Automated NGS Library Preparation with Beckman Biomek FX^P and Covaris E220

BIOGEMMA OVERVIEW

Biogemma is a European plant biotechnology company founded in 1997 by seed companies and French field crop producers. Its original mission was to create new genetic variability via GM traits to be exploited by its shareholding companies.

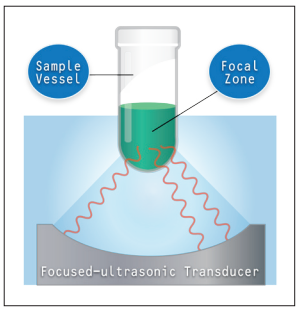
With boom of plant genomics in 1999-2000, Biogemma expanded to include the development of genomics tools for its main target species and the production of informative markers for genes identified in the genomics projects. These marker-trait associations support the marker-assisted breeding programs of their shareholders.

To this aim, Biogemma uses different technologies optimized by in-house R&D teams. The collaboration with technology providers on specific and custom developments is a key of the genomics platform's performance and contributes to better fit its shareholders' needs and to improve the overall competitiveness of the company.

Today, Biogemma has approximately 80 staff across 4 sites. with research programs focused on maize (corn), wheat, oilseed rape (canola) and sunflower.

COVARIS OVERVIEW

Covaris provides tools and technologies to improve pre-analytical sample preparation, enable novel drug formulations, and manage compounds in the drug discovery process. Founded in 1998, Covaris built upon its team's deep knowledge of fields ranging from acoustic physics and mechanical engineering to biophysics and molecular biology with nearly 80 patents granted and pending.



The Covaris technological foundation is based on its proprietary and patented Adaptive Focused Acoustics (AFA™) technology. AFA-energetics™ uses controlled bursts of high-density acoustic energy to enable highly controlled sample processing in a temperature-controlled, non-contact, closed vessel environment. Uniquely, all AFA Focused-ultrasonicators are calibrated to NIST traceable standards, ensuring highest quality and standardized results. This is a criteria to enables the development of robust, industrialized approaches to high-throughput analytical biology.

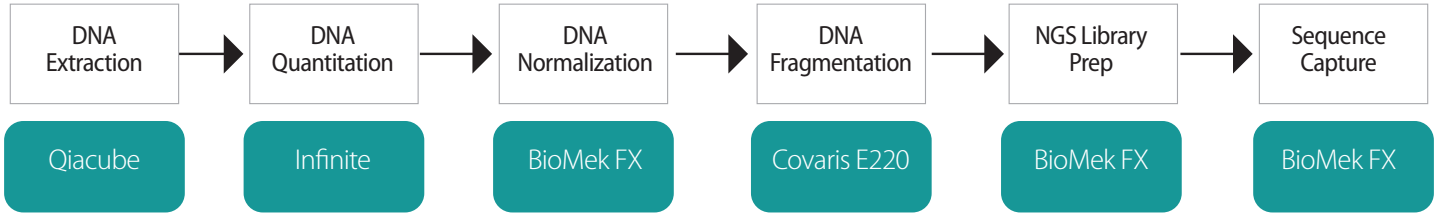
DESCRIPTION OF NGS WORKFLOW AT BIOGEMMA

Since 2009, Biogemma's Genomics team has developed a portfolio of applications exclusively based on Sequence Capture and NGS technologies. The main activity is focused on Genetic markers discovery. Nevertheless, with the improvement of technologies and tools, the Genomics team works also on new applications like Epigenetics, RNAseq and Whole Genome Sequencing.

Sequence capture, based on Roche Nimblegen technology, has been optimized though a collaboration with Roche[1]. The size of the DNA inserts are application- and project-specific and can range from 200 to 800 bp. For Sequence Capture projects, the sequencing effort is well calibrated to obtain the maximum of sequences for targeted regions. Two key parameters for high quality library preparation are the homogeneity of the library insert size and the un-biased fragmentation profile, which is why Biogemma chose to use Covaris AFA technology to fragment the DNA.

Since 2012, there has been a sharp increase in the number of samples, requiring full process automation depending on the type of the project. Therefore, to maintain a high throughput, the Genomics team has established a straightforward, automated workflow from the DNA extraction up to the Sequence Capture. All steps are managed in 96-well plates.

FIGURE 1 - Detailed workflow at Biogemma Genomics Group—A specific robotic method has been developed for transferring the DNA into the Covaris 96 microTUBE Plates and vice versa.



1. Roche NimbleGen and Biogemma Develop Enhanced Sequence Capture Technology for Wheat and Canola, http://454.com/resources-support/news.asp?display=detail&id=146

REQUIRED PARTS

Liquid Handling Robot

Beckman Coulter Biomek FXP Dual Multichannel and Span-8 Workstation – SPRIworks HT (B05450) with Biomek FX/NX Universal Clamping ALP Europe (A88294)

- Tips for or piercing the foil : Span-8 p250 Tips, Non Sterile (379501)
- Tips for transferring the DNA : Biomek AP96 P250 Tips, Sterile with Barrier (717253)

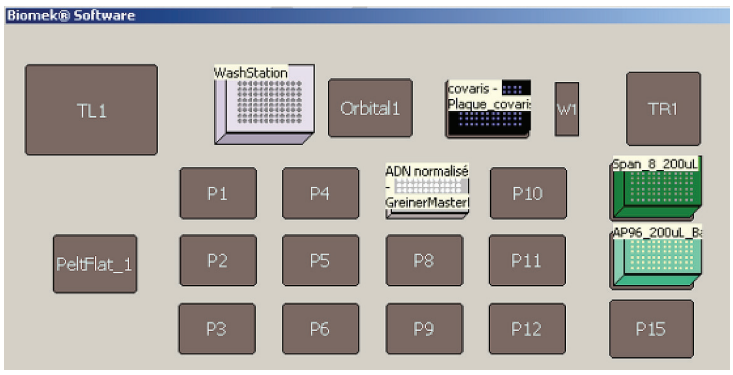
Focused-ultrasonicator for mechanical DNA Shearing

Covaris E220 Focus-ultrasonicator with 96 microTUBE-Plates PN 520078

DETAILED DESCRIPTION

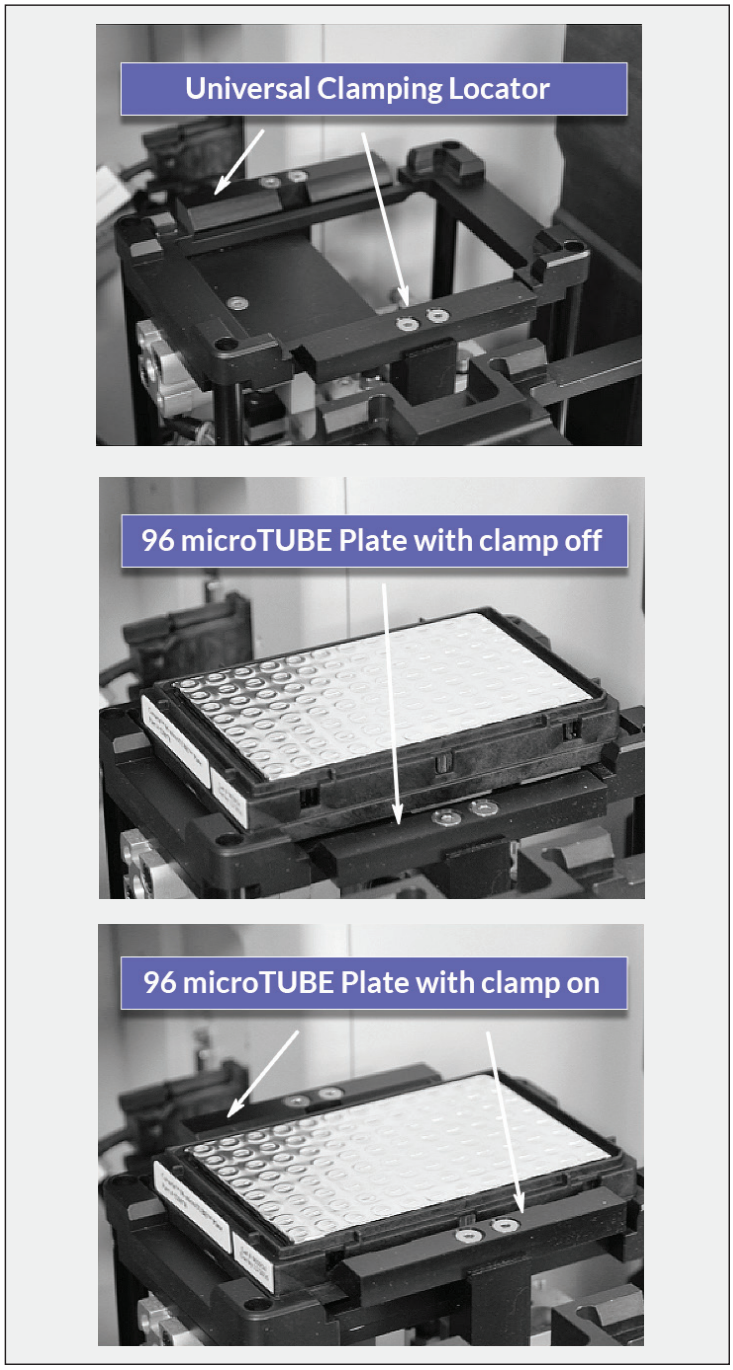
For DNA transfer to and from the Covaris 96 microTUBE plate, Beckman Biomek deck is organized following a simple layout as described below. A 96 microTUBE Plate is placed in the universal active locator position, while a normalized DNA/un-fragmented DNA is placed in a regular position.

FIGURE 2- Biomek deck layout



A universal clamping active locator is directly controlled by Biomek Software and is pneumatically activated. It can hold or release the Covaris 96 microTUBE Plate on the Biomek deck on-demand.

It is possible to perform complete or partial loading of the Covaris 96 microTUBE Plate, which is easily controlled by the Biomek software. Partial loading is used for transferring incomplete plates with less than 96 samples and is also useful to manage partially used Covaris 96 microTUBE Plate. As shown in Figures 3 and 4, the pattern of wells must be similar between the DNA plate and the Covaris plate but the location of the patterns can be different.



RESULTS

Transfer of normalized/ un-fragmented DNA

Transfer steps are completely automated and controlled by Biomek software through a user-friendly interface. 96 samples are transferred in less than 10 min. An exact volume of 130 µl of normalized DNA is transferred into the Covaris 96microTUBE Plate, the volume in the DNA input plate is 150 µl to allow for some excess.

Mechanical DNA Shearing

The mechanical DNA shearing settings and protocols are based on Covaris standard parameters. Slight improvements have been done

Figure 3. Normalized DNA/un-fragmented DNA input plate pattern definition step

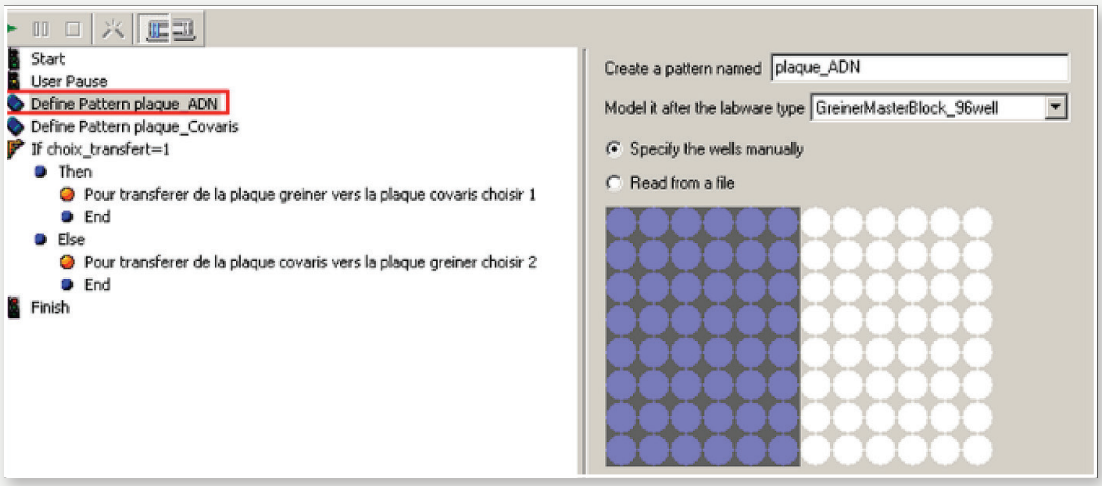
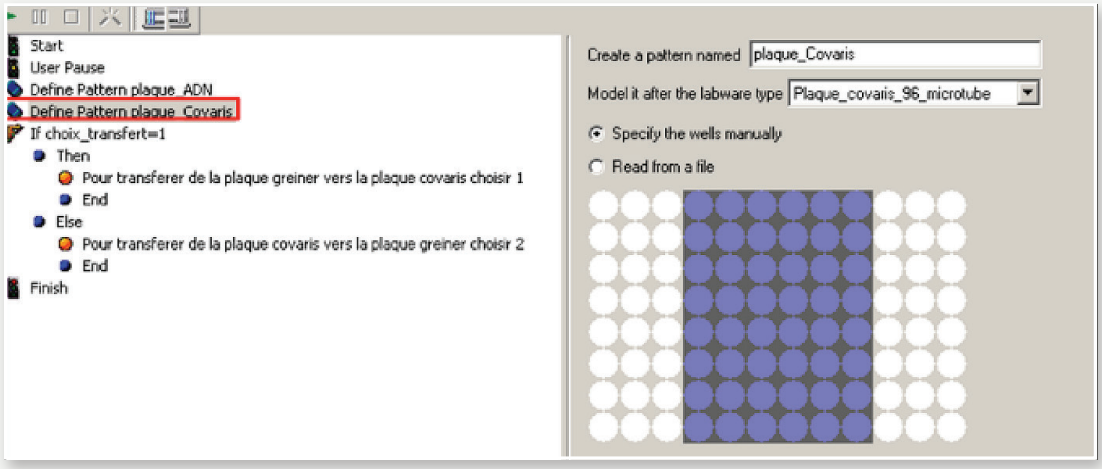


Figure 4. Covaris microTUBE Plate pattern definition step



STEP #1—Foil piercing Similar for each DNA transfer (normalized DNA/ un-fragmented DNA input Plate -> Covaris microTUBE Plate and Covaris microTUBE Plate -> fragmented DNA Plate) microTUBE-130 AFA Fiber				
Function	**Aspirating/ dispensing speeds**	**Volume transferred**	**Pipetting height**	**Comments**
Transfer	100% speed	0 µl	-5 mm from the top of the plate	Unload tips each time (the tips are changed for each well)
STEP #2 —Transfer of normalized/ un-fragmented DNA input Plate towards the Covaris microTUBE Plate				
Transfer	5% speed	130 µl	1 mm from the bottom and option Follow liquid level activated	
STEP #3 —Transfer of the fragmented DNA from the Covaris microTUBE plate towards fragmented DNA Plate				
Transfer	5% speed	130 µl	-2 mm from the liquid and option Follow liquid level activated	The aspiration is divided in tow steps of 65 µl. Between the two aspirations the probes go back up at 5 mm of the top

to optimize DNA fragmentation for specific genomics applications. The same settings are used to fragment DNA from different species such as wheat, corn, canola, etc., with various working concentrations.

Precision

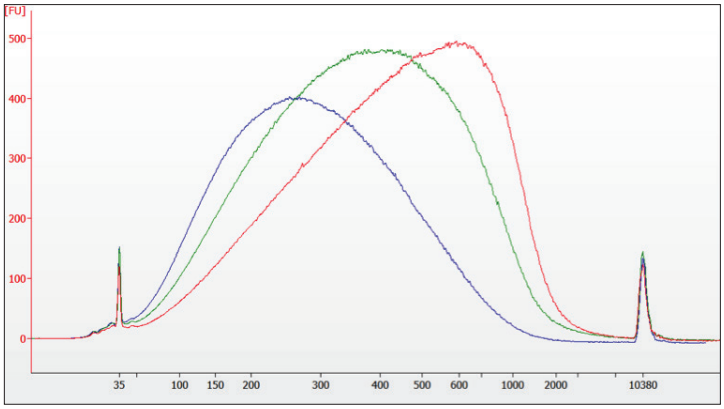


Figure 5. Examples of DNA Shearing optimization for inserts size of 250, 400 and 800 bp respectively. DNA fragment size distribution determined on Agilent Bioanalyzer - High Sensitivity DNA Chip

CONTACT:
Hervé Duborjal
Biogemma
Genomics Team Leader - Plant Engineering Platform
63720 Chappes
France
http://www.biogemma.com/

Reproducibility

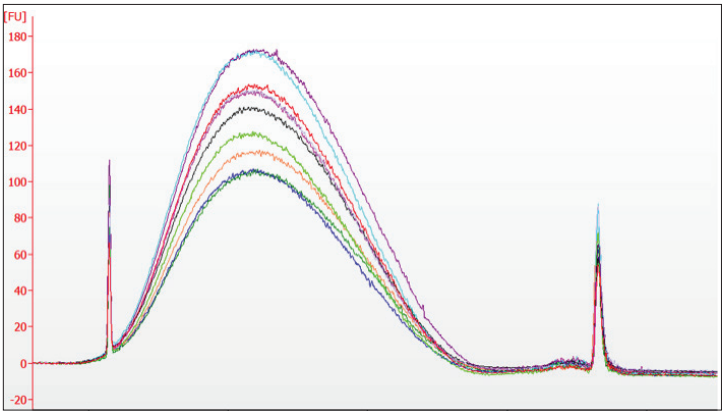


Figure 6. Overlay of 10 samples from 10 different plates for a targeted size of 250 bp. DNA fragment size distribution determined on Agilent Bioanalyzer - High Sensitivity DNA Chip

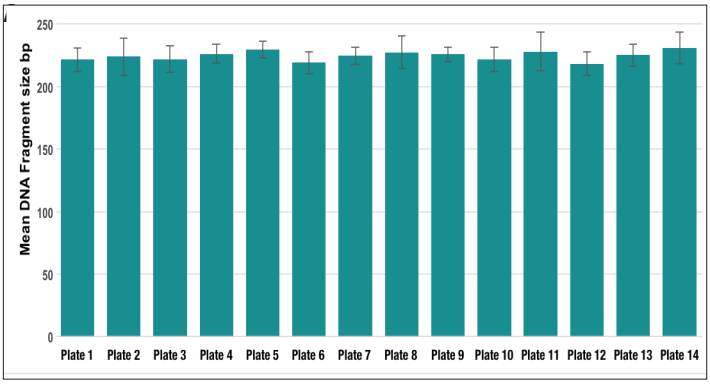


Figure 7. Average of the maximum size observed + standard deviation for each plate. Targeted size = 250 base pairs, 14 complete 96 well plates fragmented. Per plate, 11 samples have been analyzed on Agilent BioAnalyzer High Sensitivity DNA Chip.

Transfer of fragmented DNA to PCR Plate

96 samples are transferred in less than 10 min. Average volume transferred is 124 µl with a standard deviation of 4 µl. To optimize robustness of this step, this aspiration is done by successive two steps of 65 µl.

CONCLUSION

Genomics Group at Biogemma has developed an automated workflow for NGS library preparation that combines high versatility with robustness. Covering all steps of NGS library preparation, it dramatically reduces hands on time while maintaining a high level of flexibility in DNA input and type of library prepared.

For the DNA fragmentation step, the combination of the Covaris E220 with Beckman FX^{P} liquid handler enables an automated DNA transfer both before and after the mechanical DNA shearing step. Combined with the high level of performance in precision, reproducibility and accuracy provided by Covaris Focused-ultrasonicators, it robustly integrates DNA fragmentation into the overall workflow.