For research use only.
Not for use in diagnostic procedures.
For *in vitro* use only.

*Ampli*1™ LowPass Kit for Ion Torrent™

Whole Genome Library preparation for NGS Ion Torrent platforms

USER MANUAL

Version 5.0

REF		**80 reactions**
		Storage
KI0053	*Ampli*1 LowPass Kit for Ion Torrent - Set A	-25°C…-15°C
KI0052	*Ampli*1 LowPass Kit for Ion Torrent - Set B	-25°C…-15°C
KI0051	*Ampli*1 LowPass Kit for Ion Torrent - Set C	-25°C…-15°C

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Table of Contents

1. Kit Contents

***Ampli*1™ LowPass Kit for Ion Torrent**, thereinafter called ***Ampli*1 LowPass Kit**, consists of three Sets:

- Set A → ref. KI0053
- Set B → ref. KI0052
- Set C → ref. KI0051

Each Set of *Ampli*1 LowPass kit provides up to 80 reactions and is composed by one reagent box:

***Ampli*1 LowPass kit reagents**	
Reagent Name	**Content**
Ampli1 PCR Water	2 x 1100 µl
Ampli1 PCR dNTPs	1 x 200 µl
Ampli1 PCR BSA	1 x 250 µl
Ampli1 PCR Reaction Buffer	1 x 1100 µl
Ampli1 PCR Taq Polymerase	1 x 20 µl
*Ampli1 Adapter Barcodes (A-BC-XX)**	16 x 25 µl
Ampli1 Adapter P1	1 x 100 µl

***** *Ampli*1 LowPass Kit Set A provides a set of unique ***Ampli*1 Adapter barcodes (01-16)**.
*Ampli*1 LowPass Kit Set B provides a set of unique ***Ampli*1 Adapter barcodes (17-32)**.
*Ampli*1 LowPass Kit Set C provides a set of unique ***Ampli*1 Adapter barcodes (33-48)**.

2. Storage and Handling

Upon receipt, store the *Ampli*1 LowPass kit reagents at -25°C…-15°C.
Please read these instructions for use carefully before starting.
Always add reagents to the master mix in the order specified in the protocol.
When stored and handled under these conditions the kit components are stable until the indicated expiration date. Handle and store reagents with the appropriate attention and care, and set up reactions according to good laboratory practices.
Freeze-thaw reagents no more than 6 times.
It is recommended that the user follows the Guidelines for Research involving Recombinant DNA Molecules (NIH guidelines) Federal Register, July 5, 1994 (59 FR 34496) and any amendments thereto. Menarini Silicon Biosystems SpA disclaims all responsibilities for any injury or damage, which may be caused by the failure of the user to follow said guidelines.

3. Safety Information

When working with chemicals always wear suitable personal protective equipment such as a lab coat, disposable gloves, and protective goggles. For more information consult the appropriate material safety data sheets (MSDS).
MSDS of each Menarini Silicon Biosystems kits and components are available online at http://www.siliconbiosystems.com/msds-documents.

4. Technical Assistance

For technical assistance and additional information, contact the Customer Support:
E-mail: ampli1.support@siliconbiosystems.com
Telephone: (+39) 051-9944100, Mon-Fri, 9 am – 18 pm CET +01:00

5. Intended Use

***Ampli*1™ LowPass Kit for Ion Torrent** is intended to generate multiplexed, sequencing-ready libraries in a streamlined single-day protocol, to detect chromosomal aneuploidies and copy number alterations (CNA) by low-pass whole genome sequencing using Ion PGM™ and Ion S5™ systems.
*Ampli*1 LowPass Kit product applications include the characterization and investigation of genomic heterogeneity and evolution in tumor cell populations such as individual circulating tumor cells (CTCs).
*Ampli*1 LowPass Kit offers a robust and reproducible method for reduced-representation sequencing in order to assess genome-wide copy-number alterations, and is not intended for Whole Genome applications.
The *Ampli*1 LowPass Kit is intended for research use and for *in vitro* use only. No claim or representation is made for any intended use to provide information for the diagnosis, prevention, or treatment of a disease.

6. Product Use Limitation

The *Ampli*1 LowPass kit is intended for research use and for *in vitro* use only.
No claim or representation is made for any intended use to provide information for the diagnosis, prevention or treatment of a disease.

7. Product Description

The *Ampli*1 LowPass kit exploits the deterministic nature of ***Ampli*1 WGA Kit** (Menarini Silicon Biosystems ref. WG001R or KI0030) to generate sequencing-ready libraries through a single-reaction step.
The ***Ampli*1 LowPass Kit** is a library preparation kit designed to generate up to 48 barcoded libraries (Set A, Set B, Set C) even with more limited amounts of starting material and is suitable for sequencing on Ion PGM™ and Ion S5™ platforms.
The *Ampli*1 LowPass kit allows saving time compared to other procedures by avoiding the laborious fragmentation step and preparation of several enzyme reactions.

8. Additional Required Materials & Equipment

- SPRIselect beads (Beckman Coulter, Ref. B23317 or B23318);
- Qubit® dsDNA HS Assay Kit (Agilent Technologies, Ref. Q32851) or similar (e.g. Thermo Fisher Quant-iT™ PicoGreen® dsDNA Assay Kit, Ref. P11496);
- Agilent High Sensitivity DNA Kit (Agilent Technologies, Ref. 5067-4626);
- Size selection gel-based systems (Recommended E-Gel™ SizeSelect™ II Agarose Gels, 2% (Thermo Fisher Scientific, Ref. G661012); 50 bp DNA Ladder (Thermo Fisher Scientific, Ref. 10416014) or E-Gel™ Sizing DNA Ladder (Thermo Fisher Scientific, Ref. 10488100));
- NaOH for molecular biology;
- Ethanol BioUltra, for molecular biology, ≥99.8%;
- Nuclease-free water (molecular biology grade);
- LowTE (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA; suggested: ThermoFisher TE Buffer, Ref. 12090015);
- 1.5 ml tubes;
- 0.2 ml PCR tubes (Recommended: Axygen® 0.2mL Maxymum Recovery® Thin Wall PCR Tubes with Flat Cap; Ref. PCR-02-L-C), 8-tube strips or 96-well plate (Recommended: Eppendorf twin.tec PCR 96 DNA LoBind semi-skirted, Ref. H0030129504);
- Filter tips (Recommended: Gilson Diamond Filter tips in sterilized TIPACK™ or Eppendorf Dualfilter T.I.P.S., PCR Clean);
- Dedicate micropipette sets for pre-PCR and post-PCR area;
- Programmable thermocycler with adjustable ramp rates, operating within manufacturer's specifications;
- Invitrogen DynaMag™, Agencourt SPRIplate or similar magnetic rack for 0.2 ml tubes (Recommended: DynaMag™-96 Side, Ref. 12331D or DynaMag™-96 Side Skirted, Ref. 12027D);
- Mini centrifuge for 0.2 ml tubes or 96-well plate and Centrifuge for 1.5 ml tubes;
- Vortex-mixer;
- Qubit® 2.0 or 3.0 Fluorometer (Thermo Fisher);
- Agilent 2100 Bioanalyzer System (Agilent) ;
- -25°C…-15°C Storage Freezer;
- +2°C…+8°C Storage Refrigerator.

9. Sample Specifications

*Ampli*1 LowPass kit for Ion Torrent **works exclusively with *Ampli*1 WGA product**.

10. What to Know Before Starting

Laboratory setup

- Use good laboratory practices to minimize cross-contamination of products and reagents.
- Use 0.2 ml PCR tubes, 8-tube strips or 0.2 ml PCR plates (96-well format) according to the labware and sample throughput.
- Verify if 96-Well Tray is needed on the thermocycler to load tubes and 8-tube strips, in order to provide the thermal contact necessary for high-performance thermal cycling.
- To save time, pre-program the Barcoding reaction thermocycler program.
- Use aerosol-barrier pipette tips: Gilson Diamond Filter tips in sterilized TIPACK™ or Eppendorf Dualfilter T.I.P.S., Low Retention PCR Clean are recommended.
- Before starting, prepare a fresh 80% ethanol solution using absolute ethanol BioUltra and nuclease-free water (approximately 1ml per sample).

Working Area Organization

- Wear nitrile/powder-free gloves for all protocol steps, and use only newly opened kept or sterile packages of plastic ware (e.g. reaction tubes and Dualfilter Low Retention Filter tips). Do not use autoclaved plastic ware.
- Perform *Ampli*1 WGA clean up and Barcoding reaction in a separate working area or room from Library clean up step and downstream processing.
- Use a separate set of pipettes for *Ampli*1 WGA clean up and Barcoding reaction from those used for Library clean up step and downstream processing.
- Before beginning and after use, wash the working surface with 10% bleach followed by water rinses.

Pipetting recommendations

- In order to compensate volume losses during pipetting, it is suggested to include a 5% excess in each reaction mix.
- When handling barcodes (*Ampli*1 Adapter BCs), open one tube at time to prevent any cross-contamination.
- Visually check tips to ensure volumes are equivalent if using a multi-channel pipette.
- Touch tip to side of well and slowly dispense reagent on the side of the well/tube to form a droplet. This enables you both to pipette small volumes accurately and to see that you added reagent to the well.
- When performing purification, SPRIselect beads drying time is dependent on temperature and humidity of the working area. Elution reaction mix/buffer should be added when observing bead pellet lack of shininess.
- Be sure to remove all of the ethanol from the bottom of the well as it is a known PCR inhibitor, during the washing step of clean up procedures.
- When performing elution from beads dispense reagents directly on the beads pellet (dispensing with the tip touching the side of the tube/well at an angle and dragging it slightly along the wall where the beads pellet is).

- Before vortexing and spin down samples and reagents, ensure that the tubes and plate-wells are correctly closed.
- Verify that accurate amounts of reagents are dispensed in each tube/well, and prevent excess carryover from droplets adhering to the tip.

Tips and recommendations

- We recommend evaluating the quality of *Ampli*1 WGA products with *Ampli*1 QC kit (Menarini Silicon Biosystems, ref. WGQC4 or KI0027). This assay is a multiplex PCR of four markers indicative of the quality of the *Ampli*1 WGA product obtained. Positivity to at least 2 markers (GII>=2) has been demonstrated to be predictive for obtained a good sequencing quality metrics.
- *Ampli*1 LowPass Kit for Ion Torrent has been validated on Ion PGM™ and Ion S5™ platforms (Thermo Fisher), using Ion 316™v2 Chip or 318™v2 Chip for Ion PGM™ platform and Ion 530™ Chip for Ion S5™ platform respectively.
- When performing two sequencing runs per initialization (200 base-read sequencing), it is suggested to combine all barcoded libraries in a unique pool, performing size selection and slit it into two sequencing chips.
- It is essential to size-select the *Ampli*1 LowPass library from 300 bp to 450 bp size, in order to perform 400-base-read emulsion PCR and sequencing on Ion Torrent platforms, according to manufacturer's instructions

11. Procedure

*Ampli*1 LowPass kit is designed to generate Ion Torrent-compatible libraries, with a hands-on time of only 2 hours and total time of 6 hours (Figure 1).

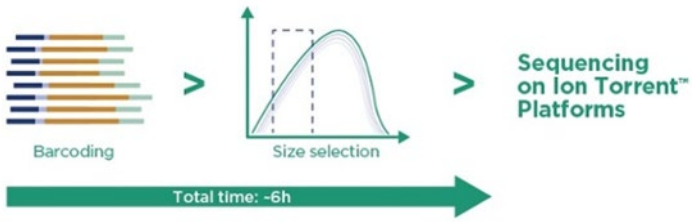

Figure 1. *Ampli*1 LowPass workflow for Ion Torrent
*Ampli*1 LowPass workflow starts from purified *Ampli*1 WGA product and consists of two main phases:
1) Barcoding reaction
2) Size-selection of final library pool
Between each reaction phase, a purification is required (bead-based clean up).

Step 1 of 5: *Ampli*1 WGA clean up

1. Transfer 5 µl of *Ampli*1 WGA product in a new tube/well plate and add 5 µl of nuclease-free water.

NOTE: *Choose performing reactions in 0.2 ml PCR tubes or 0.2 ml PCR plates (96-well format) depending on labware and sample throughput.*

Beads Binding

2. Thoroughly shake the SPRIselect Beads bottle to resuspend any magnetic particles that may have settled.

3. Add 18 µl (1.8X) of resuspended SPRIselect Beads to the diluted *Ampli*1 WGA product

4. Mix thoroughly by pipette mixing 10 times (in case of PCR plate)
or
vortex and briefly spin-down the sample to collect the liquid from the sides of the tube (in case of PCR tube).

NOTE: *The color of the mixture should appear homogeneous after mixing.*

5. Let the mixed samples incubate for 5 minutes at room temperature (RT) for maximum recovery.

Separation

6. Place the tube/PCR plate on the magnetic plate for 2 minutes to separate the beads from the solution. Wait for the solution to clear before proceeding to the next step.

7. Carefully remove and discard the cleared solution avoiding disturbing the beads. This step must be performed while the tube/PCR plate is situated on the magnetic plate.

NOTE: *Take care to not aspirate beads during this step, which have formed a pellet on the side of the tube/well.*

Washing

8. With the tube/PCR plate still on the magnetic plate, add 200 µL of 80% ethanol to each tube/well and incubate for 30 seconds at room temperature. Then, carefully aspirate out the ethanol and discard.

9. Repeat the previous step for a total of two washes.

10. Remove all of the ethanol from the bottom of the tube/well with a P10 pipette and air dry for about 1 minute

NOTE*: drying time is dependent on temperature and humidity of the working area. Take care not to over dry the beads (beads pellet should not appear cracked). When observing beads pellet lack of shininess proceed to the next step.*

11. Remove the tube/PCR plate from the magnetic plate.

Elution

12. Elute *Ampli*1 WGA product from beads by adding 12 µl of nuclease-free water directly on the beads pellet (dispensing with the tip touching the side of the tube/well at an angle and dragging it slightly along the wall where the beads pellet is).

13. Mix thoroughly by pipette mixing 10 times (in case of PCR plate)
or
vortex and briefly spin-down the sample to collect the liquid from the sides of the tube (in case of PCR tube).

14. Incubate at RT for 2 minutes.

***NOTE**: If beads pellet is difficult to resuspend, extend the incubation at RT (at least for 5 minutes) and mix frequently (vortexing thoroughly or pipetting up and down).*

Transfer

15. Place the tube/PCR plate in the magnetic plate for 5 minutes, then carefully transfer 10 µl of cleared supernatant (cleaned up *Ampli*1 WGA product) to a new PCR tube/PCR plate.

SAFE STOPPING POINT: Cleaned up *Ampli*1 WGA product may be stored at +2°C…+8°C overnight (12–16 hours). For longer periods, the cleaned up WGA products may be stored at -25°C…-15°C for up to 90 days. If stored for longer than 90 days, prepare new cleaned up *Ampli*1 WGA product.

Step 2 of 5: Barcoding reaction

1. Prepare the Barcoding reaction mix by adding all components in the order listed in the table below. Calculate the amount needed for the number of samples you are analyzing.

Reagent Name	Volume per 1 sample [µl]
*Ampli*1 PCR Water	13.50
*Ampli*1 PCR Reaction Buffer	2.5
*Ampli*1 PCR dNTPs	0.90
*Ampli*1 PCR BSA	0.65
*Ampli*1 Adapter P1	1.0
*Ampli*1 PCR Taq Polymerase	0.25
Total per reaction	**18,8**

2. Once the Barcoding reaction mix has been prepared, briefly vortex and spin it down to collect all the volume at the bottom of the tube.

3. Place on ice the master mix until you are ready to use it.

NOTE: *Choose performing reactions in 0.2 ml PCR tubes or 0.2 ml PCR plates (96-well format) depending on labware and sample throughput.*

4. Dispense 18.8 µl of Reaction Mix to each pre-labelled tube/well.

5. Add to each tube/well 5 µl of specific *Ampli*1 Adapter BC (A-BC-XX).

6. Add 1.2 µl of *Ampli*1 WGA product to each tube/well.

NOTE: *Final volume of each reaction is 25 µl.*

7. Mix by vortexing and spin down briefly. Place all samples in the thermocycler and run the following program.

Barcoding reaction - thermal cycler program		
Cycles	**Temparature [°C]**	**Time**
	95	4 minutes
1	95	30 sec
	60	30 sec
	72	2 minutes
10	95	30 sec
	60	30 sec
	72	2 minutes (+20 sec/cycle)*
	72	7 minutes
	4	∞

***NOTE**: * increase 20 seconds at each cycle.*

SAFE STOPPING POINT: Barcoding reaction samples may be stored at +2°C…+8°C overnight (12–16 hours). For longer periods, the barcoding reaction samples may be stored at -25°C…-15°C for up to 30 days.

Step 3 of 5: Purification and final elution

1. Ensure SPRIselect beads and barcoding reaction samples (prepared in the 'Barcoding reaction' step 2 of 5) are at room temperature.

Beads Binding

2. Thoroughly shake the SPRIselect beads bottle to resuspend.

3. Add 45 µl (1.8X) of resuspended SPRIselect beads to each Barcoding reaction sample.

4. Mix thoroughly by pipette mixing 10 times (in case of PCR plate)
or
vortex and briefly spin-down the sample to collect the liquid from the sides of the tube (in case of PCR tube).

NOTE: *The color of the mixture should appear homogeneous after mixing. If beads pellet is difficult to resuspend, mix frequently (vortexing thoroughly and/or pipetting up and down).*

5. Let the mixed samples incubate for 5 minutes at room temperature (RT) for maximum recovery.

Separation

6. Place the tube/PCR plate on the magnetic plate for 2 minutes to separate the beads from the solution. Wait for the solution to clear before proceeding to the next step.

7. Carefully remove and discard the cleared solution avoiding disturbing the beads. This step must be performed while the tube/PCR plate is situated on the magnetic plate.

NOTE: *Take care to not aspirate beads during this step, which have formed a pellet on the side of the tube/well*

Washing

8. With the tube/PCR plate still on the magnetic plate, add 200 µL of 80% ethanol to each tube/well and incubate for 30 seconds at room temperature. Then, carefully aspirate out the ethanol and discard.

9. Repeat the previous step for a total of two washes.

10. Remove all of the ethanol from the bottom of the tube/well with a P10 pipette and air dry for about 1 minute.

NOTE*: drying time is dependent on temperature and humidity of the working area. Take care not to over dry the beads (beads pellet should not appear cracked). When observing beads pellet lack of shininess proceed to the next step.*

11. Remove the tube/PCR plate from the magnetic plate.

Elution

12. Elute the cleaned up library from beads by adding 27 µl of LowTE buffer directly on the beads pellet (dispensing with the tip touching the side of the tube/well at an angle and dragging it slightly along the wall where the beads pellet is).

13. Mix thoroughly by pipette mixing 10 times (in case of PCR plate)
or
vortex and briefly spin-down the sample to collect the liquid from the sides of the tube (in case of PCR tube).

14. Incubate at RT for 2 minutes.

NOTE: *If beads pellet is difficult to resuspend, extend the incubation at RT (at least for 5 minutes) and mix frequently (vortexing and/or pipetting up and down).*

Transfer

15. Place the tube/PCR plate in the magnetic plate for 5 minutes, then carefully transfer 25 µl of cleared supernatant (cleaned up library) to a new PCR tube/PCR plate.

NOTE*: The cleaned up library is now barcoded, but it is not ready for sequencing on Ion Torrent platforms yet. It is essential to size-select this library from 300 to 450 bp size, in order to perform 400-base-read emulsion PCR and sequencing on Ion Torrent platforms. It is suggest to quantify and combine in a final library pool (Step 4 of 5) all libraries to be sequenced, before proceeding with size-selection procedure (Step 5 of 5).*

SAFE STOPPING POINT: Cleaned up libraries may be stored at +2°C…+8°C overnight (12–16 hours). For longer periods, the cleaned-up libraries may be stored at -25°C…-15°C for up to 90 days.

Step 4 of 5: Quantify and combine *Ampli*1 LowPass Libraries

This section describes two procedures (A or B) for quantifying *Ampli*1 LowPass libraries and guidelines for combining *Ampli*1 LowPass libraries, in order to obtain a final library pool.

A) Quantify libraries by Agilent® 2100 Bioanalyzer

This procedure provides instructions for quantifying *Ampli*1 LowPass libraries using the Agilent 2100 Bioanalyzer instrument. It is suggested to follow this procedure if the barcoded libraries were originated from *Ampli*1 WGA samples belonging to heterogeneous groups of cells, thus produced from single cells processed under the different conditions (e.g. live cells or fixed cells with 1-2 % PFA or single cells isolated from blood of several patients collected in CellSave tubes and process with CellSearch®).

***NOTE**: This procedure will lead to balanced pooling.*

- Dilute each library 1:5 with LowTE buffer (1 µl of library + 4 µl of LowTE).
- Use Agilent DNA HS Kit reagents and load 1 µl of diluted *Ampli*1 LowPass library on the Agilent® High Sensitivity DNA chip.
- Run the chip according to manufacturer's instructions.
- If the library concentration assayed is >10ng/µL, perform further dilutions of the library and run again the assay.
- Perform a region analysis, using the Bioanalyzer software, selecting the area from 300 bp to 450 bp for each sample profile. If necessary, follow the manufacturer's instruction to perform a region analysis (smear analysis) (Figure 2).

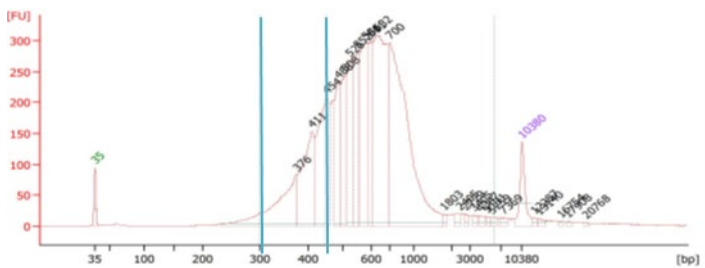

Figure 2. Example of successful *Ampli*1 LowPass library, assessed by Agilent 2100 Bioanalyzer. Typical *Ampli*1 LowPass libraries demonstrate a broad size distribution, with average fragment sizes ranging from 250 bp to 3500 bp in length. The blue bars show the region of interest (from 300 bp to 450 bp).

- Note down the concentration (ng/µl) of the selected area (from 300 bp to 450 bp) for each *Ampli*1 LowPass library.

B) Quantify libraries by Qubit®

This procedure provides instructions for quantifying *Ampli*1 LowPass libraries using the Qubit Fluorometer (Thermo Fisher). In alternative to the procedure A, it is suggested to follow this procedure if the *Ampli*1 WGA samples are produced from single cells processed under similar conditions.

***NOTE**: This procedure could lead to unbalanced pooling.*

- Analyze 2 µl of each *Ampli*1 LowPass library using the Qubit 2.0 or 3.0 Fluorometer and the Qubit dsDNA HS Assay Kit, see the Qubit dsDNA HS Assay Kits User Guide for more information.
- Note down the concentration (ng/µl) of each *Ampli*1 LowPass library.

Libraries Combination

Mix together equal amounts of each *Ampli*1 LowPass library based on procedure A or B, in order to obtain a final library pool:

- In case of procedure A, for each library use the concentration assessed by Bioanalyzer software of the selected area from 300 bp to 450 bp.
- In case of procedure B, for each library use the concentration assessed by Qubit.

NOTE: Before proceeding with barcode combination please see Sequencing Guidelines.

Quantify the final library pool using the Qubit Fluorometer (Thermo Fisher), analyzing 2 µl of the final library pool with Qubit dsDNA HS Assay kit.

NOTE: It is suggest to quantify and combine in a final library pool (Step 4 of 5) all libraries to be sequenced, before proceeding with size-selection procedure (Step 5 of 5).

Step 5 of 5: Size-selection

The final library pool shall be size-selected from 300 bp to 450 bp sizes in length.

In order to perform 400-base-read emulsion PCR and sequencing on Ion Torrent platforms, it is essential to size-select a final library pool no longer than 450 bp.

Size-selection by E-Gel SizeSelect

This section describes the procedure for performing the final library pool size-selection using the E-Gel Agarose Gel Electrophoresis System (Thermo Fisher) and the E-Gel SizeSelect II Agarose Gels, 2% (Thermo Fisher, Product #G661012).

***NOTE**: Other size-selection gel-based systems could be used. In that case, accurately follow the manufacturer's instructions.*

- One or two E-gel lanes can be loaded with the same final library pool.
- Prepare up to 25 µL of final library pool in 1X Sample Loading Buffer per lane (e.g., use 2.5 µL of 10X Sample Loading Buffer with 22.5 µL total final library pool).
- Do not exceed 500 ng of total final library pool per lane.

***EXAMPLE**: In case of loading one lane of E-Gel, if the final library pool is more concentrated than 22 ng/µl, perform a dilution in order to not load more than 500 ng of total DNA per one E-Gel sample lane.*

- Fill all wells of both rows with 50 µL of Nuclease-free Water.
- Load 25 µL of DNA Ladder into a well.
- Perform size-selection according to the manufacturer's instructions.
- Collect the size-selected library pool from the collection lane/s into a new tube.
- Clean up of size-selected library pool by SPRI beads (1.2X), according to the following step.

Clean up of size-selected library pool

1. Ensure SPRIselect beads and size-selected library pool are at room temperature.

Beads Binding

2. Thoroughly shake the SPRIselect Beads bottle to resuspend.
3. Add the volume required (µl) of resuspended SPRIselect beads to the size-selected library pool, in order to clean up with a 1.2X ratio.
4. Vortex and briefly spin-down the sample to collect the liquid from the sides of the tube.

***NOTE:** The colour of the mixture should appear homogeneous after mixing. If beads pellet is difficult to resuspend, mix frequently (vortexing thoroughly and/or pipetting up and down).*

5. Let the mixed samples incubate for 5 minutes at room temperature (RT) for maximum recovery.

Separation

6. Place the tube on the magnetic plate for 2 minutes to separate the beads from the solution. Wait for the solution to clear before proceeding to the next step
7. Carefully remove and discard the cleared solution avoiding disturbing the beads. This step must be performed while the tube is situated on the magnetic plate.

   NOTE: *Take care to not aspirate beads during this step, which have formed a pellet on the side of the tube.*

Washing

8. With the tube still on the magnetic plate, add 200 µL of 80% ethanol to each tube and incubate for 30 seconds at room temperature. Then, carefully aspirate out the ethanol and discard.
9. Repeat the previous step for a total of two washes.
10. Remove all of the ethanol from the bottom of the tube with a P10 pipette and air dry for about 1 minute.

    NOTE*: drying time is dependent on temperature and humidity of the working area. Take care not to over dry the beads (beads pellet should not appear cracked). When observing beads pellet lack of shininess proceed to the next step.*
11. Remove the tube from the magnetic plate.

Elution

12. Elute the cleaned up sample from beads by adding 22 µl of LowTE buffer directly on the beads pellet (dispensing with the tip touching the side of the tube at an angle and dragging it slightly along the wall where the beads pellet is).
13. Vortex and briefly spin-down the sample to collect the liquid from the sides of the tube.
14. Incubate at RT for 2 minutes.

NOTE: *If beads pellet is difficult to resuspend, extend the incubation at RT (at least for 5 minutes) and mix frequently (vortexing and/or pipetting up and down).*

Transfer

15. Place the tube/PCR plate in the magnetic plate for 5 minutes, then carefully transfer 20 µl of cleared supernatant (cleaned up size-selected library pool) to a new PCR tube.

SAFE STOPPING POINT: Cleaned up size-selected library pool may be stored at +2°C…+8°C overnight (12–16 hours). For longer periods, the cleaned-up size-selected library pool may be stored at -25°C…-15°C for up to 90 days. If stored for longer than 90 days, prepare new libraries.

Quantification and qualification of size-selected library pool

Check the size and quantity of the size-selected library pool by Agilent Bioanalyzer 2100.

NOTE: *The size-selected library pool must be shorter than 450 bp.*

- Use Agilent DNA HS Kit reagents and load 1 µl of diluted *Ampli*1 LowPass library on the Agilent High Sensitivity DNA chip.
- Run the chip according to manufacturer's instructions.
- Perform a region analysis, using the Bioanalyzer software, selecting the area from 300 bp to 450 bp for each sample profile. If necessary, follow the manufacturer's instruction to perform a region analysis (smear analysis) (Figure 3).

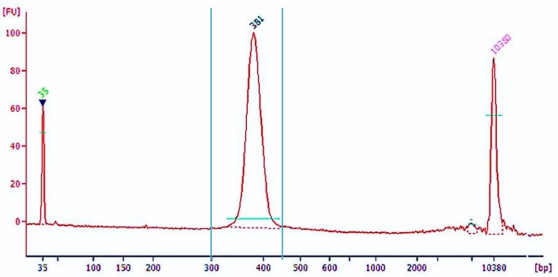

Figure 3. Example of successful size-selected library pool, assessed by Agilent 2100 Bioanalyzer. The blue bars select the region of interest (from 300 bp to 450 bp).

NOTE*: The library pool is now ready for sequencing on Ion Torrent platforms.*

12. Sequencing Guidelines

1. Dilute the final library pool according to the manufacturer's guidelines.
2. Perform a template preparation (emPCR amplification step) for ***400-base-length*** libraries.
3. Set-up 525 flows (***200-base chemistry***) run sequencing and follow the manufacturer's recommendations.
4. When performing two sequencing runs per initialization (200 base-read sequencing), it is suggested to combine all barcoded libraries in a unique pool, performing size selection and slit it into two sequencing chips.
5. When creating the template run on *Torrent Browser* (Thermo Fisher Scientific) select the *Whole Genome sequencing application* and *IonXpress* value in the Barcode set field in Kits tab.
6. In order to perform data analysis, we suggest to obtain at least 500'000 reads for each *Ampli*1 LowPass library.

13. Data Processing Recommendations

- **Library structure**

The insert of the libraries produced with Ampli1™ LowPass Kit contains an adapter added by the Ampli1™ WGA at each end of the fragment. Reads produced by sequencing of the libraries start with the adapter sequence at the 5'-end and may contain the complementary of the adapter sequence at the 3'-end:

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5'- AGTGGGATTCCTGCTGTCAGT … ACTGACAGCAGGAATCCCACT-3'
```

Following sequencing you will need to either perform alignment allowing soft-clipping on both ends of the sequence or to trim the adapter from the fragment ends before aligning to genome. Recommended methods are provided below.

- **Disabling barcode adapter validation during BaseCalling process (only for TorrentSuite v5.2 or later)**

When planning a new Template Run on the Torrent Browser select the radio button Custom in the Analysis Parameter field (Plan tab) and modify the BaseCaller command by disabling the –barcode-adapter-check option as follow:

```
BaseCaller --barcode-filter 0.01 --barcode-filter-minreads 20
--barcode-adapter-check 0
```

This setting allows a correct demultiplexing step in TorrentSuite v5.2. or later Otherwise, all sequences will be collected in a unique BAM file.

- **Aligning with soft-clipping on both sequence ends with Torrent Suite™ Software***

When planning a new Template Run on the Torrent Browser select the radio button Custom in the Analysis Parameter field (Plan tab) and modify the Alignment field by adding the –g 0 parameter as follow:

```
tmap mapall -g 0 ... stage1 map4
```

- **Trimming adapter sequences**

Alternatively, adapter sequences can be trimmed from the sequences in FASTQ format using cutadapt software (https://github.com/marcelm/cutadapt).
FASTQ files can be generated from BAM files using bedtools (https://bedtools.readthedocs.io/en/latest/) or Picard Tools Trim reads with the following command:

```
cutadapt -a ACTGACAGCAGGAATCCCACT -g AGTGGGATTCCTGCTGTCAGT –n 2 -o
output.fastq -e 0.2 input.fastq
```

- **Copy Number Alterations (CNA) Analysis**

Several software packages are available for CNA calling from whole genome sequencing data. Based on our experience, we suggest processing data with Control-FREEC software by Boeva et al 2011 which can be freely downloaded from http://bioinfo-out.curie.fr/projects/freec/.

*Torrent Suite™ is a registered trademark of Thermo Fisher Scientific.

For further assistance about bioinformatic analysis of results visit Menarini Silicon Biosystems website (www.siliconbiosystems.com) or contact technical support at customersupport@siliconbiosystems.com.

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16. Chemicals Classification

EU Regulation 1272/08 ("CLP" Regulation) art. 28 sub. 1 and sub. 3, art. 29 sub. 2 and Annex I sub. 1.5.2.1 allow to report in the secondary label only the most important information about chemicals classification.

17. Notes


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