NinthBio

EMPOWERING DNA ASSEMBLY

NinthBio Homology Path – DNA Script SYNTAX Experiment Report

FRIDAY, DEC 29, 2023

INTRODUCTION

The goal of this experiment was to assess the efficacy of the NinthBio Homology Path library synthesis design built using the DNA Script SYNTAX synthesis system. In order to do so, a library of 36 sequences with a single variable region between positions 111 to 121 was created (Figure 1). The goal was to design and synthesize these sequences with minimal cost and maximum accuracy.

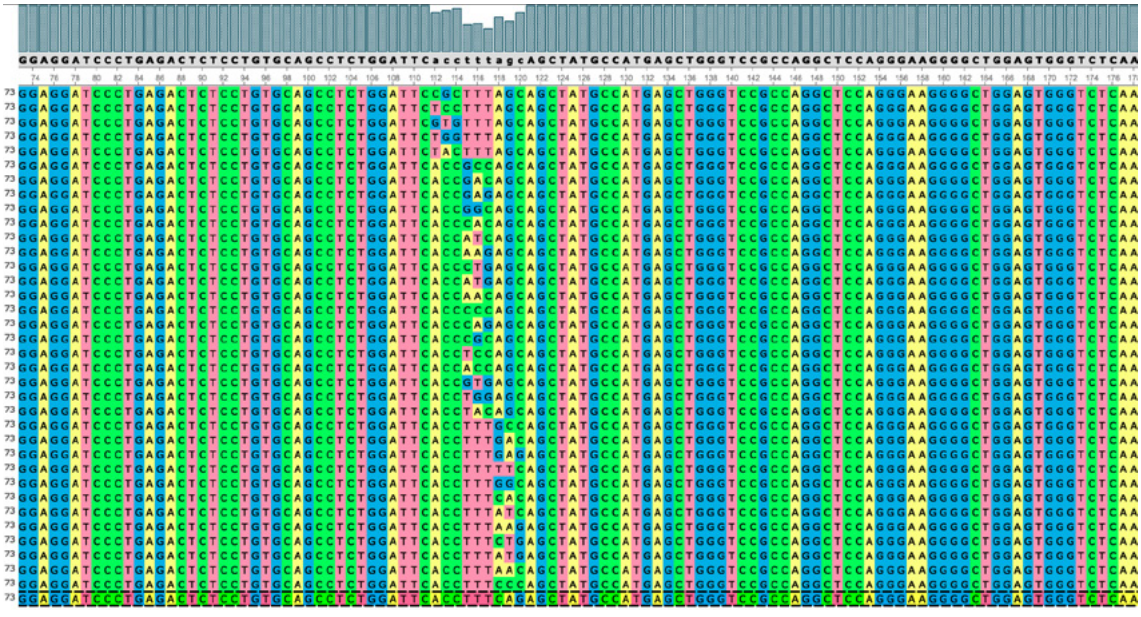

FIGURE 1.

Library of 36 sequences to be synthesized.

HOMOLOGY PATH DESIGN

The first step is to input these sequences into Homology Path Oligo Design (https://homology-path.com/OligoDesign/), with Maximize Recycling chosen as the design type. All other parameters were left as default. The resultant design had the following statistics:

Total sequences	36
Total pre-recycle oligos	792
Total post-recycle oligos	92
Total pre-recycle bases	27,216
Total post-recycle bases	3,171
Total bases recycled	24,045
Recycle efficiency	88.35 %

Notice how these 36 sequences can be built with just 92 oligos, when compared to the naïve design of 792 oligos, an **~88% savings in oligo costs.**

The data packet generated by HP included a list of all oligos necessary to assemble the dsDNA or interest, and the machine instructions needed to build each reaction pool of oligos.

DNA SCRIPT SYNTAX SYNTHESIS

We partnered with Neochromosome, Inc. to synthesize the oligos needed in a single run on their DNAScript SYNTAX system. All oligos and primers were synthesized in an overnight run with a duration of approximately 18 hours.

POOLING AND ASSEMBLY

The synthesized oligos were transferred from the source plate to a reaction plate using a Beckman Coulter Inc. Labcyte Echo 550™ acoustic liquid handler. Each oligo is transferred four times at a volume of 2.5nL for a total transfer volume of 10nL per oligo.

Oligos were assembled into dsDNA and amplified using the standard conditions outlined in the NinthBio DNA Assembly Protocol.

DNA fragments were run on a gel to assess basic synthesis success rate (Figure 2, p.3). Every sequence shows a band at the expected size. Next, Neochromosome sequenced each of the 36 samples using NGS. Thirty-four out of the 36 builds produced good sequencing data which was used for further analysis.

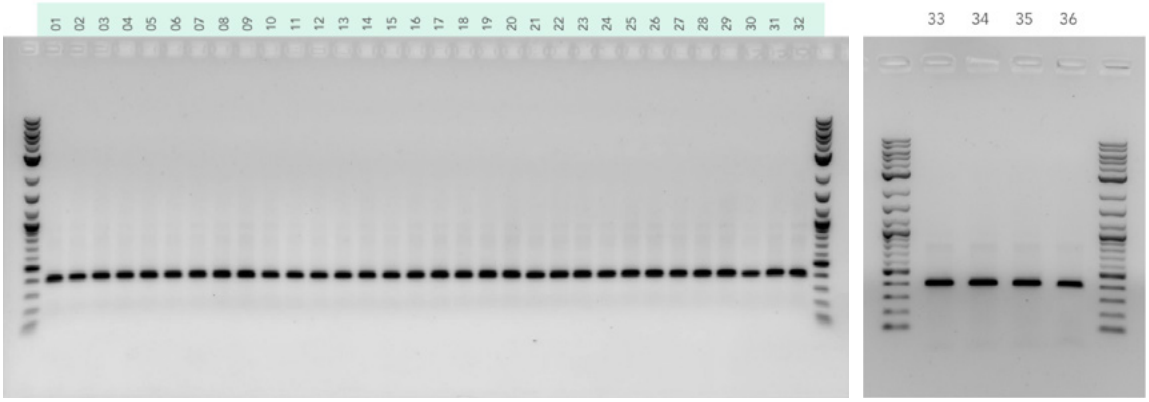

FIGURE 2.

Gel bands for the 36 fragments showing target size achieved.

NINTHBIO VARIANT ANALYSIS REPORT

The NGS data from 34 samples was run through NinthBio's variant analysis software. Sequencing coverage (Figure 3) for all samples looks consistent and valid, with sufficient coverage to ensure variants are called correctly. Since these sequences are linear products, the coverage of this NGS data falls off dramatically at the tails of each sequence. This is expected and should be considered in the analysis going forward. The conclusion from this information is that there is valid NGS sequencing data covering all bases for all sequences.

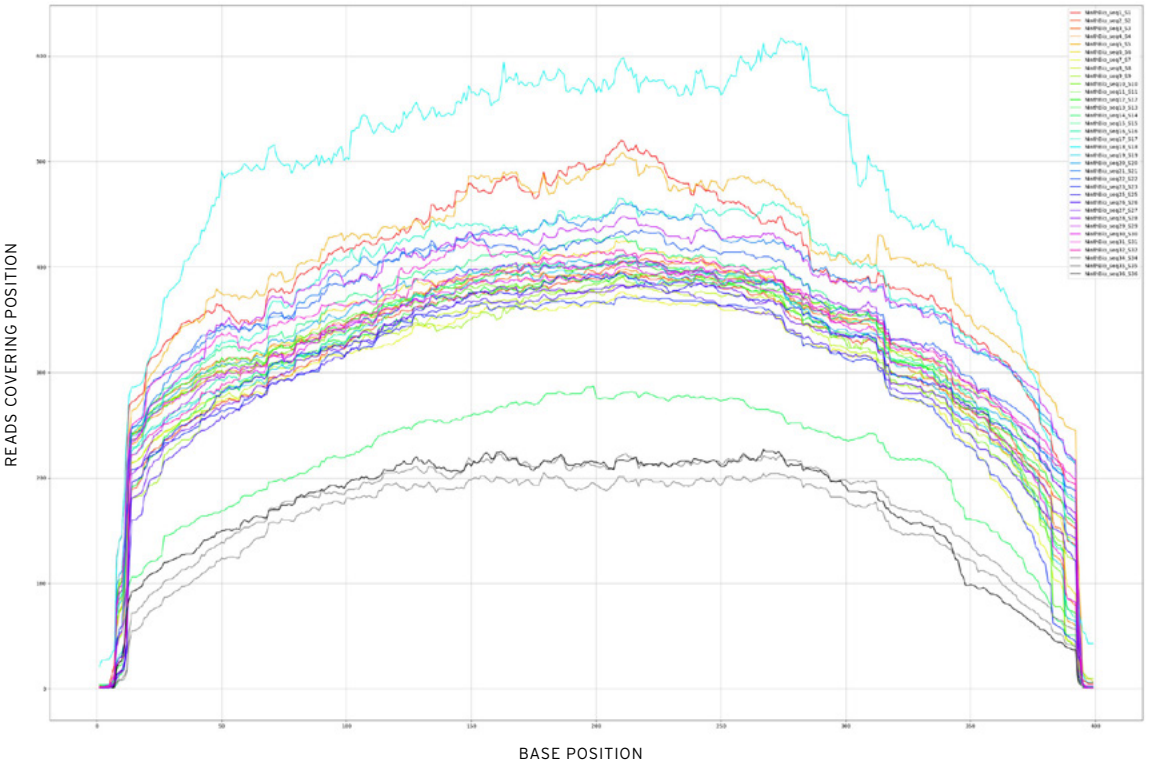

FIGURE 3.

Coverage plots for all 34 samples.

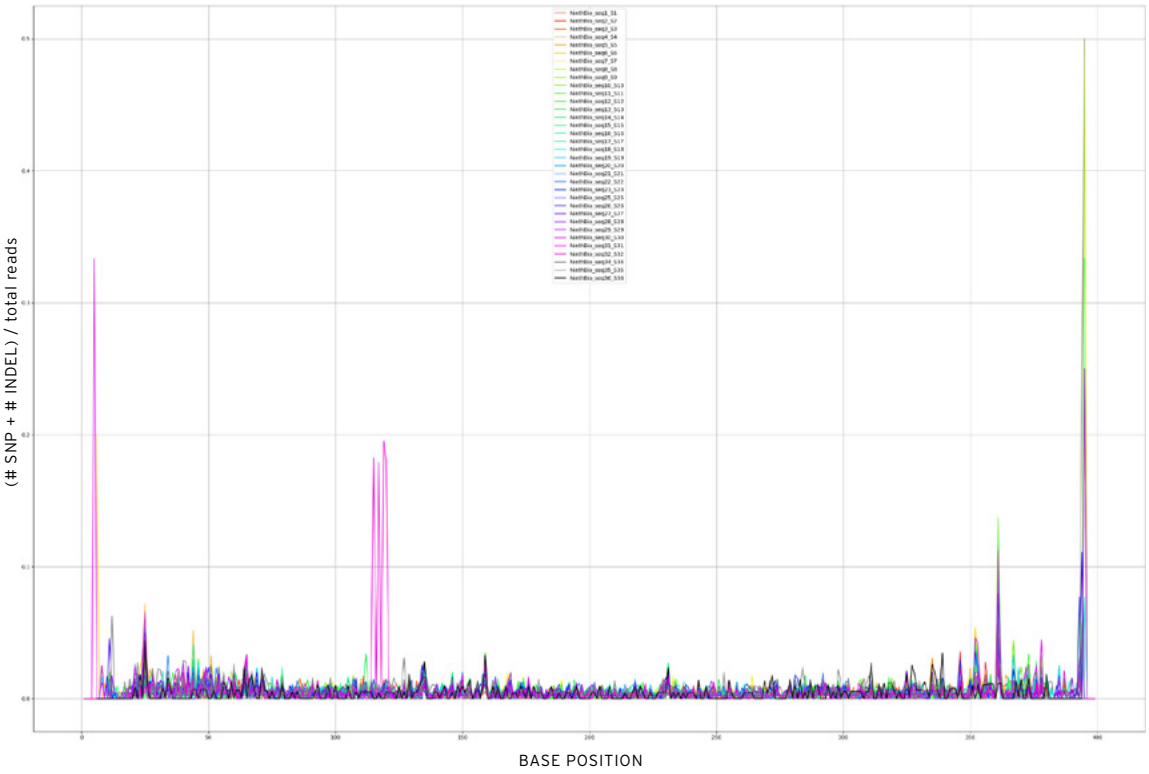

FIGURE 4.

Variant ratios plot showing (# SNP + # INDEL) / Total Reads at each base position.

The raw paired end reads were merged into single reads, deduped, and filtered. The variant ratios (Figure 4) were then calculated to assess how accurate these sequences were after synthesis. Ignoring the tail ends due to the coverage issues mentioned earlier, there were only two loci of interest. First, Ninthbio_seq31 (pink outlier in Figure 4) shows about an 18% alternate variable region. Meaning ~18% of the population showed this variant while the rest was matching the reference. Next, several sequences show a ~10% variant at position 360, with the remaining > 90% matching reference. None of these are significant products, and would not affect downstream processing or delivery of target products.

CONCLUSION

The Homology Path Oligo Design enabled DNA Script SYNTAX to create a high fidelity variant library at approximately 88% cost savings when compared to a naive design. In the worst case example (NinthBio_seq31), if one were to randomly select a sequence from the pool, there would still be an ~82% likelihood of picking the target sequence.

APPENDIX

See the spreadsheet below for the full variant report:
https://docs.google.com/spreadsheets/d/1tHQ-V1NY0tvwQFr9i8bf8NLoQC34hhhuidLGDp03-rs/edit?usp=sharing