GENE EDITING, SYNTHESIS, AND ASSEMBLY

Goal	**Breakthough Capability**	Milestone

Manufacture thousands of very long oligonucleotides with high fidelity.

Highly efficient oligonucleotide synthesis to increase the number, length, and fidelity of oligonucleotides.			
Robustly synthesize one million 200-mer oligonucleotides with a per-nucleotide error rate of fewer than one in 500 nucleotides.	Robustly synthesize 1,000-mer oligonucleotides with a per-nucleotide error rate of fewer than one in 1,000 nucleotides.	Reduce per-nucleotide error rates for 1,000-mer oligonucleotide synthesis to fewer than one in 5,000 nucleotides.	Synthesize 10,000-mer oligonucleotides at 99.99% cycle efficiency within one minute with a per-nucleotide error rate of fewer than one in 30,000 nucleotides.

Many-fragment DNA assembly with simultaneous, high-fidelity sequence validation.

Predictive design of DNA sequences for improved assembly of longer, more information-rich DNA fragments.			
Coupled design of DNA sequences to optimize nucleotide composition to support synthesis, while maintaining genetic system function.	Incorporate machine learning to identify poorly-understood problematic sequences and process conditions.	Design algorithms that identify optimal synthesis strategies for assembling megabase-length genetic systems.	Design algorithms for optimal one-pot-assembly of billions of unique genomic/chromosomal variants with defined sequences.
Methods for one-step, simultaneous assembly and sequence-verification of long DNA fragments.			
Reliable assembly of 10,000 base pair non-clonal DNA fragments.	Reliable assembly and verification of 10,000 base pair clonal DNA fragments.	Reliable assembly and verification of 100,000 base pair clonal DNA fragments.	Reliable assembly and verification of 1,000,000 to 10,000,000 base pair clonal DNA fragments.
Pipelined synthesis, assembly, and functional testing of engineered genetic systems.			
Achieve desired functionalities in lower-fidelity, error-prone genetic systems.	Achieve reliable Design-for-Testing in engineered genetic systems.	Achieve readily-swappable modules within large genetic systems.	Achieve one-month Design-to-Test cycles for megabase-length genetic systems.

Precision genome editing at multiple sites simultaneously with no off-target effects.

Ability to reliably create any precise, defined edit(s) (single nucleotide polymorphisms or gene replacement) with no unintended editing in any organism, with edits ranging from a single base change to the insertion of entire pathwa.			
Ability to generate any defined single base pair change in model organisms.	High efficiency editing (> 90%) across the genome with no off-target activity.	High-efficiency gene insertion or deletion of moderately large changes (< 10 kb) via homologous recombination.	Precise, parallel editing or regulatory modifications (10 to 1000 modifications) across model and non-model organisms, including plants and animals.
Precise, predictable, and tunable control of gene expression for many genes inside diverse cells and organisms across different timescales.			
Achieve long-lasting gene repression and activation.	Ability to regulate expression in non-model organisms.	Technologies to monitor and manipulate genetic and epigenetic mechanisms controlling tissue-wide and organism-wide expression levels over time.	Ability to precisely regulate gene expression in whole-body organisms, with single-cell resolution using dynamic or static control.
Ability to reproducibly deliver editing cargo efficiently and specifically to a given target cells or tissues, and control dosage and timing of the editing machinery.			
Improve editors to function without sequence requirements with activity comparable to 2019 state-of-the-art capabilities.	Routine use of editors without detectable off-target effects.	Enhance specificity of delivery modalities for high efficiency (>90% efficient) editing of cells in a defined tissue.	Quantitative, specific, and multiplexed editing of any site, in any cell, in any organism.
2 Years	**5 Years**	**10 Years**	**20 Years**