Ancient Genomics: Semi-Automated High-Throughput Workflow for the Preparation of NGS Libraries

Authors: Franziska Hausig-Punke[1], Anne Koch[1], Katharina Haft[1], Sajith Dass Tulasidass[1], and Charleen Gaunitz[2]

[1]Analytik Jena GmbH+Co. KG, Konrad-Zuse-Str. 1, 07745 Jena, Germany
[2] University of Copenhagen, The Globe Institute, Øster Voldage 5-7, 1350 København, Denmark

ABSTRACT

A high-throughput Next Generation Sequencing (NGS) pipeline was established at the GeoGenetics Center of the Lundbeck Foundation and 5,000 ancient genomes were analyzed to investigate the evolutionary history of neurological and mental diseases over the last 10,000 years. In the pipeline, Analytik Jena's CyBio FeliX liquid handling platform automates bead-based purification, sequencing library pooling and real-time PCR setup, increasing efficiency, reproducibility, and throughput. Thanks to the flexibility of the platform, the challenges of quantity and quality of ancient DNA were effectively overcome. The results show a comparable quality between automated and manual steps and emphasize the reliability and benefits of automation in large genome projects. The automated workflow not only streamlines laboratory processes but also contributes to the long-term well-being of employees by minimizing repetitive tasks, highlighting the potential benefits of automation in genomics research.

INTRODUCTION

To study the evolution of neurological and mental diseases over the past 10,000 years, the Lundbeck Foundation GeoGenetics Centre set up a semi-automated pipeline to prepare libraries for Next Generation Sequencing (NGS). Ancient DNA (aDNA) extracted from old material (bones and teeth) served as the starting material for the preparation of the libraries. The CyBio FeliX liquid handler from Analytik Jena automates bead-based purification steps (4), dilutes and pools the libraries (6) and prepares the quantification by quantitative (q)PCR (Figure 1). The CyBio FeliX liquid handler is flexible to enable custom configurations required for each step, assuring reproducibility and improving user convenience by reducing workload.

METHODS

Bead Purification of NGS libraries - Step 1: Set up the robot with all required materials (Figure 2). Step 2: Mixing of DNA fragments and beads. Step 3: Washing of the beads. Step 4: Drying of the beads. Step 5: Elution of DNA fragments. Step 6: Final transfer of the eluted DNA fragments.
Pooling of NGS libraries - Step 1: Import csv file; Step 2: Preparation of dilutions. Step 3: Pooling of samples.
Setup of Real-time qPCR - Step 1: Pre-pipetting of the dilution buffer. Step 2: First dilution of the samples. Step 3: Transfer of the qPCR master mix. Step 4: Second dilution of the samples and addition to prepared master mix. Step 5: Transfer of qPCR standards.

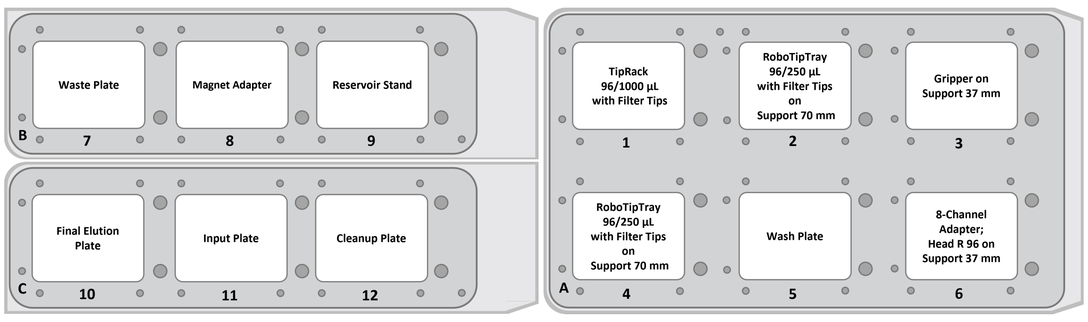

Figure 2 | Deck layout for automated bead purification.

RESULTS & DISCUSSION

Two thirds of the 22,000 libraries were generated *via* the automated workflow with CyBio FeliX, which significantly improved efficiency. Manually, it took 10 people 3 weeks to create about 24-48 libraries. With the automated workflow, only one person was needed to create 400 libraries in the same 3-week period.

Bead purification: Both the quality of the DNA libraries and the preparation time are comparable for automated and manual bead purification (Figure 3). The advantage of automating this step is the reliable reproducibility of the quality of the results. Compared to using the CyBio FeliX CHOICE head, the use of the multi-channel CyBio FeliX Head R 96/1000 µL shortened the processing time by 50% and is therefore optimal for a higher throughput.

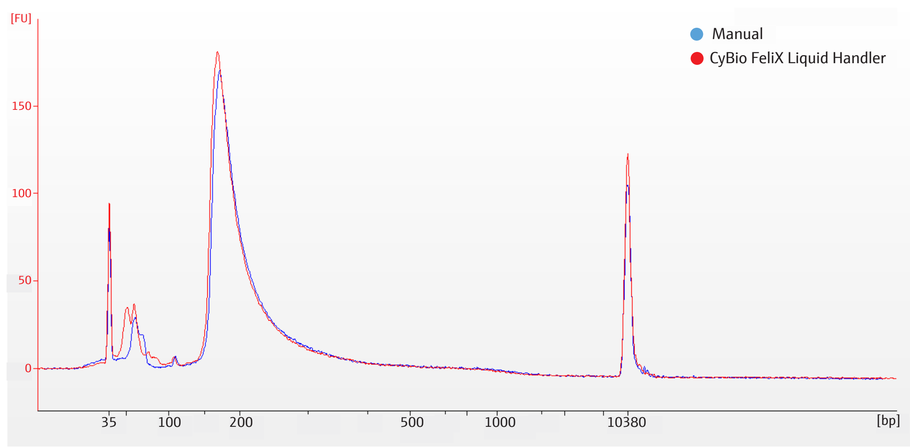

Figure 3 | Library quality control.

Pooling: Manual methods often involve individual dilution and transfer steps, which makes them particularly error-prone. To automate this step, the CyBio FeliX software imports a .csv file based on the quantification of the DNA libraries and generates the desired pooled DNA libraries without errors. Up to 192 individual DNA libraries were pooled for sequencing. Comparison of the sequence data showed that equimolarity was consistently better with the automated setup than with the manual setup (data not shown).
qPCR setup: The crossing point (Cp) deviations between different library preparations are lower when using CyBio FeliX than with manual setup, indicating the higher reproducibility of the automated workflow (Table 1).

Table 1 | Comparison of the reproducibility of the qPCR setups.

Sample		A		B		C		D		E		F		G	
Manual	Cp	13.67	12.97	14.49	13.87	13.92	13.76	13.18	12.56	11.87	11.55	11.80	11.62	13.87	13.40
		13.70	12.93	14.50	13.89	14.04	13.76	13.01	12.54	11.86	11.57	11.89	11.59	13.88	13.42
	X	49.9%	49.9%	50.0%	50.0%	49.8%	50.0%	50.3%	50.0%	50.0%	50.0%	49.8%	50.1%	50.0%	50.0%
	Y	105.7%		104.4%		101.6%		104.3%		102.6%		102.1%		103.5%	
CyBio FeliX	Cp	14.00	13.94	14.67	14.67	14.23	14.09	13.47	13.42	12.54	12.50	12.55	12.52	14.45	14.51
		14.01	13.95	14.65	14.64	14.19	14.06	13.43	13.33	12.46	12.51	12.53	12.44	14.44	14.58
	X	50.0%	50.0%	50.0%	50.1%	50.1%	50.1%	50.1%	50.2%	50.2%	50.0%	50.0%	50.2%	50.0%	49.9%
	Y	100.4%		100.0%		101.0%		100.6%		100.0%		100.5%		99.3%	

CONCLUSION

By automating NGS library preparation including bead-based library purification, qPCR setup and pooling with the CyBio FeliX benchtop liquid handling system, a single user can generate twice the amount of sequencing libraries that 10 users generate with a manual workflow. The quality of the data is better due to higher reproducibility, while the ergonomics improve the well-being of the laboratory staff. Finally, automation also saves costs, which is particularly evident in the improved reproducibility of the qPCR setup in this project.

For more information check out our related AppNote.

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