DNASCRIPT

Rapidly assemble genes in your laboratory using automated enzymatic oligo synthesis

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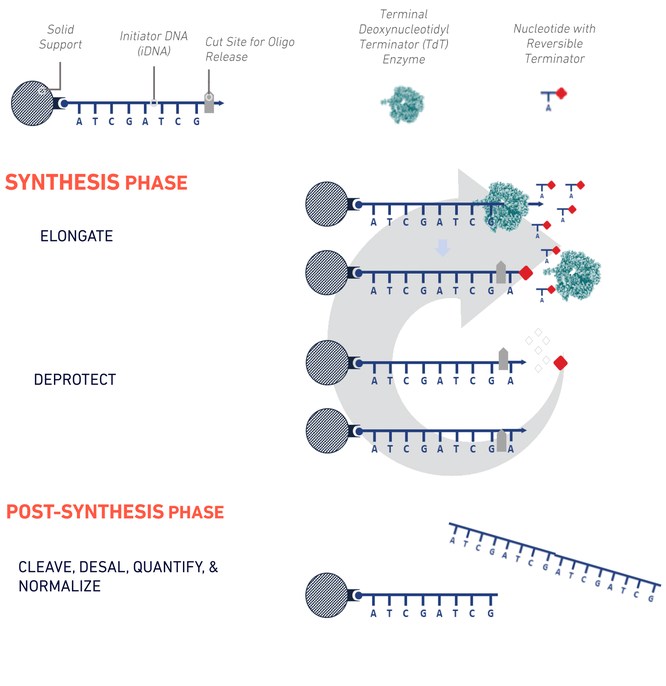
Overview of EDS Oligo Synthesis Process



INTRODUCTION

- **Gene assembly** is a central application to a wide range of scientific disciplines from vaccine development to synthetic biology.
- Current commercially available gene assembly methods utilize chemically synthesized gene fragments as a starting point, which must be ordered from a commercial vendor and can be subject to **long delays** and **uncertain research timelines.**
- The SYNTAX system, powered by Enzymatic DNA Synthesis (**EDS**) technology, provides access to **rapid, on-demand** synthesis of oligos within the convenience of a researcher's lab.
- Here we demonstrate the utility of the SYNTAX system to synthesize same-day oligos and **assemble the 1.7kb Influenza A hemagglutinin gene** using the **Polymerase Cycling Assembly (PCA)** approach
- By providing the user with **complete control** over their oligo synthesis pipeline the SYNTAX system **accelerates** the iterative design-build-test cycle and supports the assembly of 2kb genes in as little as 3 days.

Overview of Polymerase Cycling Assembly

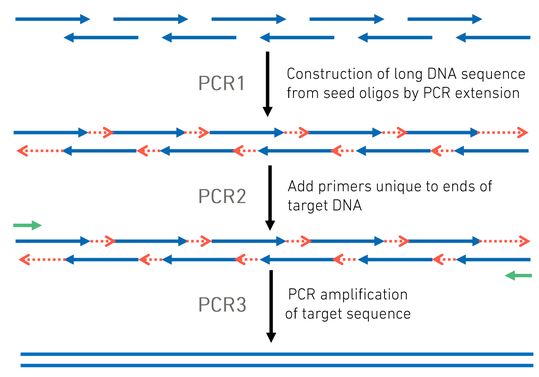


Figure 1. Polymerase Cycling Assembly (PCA) principle. The 3-step PCR process was used to assemble 60-nt or 120-nt long ssDNA oligos printed on SYNTAX 200 system into 450-650 bp dsDNA blocks for subsequent assembly into the Influenza A hemagglutinin gene (HA).

Experimental Overview

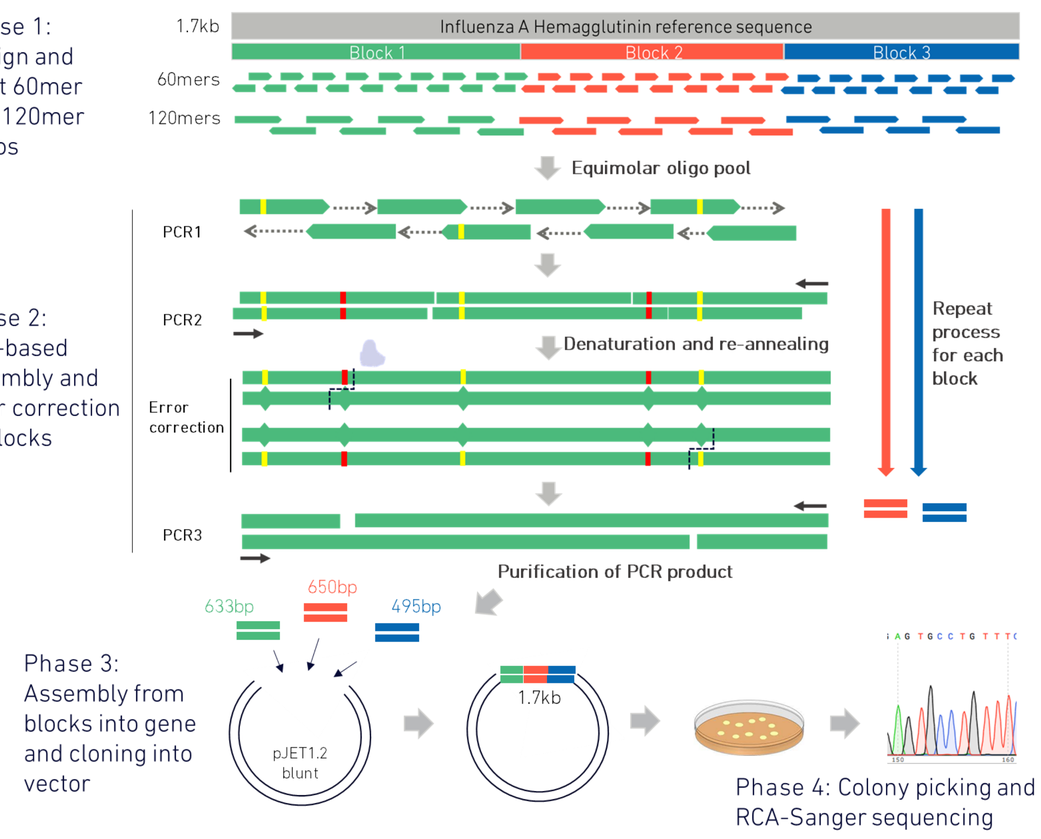


Figure 2. Hemagglutinin gene assembly experimental overview. The 1.7kb hemagglutinin (HA) gene sequence was split into 3 blocks (green, orange and blue) using NEBuilder Assembly tool. DNAWorks v3.2.4 was used to design 48 60mers or 22 120mers oligos as starting material for PCA assembly into blocks. Oligos were printed on our SYNTAX system. Each dsDNA block was then generated by PCA assembly in 3 PCR steps. Errors resulting from oligo synthesis and from polymerase extension are depicted in yellow and red respectively (for illustrative purposes only). PCR2 products were denatured and re-annealed to create mismatches at error sites. Mismatches were then identified and excised using the CorrectASE enzyme (Thermo Fisher Scientific) in an error correction step prior to PCR3 reassembly of blocks, increasing the likelihood of obtaining error-free clones. Blocks were then purified and assembled into the HA gene and cloned into the pJET1.2 blunt vector using NEB HiFi DNA Assembly kit .

- Same-day **60-mers and 120-mers** ssDNA oligos were printed on our **SYNTAX 200 System**
- Oligos were assembled into **3 dsDNA fragments using the PCA** assembly approach
- **Error correction** was performed using blocks to increase proportion of error-free clones
- Blocks were assembled into the full **1.7kb hemagglutinin gene** and cloned into vector for bacteria transformation and Sanger sequencing confirmation

From ssDNA oligos to error-corrected dsDNA blocks

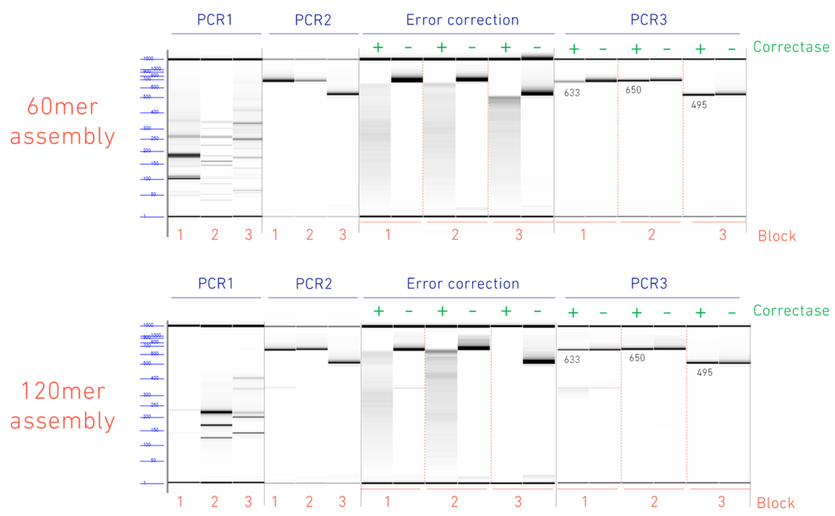


Figure 3. Reaction products generated during different stages of PCA assembly of oligos into blocks for hemagglutinin (HA) gene assembly, starting from 60 or 120-nt ssDNA oligos printed on SYNTAX**.** The dominant bands in PCR2 and PCR3 products correspond to the 3 full-length block constructs (633, 655 and 495bp) building the HA gene. Lower molecular weight bands in PCR1 products correspond to incomplete constructs/un-extended oligos. Error correction was performed after PCR2, resulting in a smear for correctASE-treated samples. Reaction products were analyzed by capillary electrophoresis using a 5300 Fragment Analyzer System and the HS Small Fragment kit (Agilent Technologies).

Influenza A Hemagglutinin gene assembly

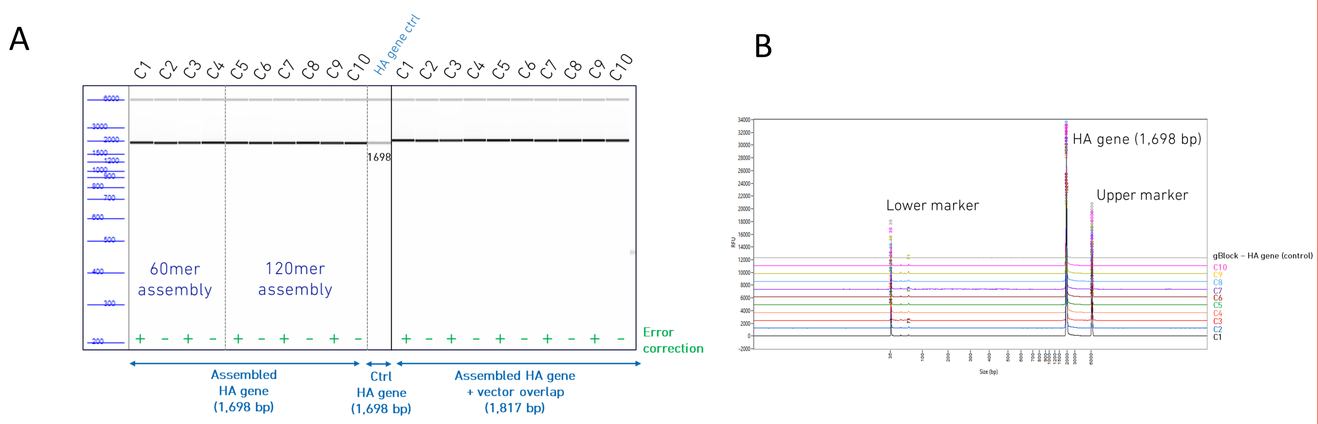


Figure 4. Hemagglutinin (HA) gene assembly from dsDNA blocks generated by PCA. The NEB HiFi DNA Assembly kit was used to assemble blocks generated by PCA (Figure 3) starting from 60mers or 120mers oligos synthesized on SYNTAX. HA gene was subcloned into pJET1.2-blunt vector for bacteria transformation. Over 40 bacteria colonies were obtained for each condition. 10 clones (labelled C1-C10) were randomly selected, miniprep cultures were grown and plasmid DNA was isolated. To verify correct product, Assembled HA gene was PCR-amplified using primers complementary to HA gene extremities and on pJET vector. Products were analyzed by capillary electrophoresis using a 5300 Fragment Analyzer System (Agilent Technologies) and the HS NGS Fragment kit. **A:** Gel product of assembled HA gene in clones C1-C10 compared to synthetic HA full gene ordered from a commercial vendor. **B:** Corresponding electropherograms showing absence of secondary non-specific assembly product.

- Successful assembly of 1.7kb Influenza A hemagglutinin (HA) gene starting from either 60 or 120nt long oligos, with equivalently high efficiency.
- Combined with error correction and low starting oligo error rates, this PCA-based protocol is highly efficient, with **100% of picked clones containing the HA gene fragment of the correct size** and an absence of non-specific assembled product (to be confirmed by sequencing).
- Assembled HA gene product length matches the size of a commercially-synthesized gBlock of the same 1,698bp sequence (Figure 4).

CONCLUSIONS

- Here we demonstrate how the SYNTAX system, powered by EDS technology, supports **efficient and rapid PCA-based assembly of 2kb genes**, taking Influenza A Hemagglutinin gene as a model.
- In a straightforward protocol combining the **simplicity** and **speed** of the **SYNTAX system** to commercially available kits and tools, full gene constructs can be rapidly **assembled** from ssDNA oligos within a **3 days**.
- **120mers** can be synthesized in as little as one day on our SYNTAX system and greatly reduce the number of oligos required to build genes, simplifying the gene assembly process and accelerating the design-build-test cycles.
- Combining the low starting error rate of EDS oligos printed on SYNTAX with the error correction step, this protocol allows **high success rates and fewer clones to be picked for Sanger sequencing confirmation.**

- Fully automated, walk-away synthesis
- Plug-and-play integration
- Flexibility for up to 96 oligos per run
- 15-minute setup time per run
- Same-day synthesis of 15 - 80 nt oligos
- Synthesize oligos overnight for next-day use
- Custom iDNA length: 1 - 45 nt
- Default 5'-phosphate