mosquito® HTS
arktic® application note

automated high-density sample storage and miniaturised liquid handling for high-throughput synthetic biology construct assembly

introduction

Synthetic biology opens many new doors for researchers. Core facilities and other dedicated synthetic biology labs now permit researchers to design large numbers of synthetic construct combinations for testing and have them made to order quicker, more easily, and to a higher standard than doing it themselves. The challenge for synthetic biology labs now is to scale up their workflows to meet the growing demand for high-throughput construct assembly and validation, whilst maintaining high quality levels.

Increasing throughput of the standard synthetic biology workflow presents a major challenge. Each constituent DNA part must be individually stored but remain accessible on demand. Core facilities typically house hundreds of thousands of components and completed constructs, storing, tracking and retrieving each one individually as required. Repeated cycles of freeze/thaw reduce sample integrity over time. The number of man-hours required for manually retrieving components, setting up assembly reactions and validating constructs is immense. Assembly time and cost is one of the main bottlenecks in synthetic biology, limiting the number of constructs it is feasible to generate per project.

automated sample storage and miniaturised liquid handling for scalable construct assembly

High-density, automated storage and retrieval of individual 2D-barcoded samples allows quick and easy access to DNA components. Automation of liquid handling enables miniaturised construct assembly to nanolitre scale. In this way, sample and reagent volumes can be reduced, and speed, accuracy and reproducibility can all be improved from manual methods.

This application note describes an automated, high-throughput workflow that has been integrated for the generation of synthetic DNA constructs at GeneMill, University of Liverpool – led by Dr James Johnson.

TTP Labtech's arktic® is an automated, high-density -80°C sample storage and retrieval system. Using 2D-barcoded tubes, arktic permits individual sample tracking and annotation in LIMS. High-speed selection and retrieval of individual tubes eliminates bulk sample freeze/thawing and allows automated cherry picking of custom tube selections into a 96-tube format straight from storage.

TTP Labtech's range of mosquito® liquid handlers (the single channel X1 and the multi-channel HTS) use highly accurate, positive-displacement pipetting to prepare miniaturised reactions with high accuracy and no cross contamination. By combining arktic automated sample management with mosquito X1 and HTS low-volume liquid handling throughout, it has been possible to streamline and accelerate GeneMill's existing manual workflow. This has expanded the synthesis capabilities of this core facility, improving access to validated tools for synthetic biology researchers.

key benefits

arktic:

- **high-density storage, small footprint - 140,000 tubes in 1.1 m²**
- **eliminate unnecessary sample freeze/thaw**
- **retrieve 96 single tubes from -80°C storage in as little as 10 minutes**

mosquito:

- **pipetting accurately at the microlitre to nanolitre scale**
- **dead volume less than 1 µl**
- **use tube racks or any plate as source**
- **increase throughput and reproducibility**
- **reduce cost through miniaturisation of reagent volumes**

case study: high-density synthetic biology component management and miniaturised construct assembly at GeneMill

The GeneMill Foundry, located at the University of Liverpool, was launched in February 2016 as an open-access academic facility providing high-throughput generation and testing of synthetic DNA constructs. It follows the, now standard, synthetic biology remit to Design-Build-Test-Learn, for synthesis and characterisation of small to large gene constructs or pathways for academic and industrial applications. The facility has a growing repository of functional synthetic biology components (promoters, ribosome-binding sites, tags, terminators etc.) and has successfully executed 65 projects in its first year.

ttplabtech

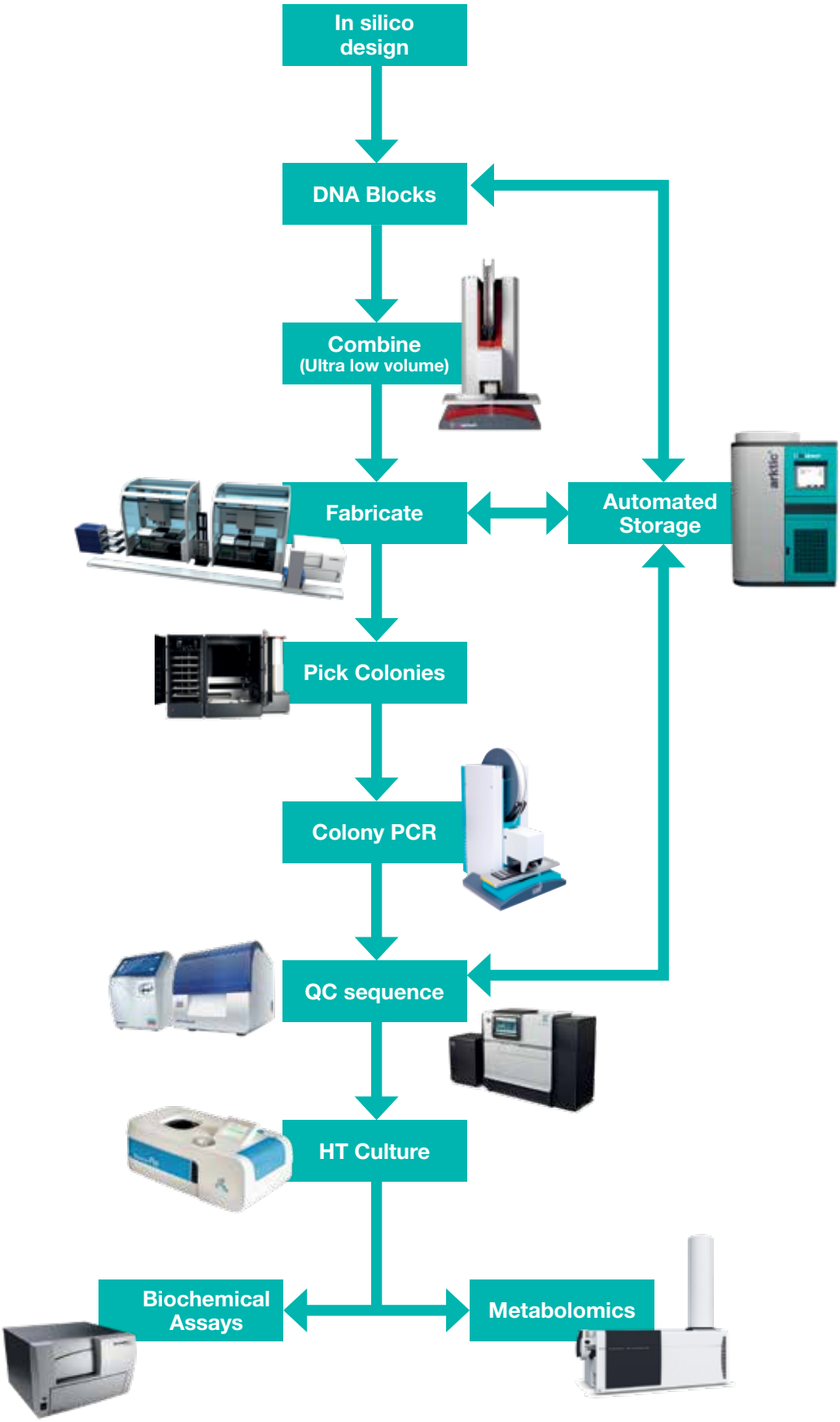

Fig 1. GeneMill workflow overview. Adapted from Johnson *et al*. 2015. DNA parts are synthesised or retrieved from arktic automated storage and then combined at the nanolitre scale with mosquito automated liquid handling (X1 and HTS). DNA assembly reactions and transformations are performed, colonies picked and positive clones are taken forward for sequencing. Constructs are then assayed for function by small-scale culture or high throughput (HT) micro-fermentation before being passed on for biochemical assays or metabolite analysis.

workflow overview

- DNA constructs are designed *in silico*;
- DNA parts are synthesised (GeneArt or Twist Bioscience) and stored in the arktic in 0.5 µL – 200 µL 2D-barcoded tubes (Micronic);
- Automated DNA part selection from arktic with custom pick lists; DNA parts are retrieved in 96-vial racks (Fig. 1);
- With the single-channel mosquito X1, DNA parts (up to 6 per well), water and assembly master mix are combined at the nanolitre scale, directly from the 96-vial racks. Multiple construct combinations are arrayed on the same 96- or 384-well plate. 96 reactions are assembled in 2 hours, each with a total volume of only 3 µL (as low as 1 µL total volume possible [Fig. 2]);
- Constructs are assembled using either NEBuilder HiFi DNA assembly (isothermal reaction) or Golden Gate assembly (PCR thermal cycling):
  - NEB: 2x master mix is combined with 50 nL vector (200 ng/µL stock) and 5 fmol of remaining construct components in a final volume of 3 µL
  - Golden Gate: 2x master mix is combined with 50 nL vector (200 ng/µL stock) and 5 fmol of remaining construct components in a final volume of 3 µL
- Automated transformation and plating of bacteria;
- Automated colony picking for culture and PCR-based QC using a K Biosystems K6-2 colony picker;
- Colony PCR procedure:
  - 384-well plates are prepared with PCR master mix (multi-channel mosquito HTS)
  - Colonies are picked and added directly from culture plates
  - PCRs are run in 5 µL final volume
  - 1 µL of reaction is then transferred from each PCR into a 96-well plate for analysis (mosquito HTS)
  - Automated electrophoresis and analysis on Qiagen QIAxcel;
- Positive clones identified from colony PCR are cultured overnight in deep-well plates;
- Purified DNA is sequence-verified by Sanger sequencing or long-read NGS;
- Verified constructs are passed to phenotypic analysis pipelines.

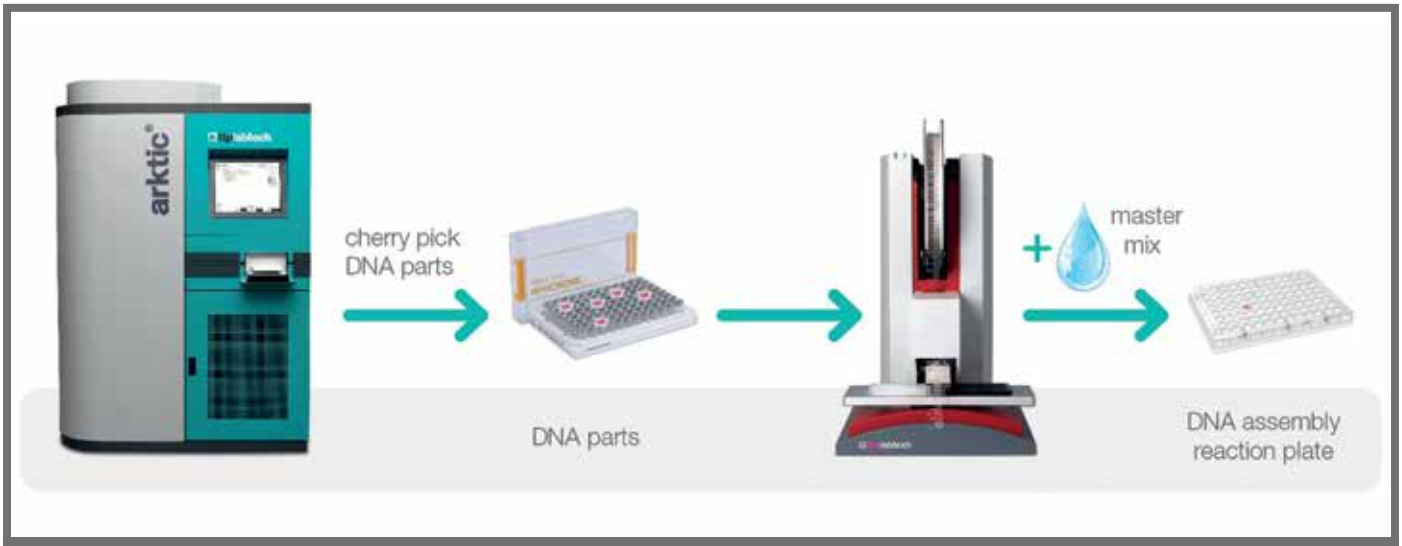

Fig 2. DNA part selection and set up of construct assembly reactions. Example shows how mosquito X1 combines several DNA parts and master mix into a single well (repeated across whole plate in practice).

results

Gibson or Golden Gate assembly reactions are equally compatible in this workflow using the mosquito X1 liquid handler. Reaction volumes are comparable for both methods. Typical reaction costs for NEBuilder are £0.97 compared to £0.43 for Golden Gate (4-fragment assembly incl. tips, plate and enzymes).

Typically, three colonies per construct are selected for QC. Of these, two or three are correct by colony PCR, and sequence verification is normally 100% thereafter.

conclusion

The work at the GeneMill facility demonstrates high-throughput, nanolitre-scale construct assembly, screening and validation and selection in a scalable, automated workflow. The process is significantly facilitated by arktic automated sample storage and retrieval, and automated liquid handling with the mosquito instruments. This workflow has improved productivity and standardisation at the GeneMill facility with reduced turnaround time and costs, increasing the number of designs possible per project.

reference

Johnson, James R., Rosalinda D'Amore, Simon C. Thain, Thomas Craig, Hannah V. McCue, Christiane Hertz-Fowler, Neil Hall, and Anthony JW Hall. "GeneMill: A 21st century platform for innovation." *Biochemical Society Transactions* 44, no. 3 (2016): 681-683.

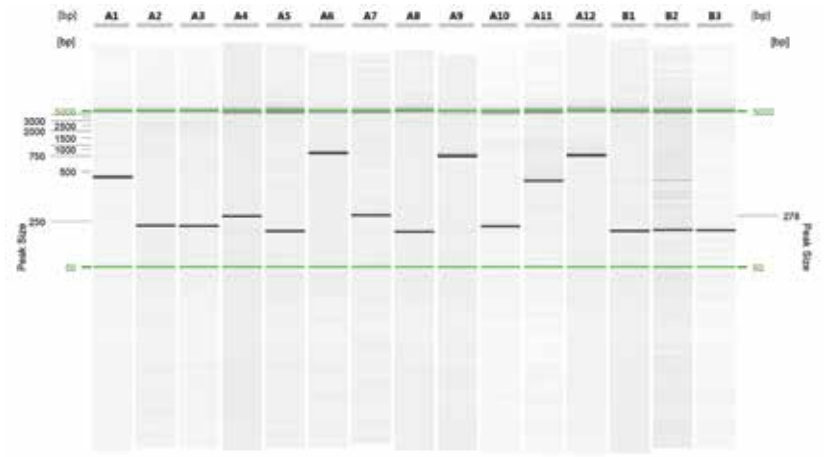

Fig 3. Automated electrophoresis (QIAxcel) confirming identity of constructs produced from low volume assembly reactions.

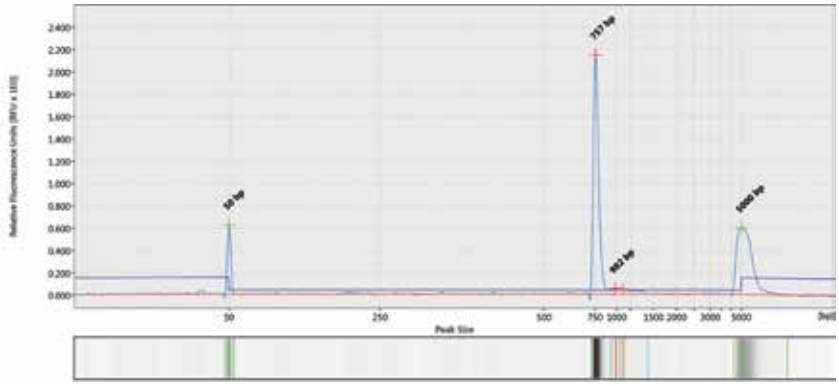

Fig 4. Electropherogram of lane A9 from Figure 3. cPCR product analysed using QIAxcel (Qiagen) screening electrophoresis cartridge. Graph shows intensity of signal from PCR product on the Y axis vs. calculated length on the x-axis.

designed for discovery

discover@ttplabtech.com
www.ttplabtech.com