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

*Ampli*1™ LowPass Kit

Low-pass WGS library prep kit for IonTorrent™ platforms

USER MANUAL

Version 2.0

Content version: January 2017

REF **WGLPTA, WGLPTB, WGLPTC, WGLPTD, WGLPTE, WGLPTF**

80 reactions

Store the kit at -25°C…-15°C

MENARINI silicon biosystems

1. Kit Contents

This Hand book	HB
Reagent Name	**Content**
Ampli1™ PCR Reaction Buffer	1 x 1100 µl
Ampli1™ PCR Taq Polymerase	1 x 20 µl
Ampli1™ PCR Water	2 x 1100 µl
Ampli1™ PCR dNTPs	1 x 200 µl
Ampli1™ PCR BSA	1 x 250 µl
*Ampli1™ Adapter Barcodes (A-BC-XX)**	16 x 5 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 and *Ampli*1™ Adapter P1.
*Ampli*1™ LowPass Kit **Set B** provides a set of unique *Ampli*1™ Adapter barcodes 17-32 and *Ampli*1™ Adapter P1.
*Ampli*1™ LowPass Kit **Set C** provides a set of unique *Ampli*1™ Adapter barcodes 33-48 and *Ampli*1™ Adapter P1.
*Ampli*1™ LowPass Kit **Set D** provides a set of unique *Ampli*1™ Adapter barcodes 49-64 and *Ampli*1™ Adapter P1.
*Ampli*1™ LowPass Kit **Set E** provides a set of unique *Ampli*1™ Adapter barcodes 65-80 and *Ampli*1™ Adapter P1.
*Ampli*1™ LowPass Kit **Set F** provides a set of unique *Ampli*1™ Adapter barcodes 81-96 and *Ampli*1™ Adapter P1.

2. Storage and Handling

Store *Ampli*1™ LowPass Kit at -25°C…-15°C. Transfer enzyme (Ampli1™ PCR Taq Polymerase) tube to ice just prior to use. Other kit components should be thawed, stored on ice and briefly vortexed before use.
Menarini Silicon Biosystems SpA recommends 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 any and all responsibility for any injury or damage which may be caused by the failure of the user to follow said guidelines.

3. Intended Use & Product Use Limitation

*Ampli*1™ LowPass kit is designed to generate multiplexed, sequencing-ready libraries in a streamlined single-day protocol, to detect chromosomal aneuploidies and copy number alteration (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.

4. 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 please 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.

5. Technical Assistance

For technical assistance and additional information, please refer to Menarini Silicon Biosystems Technical Support, Molecular Biology Department:

e-mail: ampli1.support@siliconbiosystems.com

Telephone number: (+39) 051-9944100

(Mon-Fri, 9 am – 18 pm CET +01:00)

6. Additional Required Materials *& Equipments*

- 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)
- 0.2 ml PCR tubes or 96-well plate (Recommended: Axygen® 0.2mL Maxymum Recovery® Thin Wall PCR Tubes with Flat Cap; Product #PCR-02-L-C)
- Aerosol-resistant tips and pipette ranges from 1-1000 µl
- SPRIselect reagent (Beckman Coulter, Product #B23317 or B23318)
- Magnetic rack for 0.2 ml tubes (Purification steps has been validated with DynaMag™-96 Side Magnet, ThermoFisher Scientific, Product #12331D)
- Programmable thermocycler
- Dedicated micropipette sets for pre-PCR and post-PCR reactions
- Filter tips (Recommended: Gilson Diamond Filter tips in sterilized TIPACK™ or Eppendorf Dualfilter T.I.P.S., PCR Clean)
- Mini centrifuge for 0,2 ml tubes
- Vortex
- Qubit® Fluorometer 2.0
- Qubit® dsDNA HS Assay Kit (Agilent Technologies, Product #Q32851)
- Agilent 2100 Bioanalyzer (Agilent Technologies)
- Agilent High Sensitivity DNA Kit (Agilent Technologies, Product #5067-4626)
- -25…-15°C Storage Freezer
- +2°C…+4°C Storage Freezer
- E-Gel Precast Agarose Electrophoresis System (ThermoFisher Scientific) or other size selection system gel-based could be used
- E-Gel® SizeSelect™ Agarose Gels, 2% (ThermoFisher Scientific, Product #G661002)
- 50 bp DNA Ladder (Thermo Fisher Scientific, Product #10416014)

7. *Ampli*1™ LowPass Kit Description

*Ampli*1™ LowPass kit exploits the deterministic nature of *Ampli*1™ WGA (Menarini Silicon Biosystems, Product code: WG001R (ROW), WG001U (USA)), to provide a streamlined, single-reaction protocol to generate multiplexed, sequencing-ready libraries.

Libraries are created through a single-reaction step and offer the possibility of generating up to 48 barcoded libraries suitable for both Ion Torrent NGS platforms: Ion PGM™ and Ion S5™.

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. Sample Specifications

The *Ampli*1™ LowPass Kit works exclusively with *Ampli*1™ WGA products.

9. What to Do Before Starting

1. Working Area Organization

In order to prevent any contamination, it is strongly recommended to:

- Wear nitrile/powder-free gloves for all protocol steps, and use only freshly opened plastic ware (e.g. reaction tubes and pipet tips).
- Dedicate a separate working space and use a separate set of pipettes for the pre-PCR (WGA purification and PCR set up) and post-PCR steps (barcoded libraries handling).
- Separate the Indexing PCR Reagents included SPRIselect and LowTE aliquots (keep in pre-PCR area), from the reagents necessary after barcoding reaction, including a different SPRIselect and LowTE aliquots in post-PCR area.
- Clean lab areas using 0.5% sodium hypochlorite (10% bleach).
- Use barrier tips: Gilson Diamond Filter tips in sterilized TIPACK™ or Eppendorf Dualfilter T.I.P.S., PCR Clean are recommended.

2. Tip and techniques

- All reagents, except enzymes, should be vortexed before use to ensure thorough mixing and spin down in order to collect all the volume at the bottom of the tube.
- To save time all incubation steps of the protocol should be pre-programmed on the thermal cycler.
- Before starting, prepare a fresh 80% ethanol solution using absolute ethanol BioUltra and nuclease-free water (approximately 0.5 ml per sample).

3. Recommendations and suggestions

- We recommend evaluating the quality of *Ampli*1™ WGA products with *Ampli*1™ QC kit (Menarini Silicon Biosystems, Product code: WGQC4). This assay is a multiplex PCR of four markers indicative of the quality of the DNA library obtained. Positivity to at least 2 markers has been demonstrated to be predictive of successful genome-wide analysis to detect chromosomal aneuploidies and copy number alteration (CNA).
- It is recommended to use the *Ampli*1™ LowPass Kit starting from WGAs produced from single cells processed under the same conditions. In fact, a homogeneous group of WGAs allows to obtain a balanced final pool (e.g. live cells or fixed cells with 1-2 % PFA or single cells isolated from blood samples collected in CellSave tubes and process with CellSearch®).

- If the WGAs are produced from single cells processed under different conditions, we recommend to perform the normalization step and to pool the final libraries by Bioanalyzer method as described in the Step 4b.
- The *Ampli*1™ LowPass Kit has been validated on Ion PGM™ and Ion S5™ platforms. In order to obtain at least 500K reads per sample, we suggest to multiplex up to 9 samples on 318v2 chip for Ion PGM™ and up to 30 samples on Ion 530 chip for Ion S5™.
- When performing two sequencing runs per initialization (only with 200 base-read sequencing), we suggest to combine all barcoded libraries in a unique pool, perform the size selection and distribute it into two sequencing chips.
  In this way it is possible to minimize size variation between samples and differences in chip loading.

10. *Ampli1*™ LowPass Procedure Overview

The *Ampli*1™ LowPass workflow starts from purification of a small aliquot of *Ampli*1™ WGA products, as schematically shown in Fig. 1.

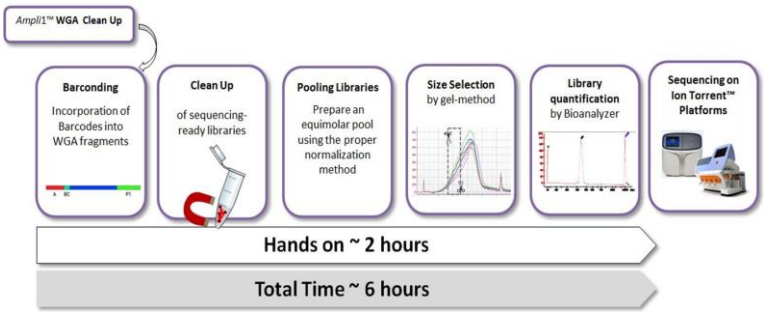

Fig. 1. *Ampli*1™ LowPass Procedure

Step 1:

Clean up of WGA product

1. Transfer 5 µl of WGA product in a new 0.2 ml tube and add 5 µl of nuclease free water.
2. Vortex SPRIselect Beads to resuspend.
3. Add 18 µl (1.8X) of resuspended SPRIselect Beads to the diluted WGA product prepared in step 1-1. Mix well by vortexing and quickly spin the sample to collect the liquid from the sides of the tube.
4. Incubate 5 minutes at RT.
5. Place the tube on a magnetic plate to separate the beads from the supernatant. When the solution is clear (about 5 minutes), carefully remove and discard the supernatant avoiding to disturb the beads that contain WGA fragments.
6. Add 200 µl of 80% ethanol to the tube while in the magnetic plate. Incubate at RT for 30 seconds, and then carefully remove and discard the supernatant.
7. Repeat the previous step for a second wash.
8. Remove any last drops of ethanol at the bottom of the tube with a 10 µl pipet and air dry for about a minute.
   Do not over-dry the beads as this will significantly decrease elution efficiency.
9. Remove the tube from the magnetic plate. Elute WGA product from beads by adding 12.5 µl LowTE.
10. Mix well by vortexing and briefly spin down to collect the liquid at the bottom of the tube.
11. Incubate at RT for 2 minutes.
12. Place the tube in the magnetic plate for about 5 minutes and carefully transfer 10 µl supernatant to a new PCR tube.

SAFE STOPPING POINT: For short-term storage keep the samples at +2°C…+4°C; for long-term storage, samples shall be kept at -25°C…-15°C.

Step 2:

Barcoding Reaction

1. Prepare the 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]
Ampli1™ PCR Water	17.50
Ampli1™ PCR Reaction Buffer	2.5
Ampli1™ PCR dNTPs	0.90
Ampli1™ PCR BSA	0.65
P1	1.0
Ampli1™ PCR Taq Polymerase	0.25
per reaction	**22.8**

2. Once the Reaction Mix has been prepared, briefly vortex and spin it down to collect all the volume at the bottom of the tube.
3. Dispense 22.8 µl of Reaction Mix to each pre-labelled tube.
4. Add to each pre-labeled sample tube 1 µl of specific *Ampli*1™ Adapter BC (A-BC-XX).
5. Add 1.2 µl of *Ampli*1™ WGA product to each pre-labeled sample tube. The final volume of each reaction is 25µl.
6. Mix by vortexing and spin down briefly. Place all samples in the thermocycler and run the following program.

Cycles	Temperature [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	∞	

SAFE STOPPING POINT: For short-term storage keep the samples at +2°C…+4°C ; for long-term storage, samples shall be kept at -25°C…-15°C .

Step 3:

Clean up of final library

1. Vortex SPRIselect Beads to resuspend.
2. Add 45 µl (1.8X) of resuspended SPRIselect Beads to each tube containing barcoded library. Mix well by vortexing and quickly spin the sample to collect the liquid from the sides of the tube.
3. Incubate 5 minutes at RT.
4. Place the tube on a magnetic plate to separate the beads from supernatant. When the solution is clear (about 5 minutes), carefully remove and discard the supernatant avoiding to disturb the beads that contain DNA library.
5. Add 200 µl of 80% ethanol to the tube while in the magnetic plate. Incubate at RT for 30 seconds, and then carefully remove and discard the supernatant.
6. Repeat the previous step for a second wash.
7. Remove any last drops of ethanol at the bottom of the tube with a 10 µl pipet and air dry for about a minute.
   Do not over dry the beads as this will significantly decrease elution efficiency.
8. Remove the tube from the magnetic plate. Elute from beads by adding 27.5 µl Low TE.
9. Mix well by vortexing and briefly spin down to collect the liquid at the bottom of the tube.
10. Incubate at RT for 2 minutes.
11. Place the tube in the magnetic plate for about 5 minutes and carefully transfer 25 µl of supernatant to a new PCR tube.

SAFE STOPPING POINT: For short-term storage keep the samples at +2°C…+4°C; for long-term storage, samples shall be kept at -25°C…-15°C.

Step 4a

Pool barcoded libraries by Qubit®

This step describes how to generate an equimolar final pooled library.
Barcoded libraries can be normalized following two options, depending on the level of homogeneity between the WGAs sample you are analyzing (see 9.3 Recommendations and suggestions).
If the WGAs are produced from single cell obtained with the same conditions, perform the normalization by quantifying each library by Qubit 2.0 Fluorometer, as follows:

1. Analyze 2 µl of each purified library using Qubit 2.0 Fluorometer and Qubit dsDNA HS Assay kit.
2. Prepare an equimolar final pool of barcoded libraries using the concentrations assessed in the previous step 4a-1.

3. The final pool should be at least 15 ng/µl and no more than 38 ng/µl in a final volume of 44 µl (needed amount for two E-Gel wells, see Step 5)
4. Perform the Size Selection by E-Gel® Agarose Gel Electrophoresis System (Thermo Fisher Scientific), as described in Step 5.

Note: The number of samples pooled depends on the Ion platform used (see 9.3 Recommendations and suggestions).

Step 4b

Pool barcoded libraries by Agilent Bioanalyzer ®

If the barcoded libraries were originated from WGAs belonging to heterogeneous groups of cells, perform the sample normalization step by Agilent® Bioanalyzer® method, as follow:

1. Dilute each library 1:5 with LowTE (1 µl library and 4 µl LowTE).
2. Load 1µl of diluted DNA library in each well of Agilent® High Sensitivity DNA chip and place the prepared chip into the Agilent® 2100 Bioanalyzer.
3. Note down the concentration (ng/µl) by selecting the area from 300 bp to 450 bp for each sample profile. If necessary, follow the Bioanalyzer® software manufacture's instruction to perform a region analysis of the selected area.
4. Prepare an equimolar final pool of barcoded libraries using the concentrations assessed in the previous step 4b-3.
5. Analyze 2 µl of the final pool using Qubit 2.0 Fluorometer and Qubit dsDNA HS Assay kit.
6. The final pool should be at least 15 ng/µl and no more than 38 ng/µl in a final volume of 44 µl (needed amount for two E-Gel wells, see Step 5); if the final pool is more concentrated than 38 ng/µl, perform a dilution using the indicated concentration range.
7. Perform the Size Selection by E-Gel® Agarose Gel Electrophoresis System (Thermo Fisher Scientific), as described in the Step 5.

The number of samples pooled depends on Ion platform type (see 9.3 Recommendations and suggestions).

SAFE STOPPING POINT: For short-term storage keep the samples at +2°C…+4°C; for long-term storage, samples shall be kept at -25°C…-15°C.

Step 5

Size selection

In this step, the final pool will be size-selected to 300-450 bp size range in order to optimize subsequent emulsion PCR performance and sequencing step.

Detailed below is a brief description of the steps for the setting up of the E-Gel® Agarose Gel Electrophoresis System (Thermo Fisher Scientific) for convenient loading, running and elution of size selected DNA. Other size selection system gel-based could be used.

Load and run the gel

8. Remove the comb from the E-Gel and place it in the E-Gel iBase system linked to the E-Gel Safe Imager Real Time Transilluminator according to the manufacturer's instructions.
9. Load 20 µl of the pooled library from Step 4 into each of the two wells in the top row of the E-Gel® SizeSelect™ Agarose Gels, 2%. When loading, skip the two wells in the center and the last two wells on each side of the gel.
10. Dilute 50 bp DNA Ladder (1 µg/µl) in LowTE buffer to 25 ng/µl (1:40 dilution). Add 10 µl of diluted DNA Ladder into the middle well, lane M.
11. Fill the empty wells in the top row with 25 µl of Nuclease-free Water.
12. Add 25 µl of Nuclease-free Water to all the large wells in the bottom row (collection wells), and add 10 µl to the center well (lane M) of the bottom row.
13. Run the gel with iBase program "SizeSelect 2%" and monitor the DNA ladder band run.
14. Stop the run when the 300 bp band of the ladder DNA reaches the reference line.
15. Discard the solution of each collection well and refill with 25 µl of Nuclease-free Water.
16. Press "Go" to restart the run and monitoring the marker (M) well frequently, stop the run when the band 450 bp of ladder reaches the reference line (Fig. 2).

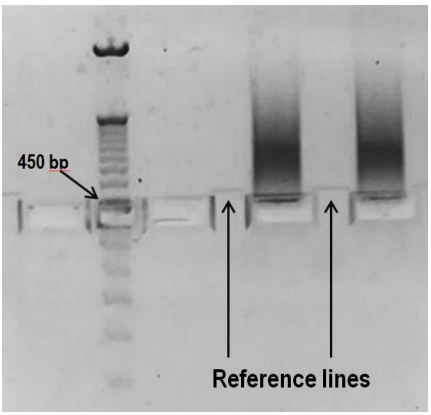

Fig. 2: Size selection with the E-Gel™ SizeSelect™ 2% Agarose Gel for 400-base-lenght libraries (400bp target peak)

Collect the sample

17. Collect the solution from the collection wells using a pipette into a new 0.2 ml tube, without touching the bottom of the well.
18. The total recovered volume should be ~ 50 µl.
19. Dispose the used gel as hazardous waste and proceed immediately to the next step.

Step 6

Cleanup of final library

1. Vortex SPRIselect Beads to resuspend.
2. Add 60 µl (1.2X) of resuspended SPRIselect Beads to the final pool library. Mix well by vortexing and quickly spin the sample to collect the liquid from the sides of the tube.
3. Incubate 5 minutes at RT.
4. Place the tube on a magnetic plate to separate the beads from supernatant. After the solution is clear (about 5-10 minutes), carefully remove and discard the supernatant avoiding to disturb the beads.
5. Add 200 µl of 80% ethanol to the tube while in the magnetic plate. Incubate at RT for 30 seconds, and then carefully remove and discard the supernatant.
6. Repeat the previous step for a second wash.
7. Remove any last drops of ethanol at the bottom of the tube with a 10 µl pipet and air dry for about a minute.
   Do not over-dry the beads as this will significantly decrease elution efficiency.
8. Remove the tube from the magnetic plate. Elute the final library pool from the beads into 22.5 µl LowTE.
9. Mix well by vortexing and quick spin the tube to collect the liquid and incubate at RT for 2 minutes.
10. Place the tube in the magnetic plate for about 5 minutes and carefully transfer 20 µl supernatant, which contains the final pool library, to a new PCR tube.

SAFE STOPPING POINT: For short-term storage keep the samples at +2°C…+4°C; for long-term storage, samples shall be kept at -25°C…-15°C.

Step 7

Final Library Quantification

Check the size and quantity of the final library pool by analyzing 1 µl on the Agilent Bioanalyzer® 2100 using High Sensitivity chip.
The final library pool must be shorter than 450bp, according to the Ion Chef™ manufacturer's guidelines for 400-base-length libraries.
Determine the molar concentration of the library pool using the Bioanalyzer® software.

If necessary, follow the manufacture's instruction to perform a region analysis (smear analysis) to place the entire distribution of library molecules within a single peak (Fig.3).

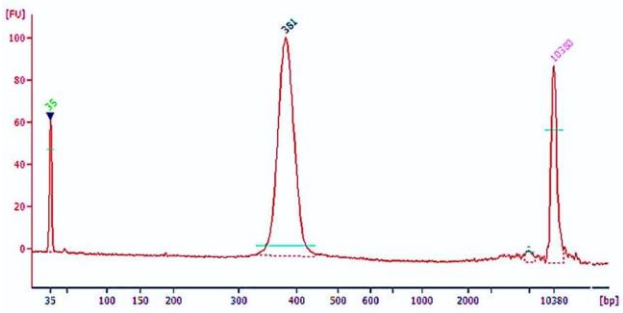

Fig.3: Final Library Size distribution using E-Gel Size Selection.
Electropherogram profile analyzed using DNA High Sensitivity Kit. The final library pool must be shorter than 450bp. Peaks at 35 bp and 10380 bp represent low- and high-molecular weight markers.

Step 8

Sequencing Step

Perform the final library dilution following the manufacturer's guidelines depending on which system it is used for template preparation:

- For template preparation using the Ion Chef™ instrument, consider the recommended concentration for library with length ≥300 bases.
- For template preparation using Ion OneTouch™ 2 Instrument treat the final pool library as "gDNA fragment or Amplicon Library" type.

Step 9

Sequencing Setup

*Ampli*1™ LowPass kit is designed to generate multiplexed, sequencing-ready libraries fully compatible with Ion PGM™ and Ion S5™ platforms.
Given library size has a median length more than 200 bp is mandatory to perform a template preparation (emPCR amplification step) for 400-base-length libraries.
Proceed with libraries sequencing setting the 525 flows (200-base chemistry) run format and following the manufacturer's recommendations.
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.
For further information on how to setup a template run on Torrent Browser consult the user manual instructions.

11. Appendix A: Data Processing Recommendations

- **Library structure**
  The insert of the libraries produced with *Ampli*1™ LowPass Kit contains an adapter added by the *Ampli*1™ 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:

  ```
  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)**
  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. 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 Picard Tools (https://broadinstitute.github.io/picard/).

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.

12. Patent & Trademark Information

This product is patent pending.
The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product. Menarini Silicon Biosystems SpA products may not be transferred to third parties, resold, modified for resale, used to manufacture commercial products without written approval of Menarini Silicon Biosystems SpA.
*Ampli*1™ is a trademark of Menarini Silicon Biosystems SpA, or its subsidiaries, which may be registered in certain jurisdictions. Other brands and product names are trademarks of their respective holders.

13. Warranty

This product is warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Menarini Silicon Biosystems SpA makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the Technical Specifications of the products. Menarini Silicon Biosystems SpA's liability is limited to either replacement of the products or refund of the purchase price. Menarini Silicon Biosystems SpA is not liable for any property damage, personal injury or economic loss caused by the product.

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14. Annex 1: 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.