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GP-write

# Pilot Project Proposal

Prepared for GP-write

**Name of Project:**

Isothermal Amplification Array (IA Array)

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**Background:**

DNA synthesis is still limited by traditional phosphoramidite chemistry, with base-by-base addition of nucleotides as the only real option for a synthetic gene sequence. While the price has dropped marginally to ~$0.10 per bp, simply refining the process will not bring about the exponential reduction in cost necessary to write an entire genome. This means alternative avenues must be explored to make long synthetic sequences a reality.

The proposal exploits isothermal amplification of short, 8-nucleotide oligos, produced by a mixture of polymerase, nicking endonuclease (nickase), and dNTPs, with template molecules immobilized on an array. After amplification, oligos are harvested and fed into a reaction step-wise, using ligase to extend a product to useable length.

**Technical Idea:**

*(Please see Figure 1 for a diagram)*

First, a short "foundation" dsDNA sequence is synthesized in bulk, matching the recognition site of the nickase. The foundation is spotted onto an array (~65,000 spots) and immobilized covalently. Each permutation of 8-mer is further attached through the techniques used for DNA microarray production. This leaves a 3'-5' template that can be amplified by a polymerase.

Once the array has been created, the enzyme mixture can be pipetted onto a given spot, allowing the reaction to proceed: polymerase will attach to the underhang and synthesize the 8-mer, and the nickase subsequently cleaves it off, allowing the polymerase to re-enter and start the cycle anew. Once sufficient 8-mer is generated, the droplet is harvested, the enzymes inactivated, and the oligo is added to the extension reaction.

Extension relies on the ability of ligase to recognize as little as 4bp of overlap and ligate the oligos together. The reaction extends like a ladder as subsequent 8-mers are fed in and incorporated into the growing strand. This has already been shown in principle (see PMC2994885).

A number of different enzymes are available for each step of the reaction:

- Nickases that leave no excess nucleotides on the 8-mer
- Polymerases with strong strand displacement activity and processivity
- Ligases able to operate efficiently with a minimum base-pair overlap

Using this technique, it should be possible to generate dsDNA fragments of reasonable length, ready to be used in downstream reactions. It relies on the well-established activity of only a few enzymes, uses no hazardous chemicals, and is error-correcting *in situ* as incorrect 8-mers are less likely to be incorporated during the ligation reaction.

**Utility:**

At today's prices, a fully synthetic human genome would cost hundreds of millions of dollars. Any reasonable approach to reduce this number merits investigation. In combination with Recombinase-Mediated Assembly *(see other proposal)*, this technique could allow for extremely long sequences to be generated in an automated fashion.

**Figure 1**