GINKGO BIOWORKS

Gene Synthesis from Oligo Pools on the Ginkgo's Reconfigurable Automation Cart (RAC) Platform

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96 genes	0	> 8 hours
Synthesized with high fidelity	runtime errors	Of walkaway time gained

Gene Synthesis from Oligo Pools on the Ginkgo's Reconfigurable Automation Cart (RAC) Platform

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INTRODUCTION

Gene synthesis - the assembly of short DNA oligonucleotides into full, gene-sized fragments (up to ~3 kb) - is a key technique that enables rapid prototyping of *in silico*-designed, custom DNA constructs. It bypasses the reliance on existing DNA templates and typically requires less time than classical molecular cloning. Researchers use gene synthesis to evaluate DNA sequence variants, tune protein expression or introduce cis-regulatory elements with precision, supporting applications ranging from therapeutic development to metabolic engineering[1-3].

This technical note describes a gene synthesis workflow on Ginkgo's Reconfigurable Automation Cart (RAC) platform, supporting Ginkgo's downstream high-throughput cell-free protein synthesis screening pipeline.

The fully automated gene synthesis workflow is cheap and scalable.
We use highly multiplexed DNA oligo pools as the starting material[4] and the polymerase chain reaction (PCR) to "fish out" target DNA oligos that are required to make particular gene-length fragments. Through a process analogous to Golden Gate assembly[5], we then assemble the gene-length fragments, clone them and verify their DNA sequence.

AUTOMATED BIOLOGICAL WORKFLOW

The automated workflow consisted of the following main protocol steps:

1. **Multiplexed PCR**: PCR reactions were set up using the 384-well format Bravo liquid handling RAC, and DNA primers specific to pre-defined sub-pools. The Automated Thermal Cycling (ATC) RAC was used to perform thermal cycling.

   When launching the multiplexed PCR protocol, **Ginkgo's Catalyst software enabled easy customization of thermal cycling profiles, necessary for ensuring uniform representation of all required DNA oligos in the PCR reactions. Real-time temperature profiles were viewable via the ATC RAC web app and were saved in the Ginkgo Catalyst time-series database, for retrospective review** (see **Figure 1**).

2. **Magnetic bead-based PCR amplicon cleanup:** To purify the output PCR

amplicons before the next processing step, magnetic bead-based cleanup was performed on the 384-well format Bravo liquid handling RAC as well. This protocol step can be repeated following any of the downstream enzymatic reactions, to purify DNA outputs as desired. Since magnetic bead-based cleanup is broadly applicable to different input sample types (linear, circular DNA / RNA and proteins), **we leveraged Ginkgo's Catalyst software ability to parametrize key protocol inputs (number of wash cycles, elution volume etc.), to ultimately build a protocol that can be easily reused in the future.**

3. **Enzymatic treatments:** Additional enzymatic treatments were performed to increase the likelihood of obtaining the desired full length DNA sequences downstream. Similarly to the multiplexed PCR protocol, the Bravo and ATC RACs were used, with Ginkgo's Catalyst software enabling the thermal cycling profile customization.

4. **DNA digestion and ligation reactions**: Same logic was followed when performing the downstream multi-piece DNA assemblies into longer gene-length fragments.

5. **Gene-length PCR:** "Finishing" PCR reactions were used to amplify the desired full length DNA products.

Figure 1: Top: Ginkgo Catalyst ATC RAC web app, with a live display of thermal cycling lid and block temperatures; **Bottom:** Example thermal cycling temperature profile captured in the Gingko Catalyst time-series database.

RESULTS

96 gene-sized fragments of unique length and sequence were successfully synthesized, using the above protocol steps (see **Figure 2**), as shown by the offline capillary electrophoresis QC results (see **Figure 3 and 4**). **No contaminating DNA byproducts were observed, proving high-fidelity of our gene synthesis workflow.**

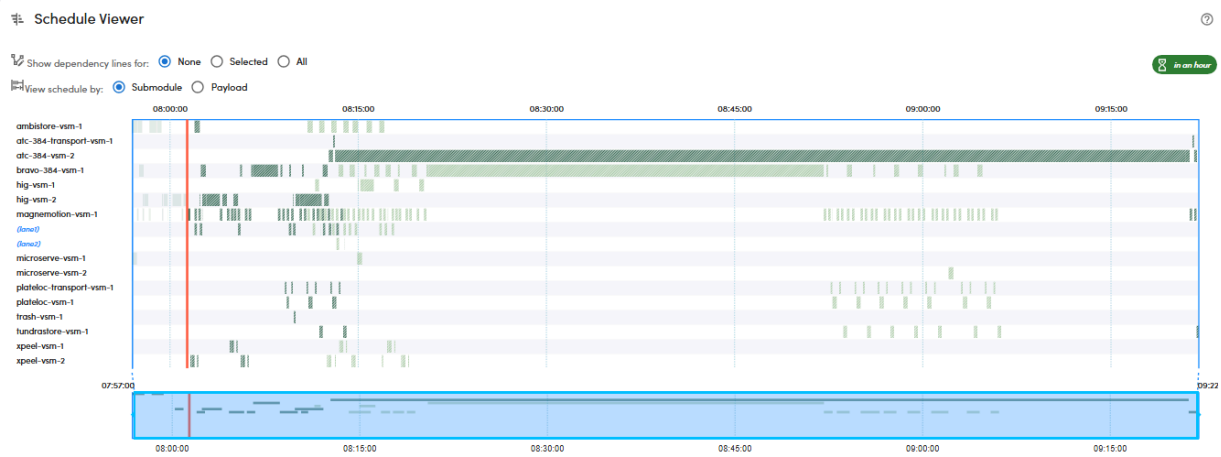

Figure 2: Example Ginkgo Catalyst broker view of the schedule for an interleaved PCR reaction setup and thermal cycling protocol with the magnetic bead-based cleanup protocol.

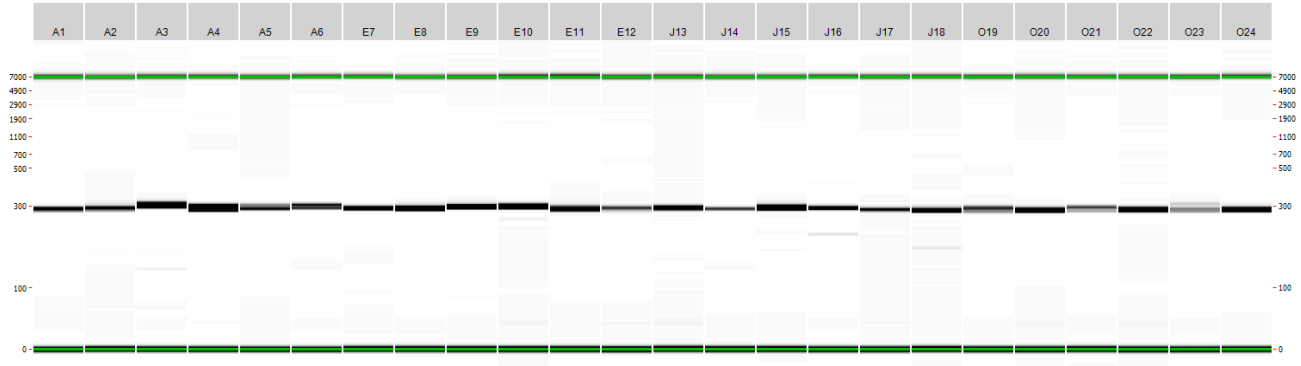

Figure 3: An in-process quality control electropherogram showing PCR results of 6-plexed 300-mer oligo pools. These reactions, which contain only the 6 oligos needed to assemble gene-length fragments, have been de-multiplexed from a pool of thousands of oligos.

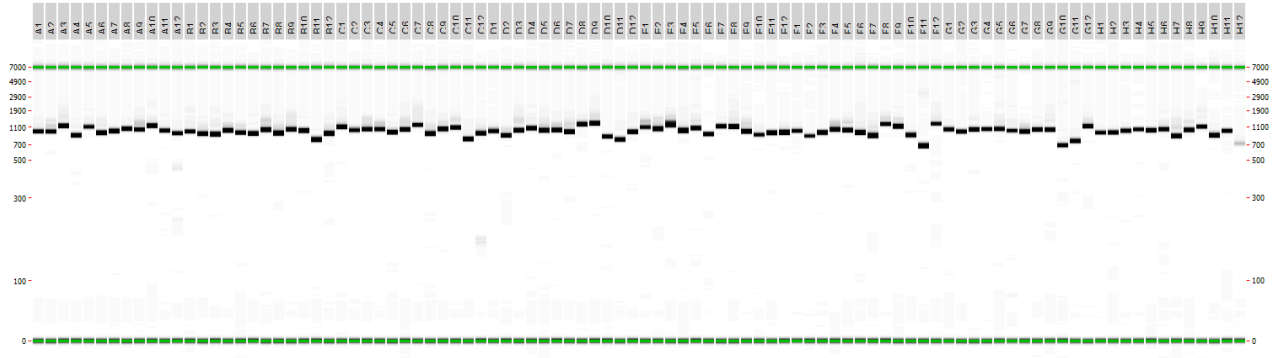

Figure 4: The final quality control electropherogram showing 96 samples (from one 384-well plate quadrant) of assembled gene-length fragments made on Ginkgo's RAC system. No contaminating DNA fragments were observed, indicating high fidelity DNA assembly across a range of lengths and sequences.

CONCLUSIONS

This technical note demonstrates how the RAC platform can be successfully leveraged to execute complex workflows, here gene synthesis, yielding high-quality results.

Complex, multi-step workflows can be easily set up via the protocol chaining functionality of Ginkgo's Catalyst software, and key individual protocols, here magnetic bead-based cleanup, can be easily reused, thanks to the built-in parametrization features. Multiple, different protocol runs can be interleaved to increase throughput, while still ensuring high-quality results and end-to-end traceability via key metadata capture (e.g. thermal cycling profile metadata).

In the future, further walkaway time gains can be achieved via a simple capillary electrophoresis RAC addition, and further conditional chaining, based on upstream workflow QC results, with downstream workflows, using newly built gene-sized fragments.

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