non-covalent intramolecular self and intermolecular genomic interactions. Findings indicate constraints and nuances for the design of the donor DNA molecule particular to a genomic editing site and, analogously, of the triplex-forming molecule particular to a genomic targeting site.

Keywords — recombinagenic donor DNA, mutagenic triplex-forming molecule, genome engineering, precision medicine

I. PURPOSE

SYNTHETIC oligo- and peptide- nucleic acid mutagenic molecules have been more predictably designed and externally delivered into the intracellular milieu. These deployed technologies interact with, and influence, the cytoplasmic and nuclear molecular machinery in order to regulate potentials involved in genomic targeting and editing. By regulating these potentials, silenced yet functional genes can be reactivated, as well as exquisitely controlled by external and environmental stimuli, thus modulating the cellular regulatory hematopoiesis system [1].

It is demonstrated that synthetic nucleic acid nanostructures composed of various nucleobase and backbone modifications can regulate the genomic recombination rate, the sequence-specific restriction of a locus, and the endogenous repair pathways. The formation of a triplex nanostructure (Fig. 1), by exogenously introduced PNA molecules with the duplex chromosomal and episomal DNA, is shown to elevate the cell's targeted recombination potential [2].

Recombinagenic donor DNA molecules co-opt these

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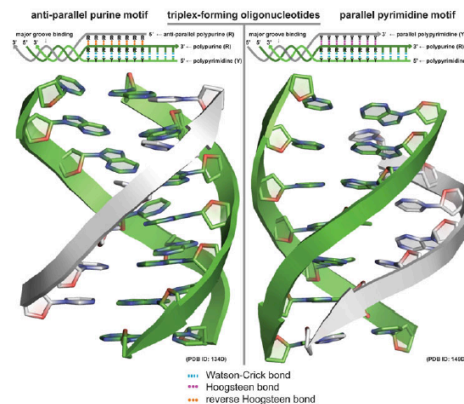


Figure 1. Triplex-forming molecule genomic motifs.

elevated recombination or initiated restriction potentials to form competing nanostructures that act as homology-dependent templates, sans edits to be introduced, thus potentiating repair [3]. Safety and efficacy of these nanostructures is achieved by leveraging the performance profile of the cell's own endogenous recombination, restriction, and repair machineries in concert with these sequence-specific and localizing-in-tandem molecules. Progenitor cells drugged with designed molecules, and primed with chemical cell modulators, safely and effectively redesign the genome, which are then propagated to cellular progeny.

These molecular technologies are developed to remediate the underlying genomic causes of monogenic human diseases such as hemaglobinopathies, engineer living genetic codes, improve crop characteristics, and defend against outbreaks, through quantitative modeling and elucidation of cellular genomic recombination, and thus has well-positioned technology profiles for healthcare, biotechnology, agrotechnology, and national security.

REFERENCES

- [1] Chin JY, Reza F, Glazer PM (2013) Triplex-forming peptide nucleic acids induce heritable elevations in gamma-globin expression in hematopoietic progenitor cells. *Molecular Therapy* **21**, 580-587.
- [2] Reza F, Glazer PM (2014) Triplex-mediated genome targeting and editing. *Methods in Molecular Biology* **1114**, 115-142.
- [3] Reza F, Glazer PM (2015) Therapeutic genome mutagenesis using synthetic donor DNA and triplex-forming molecules. *Methods in Molecular Biology* **1114**, 115-142.