Automated Clinical Rapid Whole Exome Sequencing with the Covaris R230 Focused-ultrasonicator

- Jayne Simon, Rebecca Callard, and Eugenio Daviso - Covaris, Inc., Woburn, Massachusetts 01801
- Fallon Berkhout, Marcel Nelen, and Tom Hofste - Radboudumc, Nijmegen, The Netherlands
- Birgit Ottenwälder - Hamilton Bonaduz AG, Switzerland

Abstract

DNA fragmentation is a critical step in the preparation of good quality libraries for next-generation sequencing (NGS). Covaris Adaptive Focused Acoustics® (AFA®) technology remains the gold standard in reproducible and robust fragmentation, especially relevant while working with precious clinical samples. Automated workflows are needed more than ever to meet the increasing demand in sample throughput and sequencing capacity, whilst minimizing the risk of human errors. The newest line of Covaris AFA-TUBE consumables allows easy automation integration and the R230 Focused-ultrasonicator can integrate with any liquid handler on-deck or deck-adjacent. Here we present a case study with Radboudumc using our next generation of plate-based Focused-ultrasonicator, the R230, and a streamlined approach to high-throughput NGS library preparation.

Introduction

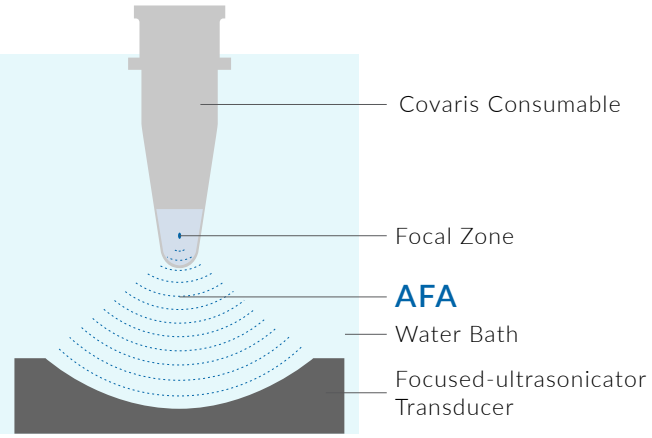
Radboudumc Background

The Genome Diagnostics division of the Human Genetics department is responsible for all clinical genetic testing at the Radboud University Medical Center (Radboudumc). It is one of eight Dutch UMC-based centers for genetic testing and is granted an ISO 15189 accreditation by the Dutch Accreditation Council (RvA). The Radboud Genome Technology Center (RGTC) is a core facility embedded in the Genome Diagnostic division, which positions the RGTC in a unique and highly innovative environment with a strong focus on clinical genetic testing and clinical research. RGTC uses the overcapacity of their systems to provide research groups access to the same standardized high throughput sequencing workflows. A high performance compute cluster is in place to support data analysis and data storage. Late 2010, RGTC was the first laboratory to use whole exome sequencing (WES) in a clinical setting and has since established the new standard in genetic testing with over 45,000 clinical exomes sequenced. RGTC implemented a rapidWES workflow in 2017 to provide NICU patients a quick diagnosis with a turnaround time of five to seven days. As the number of tests increase, the manual workflow is moved towards an automated library preparation using Hamilton Microlab® STAR Liquid Handling Platforms.

R230 at Radboudumc

A major goal of the Genome Diagnostics division at Radboudumc was to ramp up the sample volume in the clinical rapid WES service while maintaining the turnaround time. The integration of mechanical shearing using the R230 on-deck in Radboud's fleet of Hamilton liquid handlers, has enabled high-quality exome data. The average turnaround time from sample receipt to patient report is maintained at five to seven days. Patient DNA samples arrive from a large number of national and international laboratories, often varying in concentration, volume, and buffer composition. Being a core facility, Radboudumc noticed that variation in sample composition, quality, and concentration has a significant impact on enzymatic fragmentation showing more vulnerability to failures. The R230 eliminated concerns around sample quality, by enabling standardized and robust shearing, regardless of sample input. The R230 uses AFA-TUBE TPX Plates, which allows for flexible shearing volumes (5 to 50 µL) with no minimum DNA input required, ideal for processing precious clinical and low input samples. The AFA-TUBE TPX consumables meet ANSI SLAS plate standards and are compatible with most thermal cyclers and heat blocks, allowing libraries to be prepared in one tube, reducing transfer steps, and minimizing sample loss.

An important requirement in Radboud's clinical WES workflow is sample traceability and tracking through their Laboratory Information Management System (LIMS). The R230 records sample and consumable information through RFID scanning and the creation of a LIMS file. This ability to track samples along with the use of automation creates a workflow with reduced hands-on time and lessens the potential for human errors and technical mistakes.



Adaptive Focused Acoustics (AFA) is an advanced acoustic technology which enables the mechanical processing of samples through focused-ultrasonication. AFA employs highly controlled bursts of focused high-frequency acoustic energy to efficiently and reproducibly process samples in a temperature-controlled and non-contact environment.

The Covaris R230 Focused-ultrasonicator is the next generation AFA powered instrument, seamlessly integrating on-deck in most liquid handler platforms such as Hamilton. When coupled with the 96 AFA-TUBE TPX Plate it provides a cost-effective and streamlined workflow for NGS library preparation.

Workflow

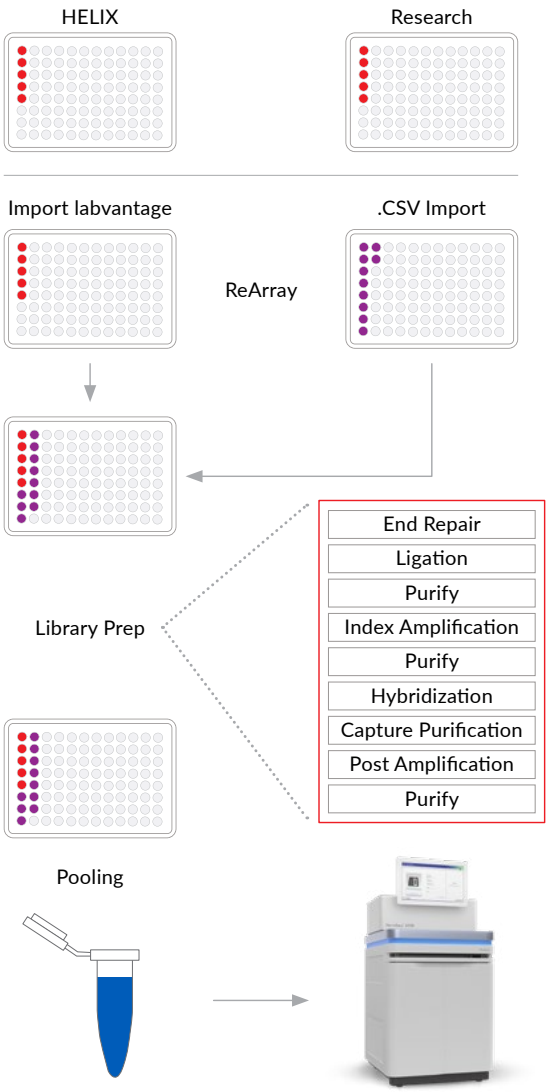


Figure 1. Experimental diagram of the library preparation workflow on the Hamilton STARplus. 32 samples are processed per run, with the ability to increase to 64 samples.

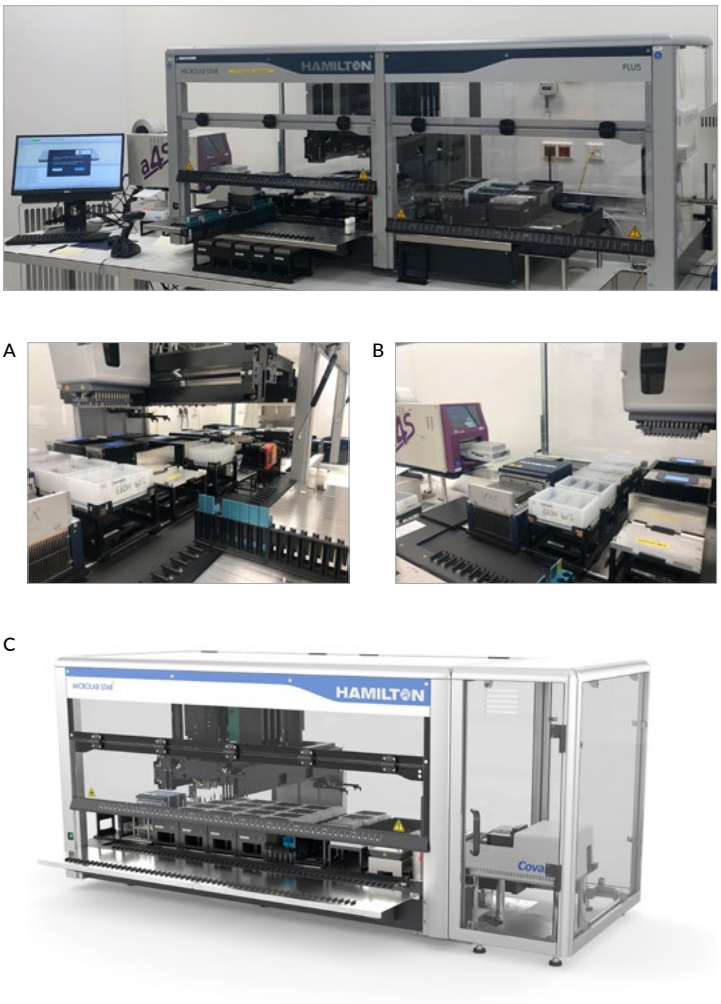


Figure 2. Automated library preparation on the Hamilton STARplus with (A) 96-well pipetting heads, (B) on-deck thermocycler and (C) Covaris R230 Focused-ultrasonicator.

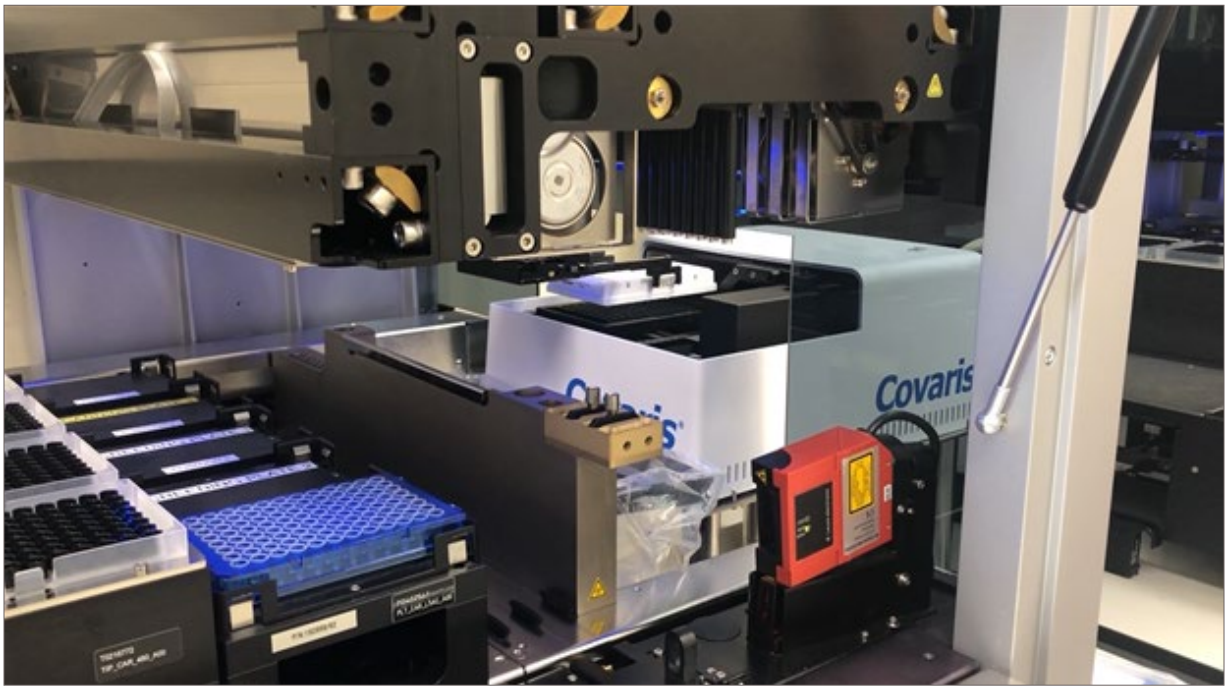


Figure 3. Picture of the R230 Focused-ultrasonicator integrated on deck with the Hamilton STARplus. This R230 is integrated onto the deck of the STARplus used for sample normalization

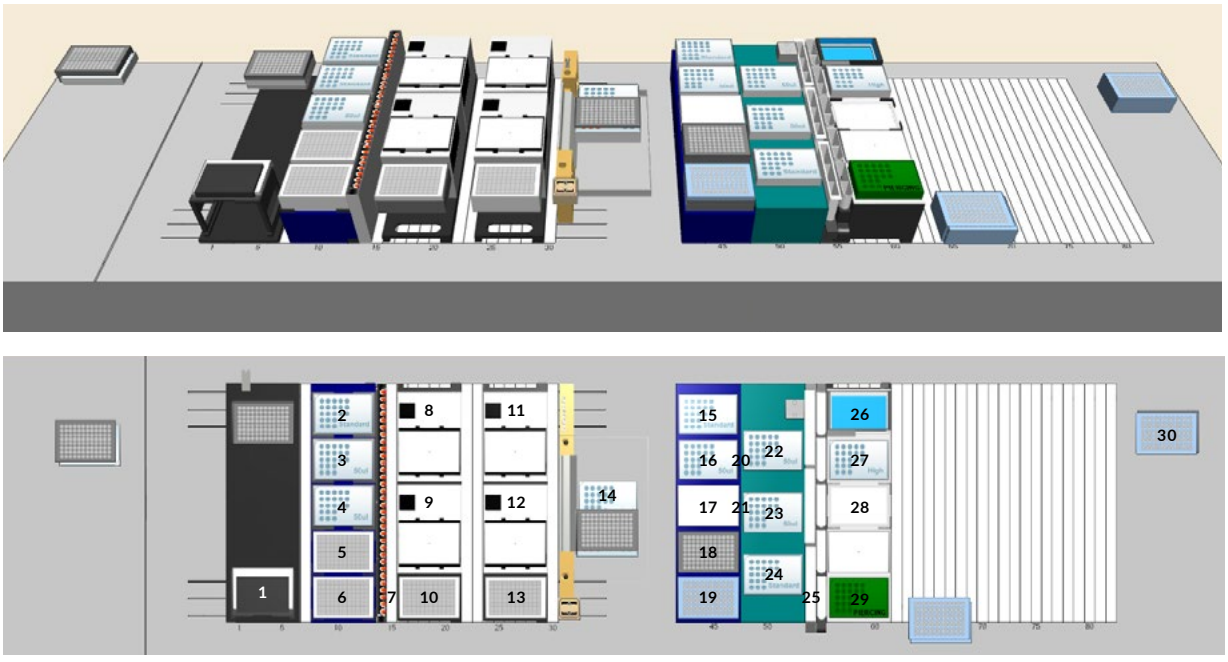


Figure 4. Deck Layout: 1 – ODTC, 2 – Loading position for array plate/Block for 50 µL tips, 3 – Block for 300 µL tips, 4 – Block for 300 µL tips for waste, 5 – 0.1x TE -MQ Elution Buffer/Binding Buffer, 6 – Ethanol preparation position/Wash buffer 1, 7 – Hybridization Mix, 8 – Heating unit for Wash buffer 2, 9– Heating unit for Wash buffer 2 incubation in Deep Well (DW) block, 10 – Vapor, 11 – Cooling block for End Repair -Atailing, Ligation, Universal Primers, Amplification, and Blocker Mix, 12 – Cooling unit for armadillo plates, 13 – Purification Beads, 14 – Waste for tips, armadillo plates, and DW-plates, 15 – NTP 300 µL Tips, 16 – NTR 50 µL Tips, 17 – Home position for work plate, 18 – Magnet Block, 19 – Store position for 2nd plates and downholder, 20 – 100% Ethanol, 21 – MQ, 22 – Tipfeeder NTP 300 µL Tips without filter, 23 – Tipfeeder NTR 50 µL Tips with filter, 24 - Lid of Hamilton for ODTC plates, 25 – Streptavidin Beads, 26 – Liquid Waste dispenser, 27 – NTR 1000 µL Tips with filter, 28 – Piercing position, 29 – piercing tips, 30 – Cytomat.

Materials and Methods

- Covaris R230 Focused-ultrasonicator (PN 500620)
- 96 AFA-TUBE TPX Plate (PN 520291)
- R230 Rack 96 AFA-TUBE TPX Plate (PN 500668)
- Samples: blood, saliva, amnion, chorion, cell line, and FFPE extracted via ChemagicSTAR with Chemagen extraction kits
- Agilent 4200 TapeStation (PN G2991AA)
- Agilent High Sensitivity D1000 ScreenTape (PN 5067-5584)
- Agilent High Sensitivity D1000 Reagents (PN 5067-5585)
- Twist Bioscience Human Core Exome Kit

Base Pair Mode (bp)	350
Sample Volume (µl)	35
Dithering Range, y-axis (mm)	3
Dithering Speed (mm/s)	10
Temperature (°C)	10
Peak Incident Power (W)	280
Duty Factor (%)	25
Cycles per Burst (n)	50
Pulsing (sec)	10
Treatment Time (sec)	100
TAT/Plate (min)	42

Table 1. AFA treatment settings used on the R230 Focused-ultrasonicator for Twist exome library construction.

Results and Discussion

Mechanical fragmentation using the R230 Focused-ultrasonicator produced fragments with a peak at 350 bp across various sample types. Fragment sizing analysis was performed using the Agilent 4200 TapeStation with the High Sensitivity D1000 ScreenTape. Across 64 reactions containing various sample types and quality, genomic DNA was sheared to an average of 369 bp with 4.9% CV, showing robust and reproducible fragmentation.

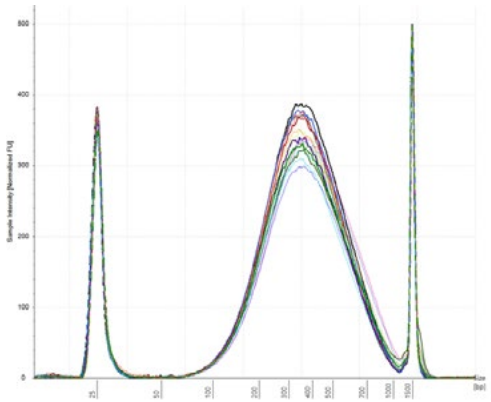


Figure 5. DNA fragment size distribution profiles of 16 overlaid samples using settings listed in Table 1. measured by Agilent TapeStation® showing reproducible fragmentation.

Sequencing metrics from over ten runs (n=339 samples) show high quality libraries produced with the Covaris-fragmented samples. This workflow performs best with a target insert size (measured from the sequencer) of 200 bp. The insert size across the sample cohort achieved an average of 199 bp with 8.1% CV and only 2.9% of samples below the 150 bp insert size cut-off.

For high quality data without bias from library preparation (such as during the strepdavidin bead wash step during hybridization), the GC content should span 40 to 60% of the sequenced basepairs. Average GC content for these sample were at 54% with no samples failing due to GC or AT drop-out. Note, the Twist exome is GC rich, so it is important to have a higher GC content across the panel.

Duplicate reads were around 10%, showing efficient library construction and good clustering on the flow cell. The average sample coverage was above 100x and only 1.8% of DNA samples required rework to increase depth of coverage. In addition, all but one sample met the QC cut-off for 20x coverage (ideal = 99%, cut-off < 95%, failing sample had 94.8%).

The average percent of on-target reads was 73% (ideal range 70 to 75%, < 65% cut-off), which meets the QC mark. Although the percent on-target goal is low, the target panel used for hybridization has a greater content than most other commercially available human exome kits, and therefore more data is generated from a lower amount of on-target reads than other kits with a higher percent on-target rate.

Trends Over Time

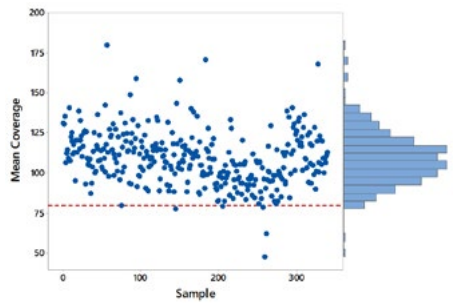


Figure 6. Marginal plot of the Mean Coverage as a function of Sample ID, showing 98.2% of the samples (333/339) with 80X Coverage or more. The average coverage is 109X.

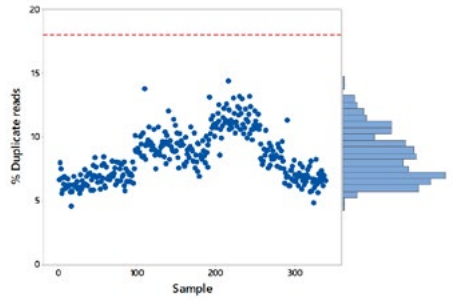


Figure 7. Marginal plot of the % Duplicate reads. All samples are below the QC threshold of 18%.

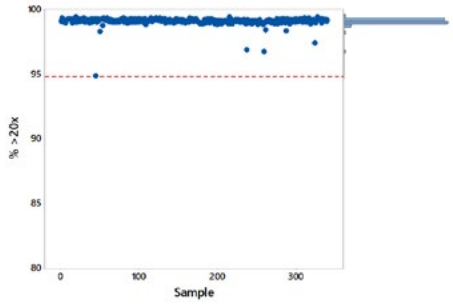


Figure 8. Marginal plot of the % reads with at least 20X coverage. All samples are above the 98.4% QC threshold.

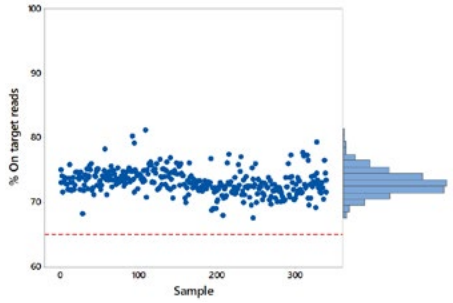


Figure 9. Marginal plot of the % On target reads. All samples have >65% or reads on target.

Conclusions

The Covaris R230 Focused-ultrasonicator combined with a liquid handler, such as the Hamilton STARplus, enables automated library preparation with high-quality sequencing performance as described in this case study. Using this automated library preparation workflow, Radboudumc has been able to expand their clinical rapid WES service through increased sample throughput, whilst maintaining the turnaround time to patient report as five to seven days.

In summary, using mechanical fragmentation in library preparation with the R230:

- Standardizes workflow for multiple sample types, such as blood, saliva, amnion, chorion, cell line, and FFPE.
- Enables processing of samples of varying concentrations and buffer types through the same pipeline (especially relevant if samples are received from external sources with different extraction procedures).
- Eliminates lot-to-lot variability by reducing re-optimization steps that can occur with enzymatic fragmentation and reducing QC steps due to the robustness of mechanical shearing methods (AFA).
- Reduces hands-on time with high-throughput processing in 96-well plates and allows increase of sample throughput with automated pipelines.
- Decreases human error potential with end-to-end sample tracking in an automated workflow.

In conclusion, the R230 features all the benefits of Covaris AFA technology for robust, reproducible and confident fragmentation, and can integrate into most library preparation workflows, whether on-deck with a liquid handling robot, or used as a standalone benchtop instrument.

In collaboration with:

HAMILTON®

USA: Tel: +1 781.932.3959 | Email: customerservice@covaris.com | Europe: Tel: +44 (0)845 872 0100 | Email: emeacustomerservice@covaris.com | APAC: +86 137 6427 6714 | Email: APACcustomerservice.com
Web: www.covaris.com | Applications: applicationsupport@covaris.com | Service and Support: techsupport@covaris.com | M020151_RevC_Mar2021 | 2021© Covaris, Inc.