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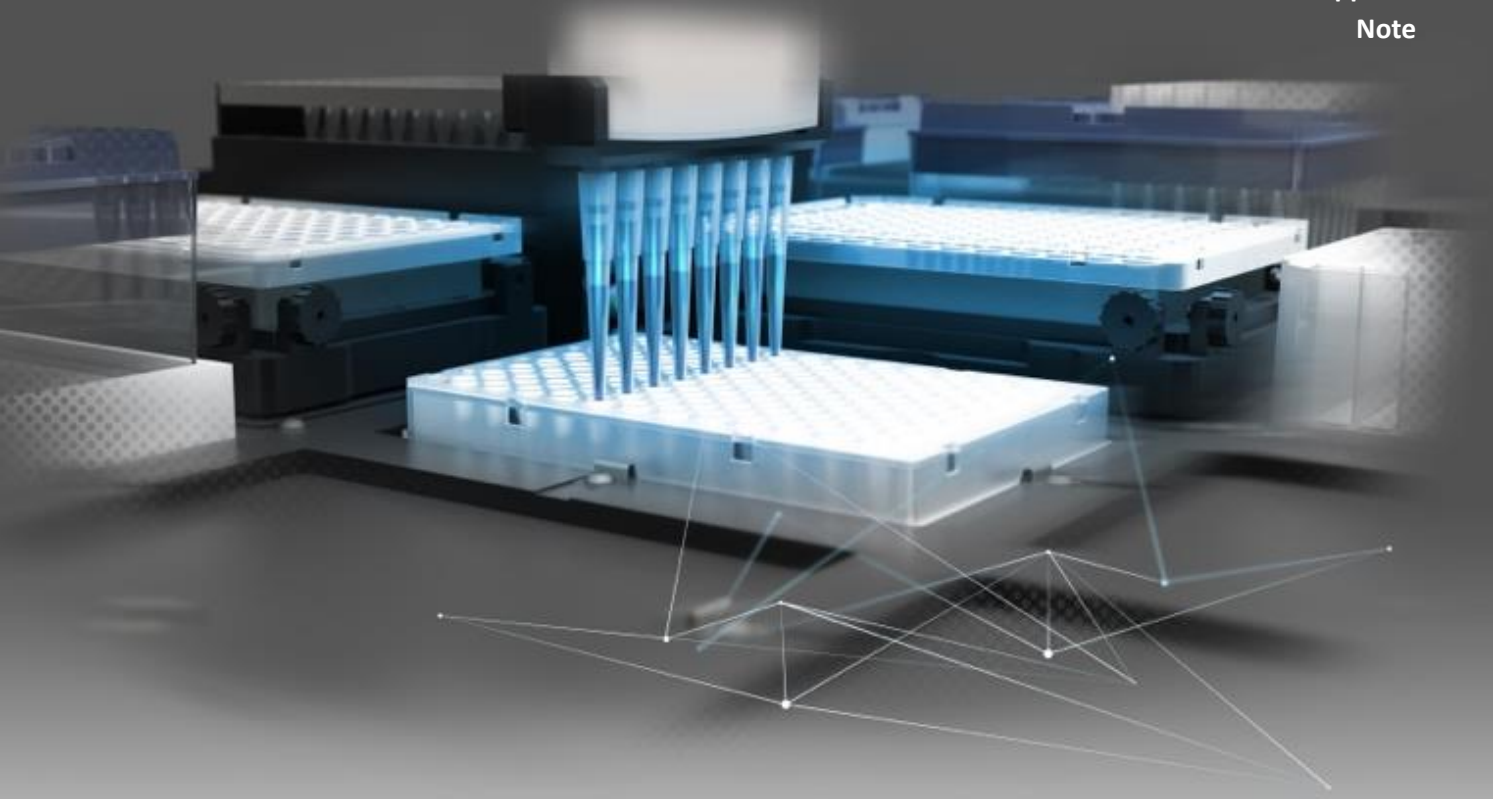
Application Note



Automated library preparation using KAPA Library preparation kits on the ACSIA NGS^{LibPrep}Edition

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Abstract

Being aware of the bottleneck of libraries preparation of next generation sequencing protocols, PrimaDiag developed the ACSIA NGS^{LibPrep}Edition automated pipetting platform. With the ACSIA platform, the library preparation using KAPA kit provided by Roche Diagnostics is **fully automated** with a very cost-effective methods. In this document, the technique used on the ACSIA NGS^{LibPrep} workstation is described and some comparative efficiency results between automated and manual experiments are given.

Keywords:

ACSIA, PrimaDiag, Roche, automation, liquid handling, automated pipetting platform, NGS, library preparation, target enrichment, Capture, Exome, SeqCap, KAPA.

Materials :

- ACSIA NGS^{LibPrep}Edition
- Caliper (Qiagen)
- CLARIOstar (BMG Labtech)
- Human DNA (Provided by Metabolic Unit of the Genetic Center at the Pitié-Salpétrière hospital)
- KAPA Library Preparation kit (Kapa Biosystems)

Introduction & Hardware description

High-throughput sequencing, also known as Next Generation Sequencing (NGS), well described by its name, permits sequence a very large number of genes of many individuals per run. It is now a major driver in genetics research, providing a powerful tool to study DNA and RNA samples. In spite of the technological advancement in NGS, the library preparation is still time-consuming and repetitive. Because of a critical role of the library quality and several important considerations, the whole process requires the technician to be highly concentrated during the manipulations. The automation of this preparation permits to avoid any human error and improve the quality of the library thanks to repeatability and accuracy pipetting.

The **NGS^{LibPrep}** system developed by PrimaDiag uses the patented technology which allows the handling actively of magnetic beads, the strength of the magnetic field is controlled. This system is fit with any kind of plate and deepwell available on the market (no skirted, semi skirted, skirted plates and so on).

Moreover, all liquid handling parameters are carefully optimized for **minimal reagents consumption** and the protocols are optimized to use a minimum **number of tips** (3 tip boxes for preparing 48 libraries instead of 14).

In this document, we describe the methods used and demonstrate the efficiency of the library preparation with the **KAPA Library Preparation kit** on the **ACSIA NGS^{LibPrep} Edition system** when compared to the manual method.

Methods

1. Fragmentation and quality control

After the fragmentation of samples using a Bioruptor system, the size distribution of the fragment is validated by the Caliper machine. Once the quality control is validated, samples are transferred to the ACSIA machine for library preparation.

2. Library preparation

First of all the PrimaController®II software named "KAPALibPepDNA.prws" is loaded on to the control computer.
Secondly, the necessary labwares and reagents are placed on the ACSIA worktable. A click to the validate button permits to choose the number of samples to be processed in the run (from 1 to 48 samples), and the number of indexes used from kit A and/or kit B (from 1 to 12),

- ✓ If the number of samples and the number of index to be used are the same as those indicated in "My parameters" of the chosen protocol, click on "Continue & Ignore",
- ✓ If the number of samples and the number of indices to be used index are not changed the same as those indicated in "My parameters" of the chosen protocol, choose click on choose "Continue & Update".

The system gives the information about the required quantity of each of the necessary reagents in the rack/reservoir for performing this protocol with the selected number of samples. (**Figure 2**)

Then the method runs according to the desired values. In the following application the reaction volume is 50µl.

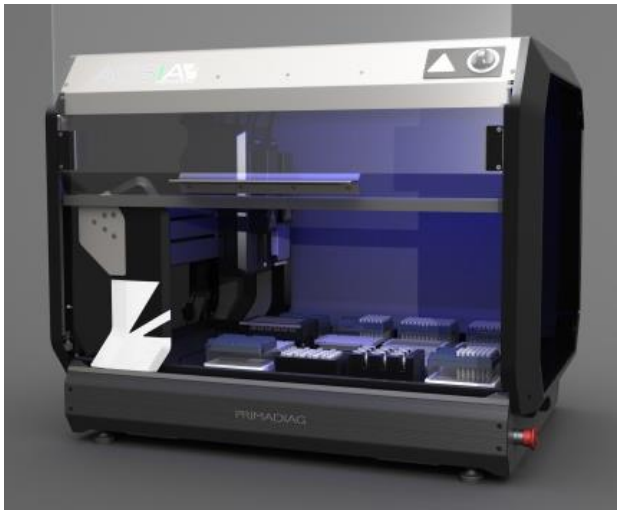

Figure 1: ACSIA NGS^{LibPrep}Edition

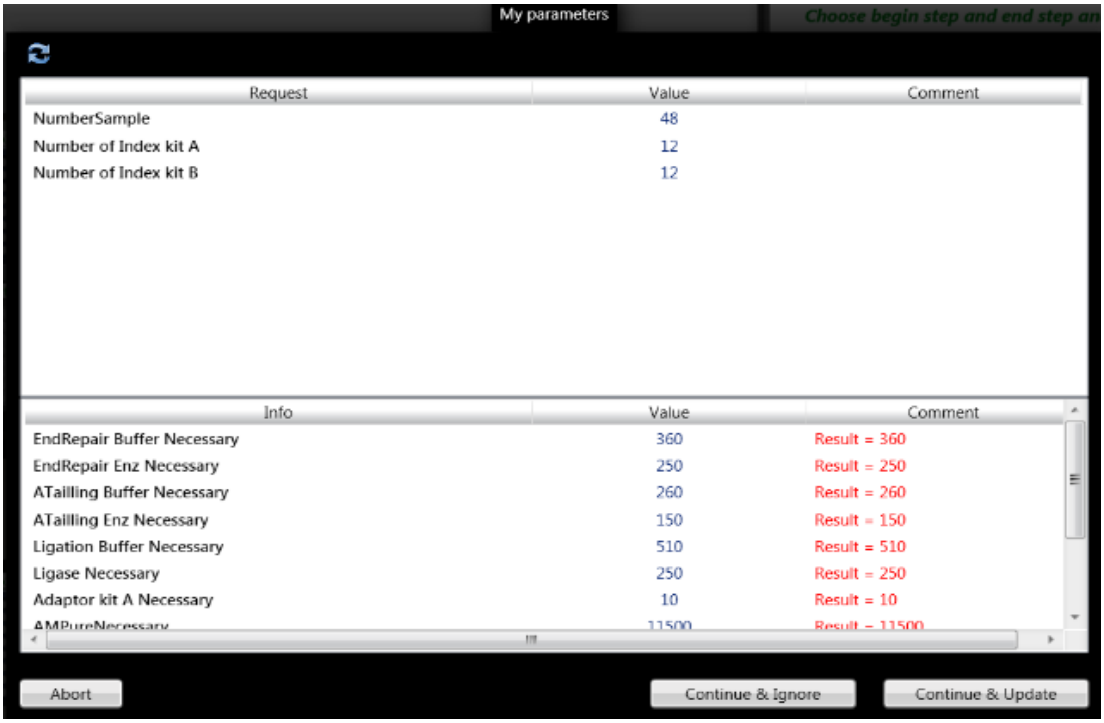

Figure 2: Parameters for library preparation by KAPA

Firstly, the system prepares **End Repair** mix and distributes to each well of the plate containing fragmented DNA. Then the plate goes through 30 minutes of incubations at 20°C. A purification of End-repaired DNA with an adequate quantity of magnetic beads is then processed.

During the purification post-End-repair, the ACSIA system prepares the **A-tailing** mix for saving time. Once purification finished, a quantity of A-tailing mix is added to each well. The sample plate goes through 30 minutes of incubation at 30°C.

For A-tailed DNA clean-up, a quantity of PEG/NaCl SPRI solution is used and a classical purification by magnetic beads is performed.

During the post-A-tailing clean-up, the **ligation** mix is prepared. An adequate quantity of mix is added to each well contained 3'-tailed DNA. Then, 5µL of Adapter from kits A or kit B is added in each well in order to index samples. The sample plate is incubated at 20°C for 15 minutes then a clean-up post ligation is proceed for removing excess unligated adapter and adapter-dimer molecules from the library.

In order to eliminate fragments >450bp and <250bp, a **size selection** is performed by using PEG/NaCl SPRI solution and magnetic beads.

Finally, DNA target is eluted from beads in 25µL and purified libraries is transferred to a new PCR plate.

A LM-PCR for amplifying the libraries is performed in a thermocycler following by a post-PCR clean-up by magnetic beads on ACSIA machine.

	Hands-on time for 48 samples	Number of tips used by sample
ACSIA	**15 minutes**	**6**
Manual	**5 hours**	**28**

3. Quality control of libraries

The quality of the library preparation is checked using a **quantification by CLARIOstar** and then by Caliper to ensure that the size distribution of fragments is coherent.

Results & discussions

For this experiment, 12 samples are prepared in parallel by ACSIA robot and manually for comparison. The tables above show the obtain concentration of these samples after library preparation using KAPA kit.

Sample	Concentration (ng/µL)	Sample	Concentration (ng/µL)
1	22,93	7	26,24
2	25,31	8	24,32
3	24,17	9	25,30
4	24,44	10	27,83
5	24,70	11	25,56
6	24,10	12	25,76

Mean ± 1SD : 25,06 ± 1,25

Sample	Concentration (ng/µL)	Sample	Concentration (ng/µL)
1	36,96	7	31,61
2	39,53	8	44,41
3	23,88	9	35,88
4	22,52	10	47,73
5	27,81	11	40,25
6	25,16	12	29,42

Mean ± 1SD : 33,76 ± 8,29

Figure 3: Quantification results of samples prepared respectively on ACSIA (above) and manually (below)

Regarding the manual preparation, the concentration of the samples varies from **22.52 to 47.73 ng/µL**. Related to the automated preparation, the obtained concentrations vary from **22.93 to 27.83 ng/µL**. We observe that the coefficient of variation is clearly lower with the automated preparation. We obtained **1.25** compared to manual preparation which is **8.29 (Figure 3)**.

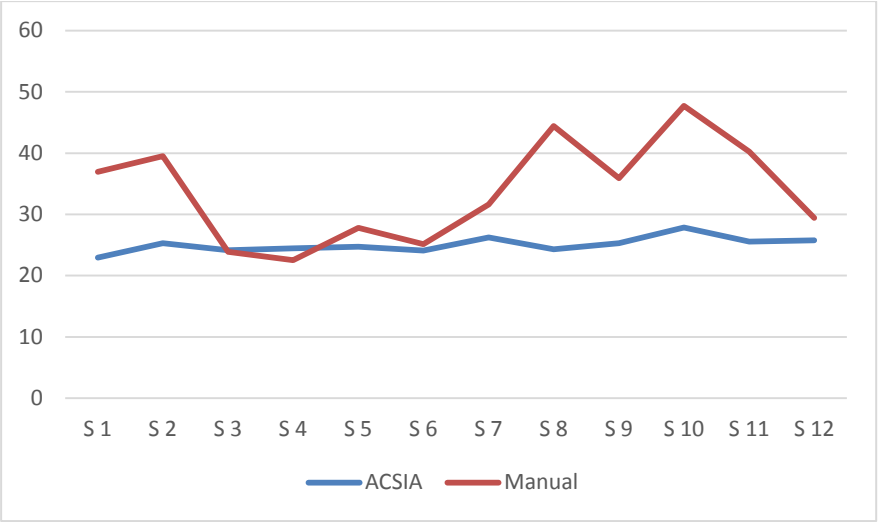

Figure 4: Variation of concentration between 12 samples prepared on ACSIA (blue) and manually (red)

This result shows that the automated KAPA preparation has a best homogeneity between treated samples, and it makes easier the pool before the sequence or hybridization. The variation of the concentration of 12 tested samples is compared between automated and manual preparation. This comparison is showed in **figure 4**.

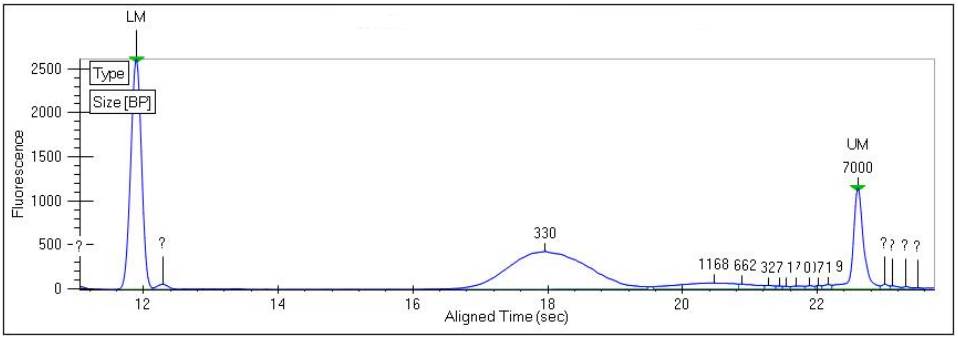

Figure 5: Size distribution of sample prepared on ACSIA

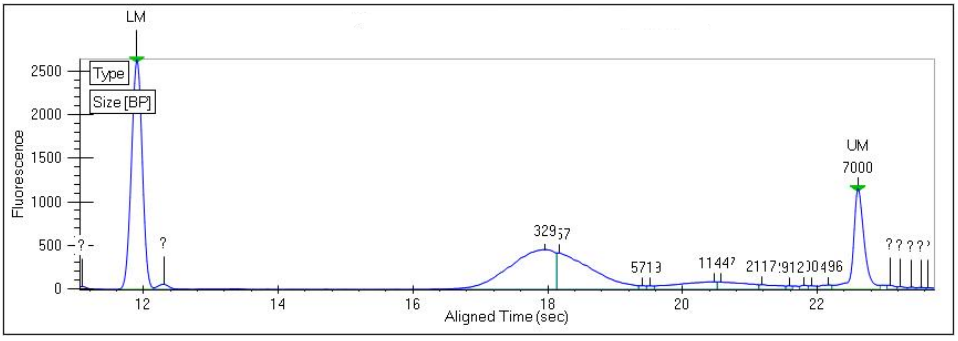

Figure 6: Size distribution of sample prepared manually

Figure 5 and **figure 6** show the size distribution of samples prepared respectively by ACSIA and manually automated library and manual. We observe that the **efficiency is similar** between automated and manual method, expected size distribution is obtained in both methods.

CONCLUSION

Thanks to ACSIA NGS^{LibPrep} platform, the biggest and most laborious steps of the libraries preparation then clean-up can be efficiently automated. These automations permit you **to save up to 5 hours**. The number of tips used for each sample is divided by 5 (**3 tip boxes** are used for automated protocol compared to 14 tip boxes for manual protocol). The obtained results from automated and manual preparation are quite similar. Nevertheless, the automated result shows a **slightly better profile post-capture and sequencing**.

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Definitions/Abreviations :

MWU: Magnetic Work Unit

DNA: Deoxyribonucleic Acid

PCR: Polymerase Chain Reaction

SPRI: Solid Phase Reversible Immobilization

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