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

*Ampli*1™ WGA Kit

Whole Genome Amplification for Single Cells

USER MANUAL

Version 01

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REF WG 000 050 R01

50 reactions

Store the kit at −20°C

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1. Kit Contents

	Vial	Label	Cap Color	Contents
Regents	R1	Reaction Buffer 1	white	▪ 1 vial/90 µl
	R2	Reagent 2	blue	▪ 1 vial/50 µl
	R3	Reagent 3	blue	▪ 1 vial/50 µl
	R4	Reagent 4	yellow	▪ 1 vial/35 µl
	R5	Reagent 5	yellow	▪ 1 vial/35 µl
	R6	Reagent 6	green	▪ 1 vial/70 µl
	R7	Reaction Buffer 7	purple	▪ 1 vial/1,000 µl
	R8	Reagent 8	purple	▪ 1 vial/200 µl
	H_2O	Water	colorless	▪ 3 vial/1,000 µl
Enzymes	E1	Enzyme 1	blue	▪ 1 vial/30 µl
	E2	Enzyme 2	black	▪ 1 vial/15 µl
	E3	Enzyme 3	green	▪ 1 vial/60 µl
	E4	Enzyme 4	purple	▪ 1 vial/70 µl

2. Storage and Handling

Store the *Ampli*1™ WGA Kit at –20°C; ship at –20°C. Transfer Enzymes 1, 2, 3, 4 tubes to ice just prior to use. Other kit components should be thawed on ice and briefly vortexed before use. When stored and handled under these conditions the kit components are stable through the expiration date specified. Handle and store reagents with the appropriate attention and care, and setup reaction according to good laboratory practices for PCR.

Silicon Biosystems SpA recommends that the buyer and other persons using this product follow the Guidelines for Research involving Recombinant DNA Molecules (NIH guidelines) Federal Register, July 5, 1994 (59 FR 34496) and any amendments thereto. Silicon Biosystems SpA disclaims any and all responsibility for any injury or damage which may be caused by the failure of the buyer or any other person to follow said guidelines.

3. Intended Use & Product Use Limitation

The *Ampli*1™ Whole Genome Amplification Kit is intended for research use only. The *Ampli*1™ Whole Genome Amplification Kit is for *in vitro* use only. No claim or representation is made for an intended use to provide information for the diagnosis, prevention, or treatment of a disease.

It is normal that some background of bacterial DNA will be present in the *Ampli*1™ product at the end of the reaction (even in no-template controls). *Ampli*1™ WGA should not be used for bacterial samples. The *Ampli*1™ WGA Kit is not recommended for downstream analysis with BAC Array.

4. Safety Information

When working with chemicals always wear suitable lab coat, disposable gloves and protective goggles. For more information please consult the appropriate material safety data sheets (MSDSs).
MSDS of each Silicon Biosystems kit and components are available online at http://www.siliconbiosystems.com/mSDS.page.

5. Technical Assistance

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

e-mail: ampli1.support@siliconbiosystems.com

Telephone number: +39 051-40.71.300;

6. Additional Required Materials

- Thermal Cycler
- Dedicated pipette set
- PCR microcentrifuge tube 0,2 ml. Recommended: MicroAmp Reaction tube with cap (0,2 ml) (Applied Biosystems, Part No.: N801-0612)
- Barrier tips
- Mini Centrifuge suitable for PCR tubes
- Laminar flow hood
- -20°C Storage Freezer
- Vortex

7. *Ampli*1™ WGA Kit Description

The *Ampli*1™ Whole Genome Amplification kit has been specifically developed and optimized for the amplification of the total DNA content of a single cell.

The *Ampli*1™ WGA procedure is based on a ligation-mediated PCR following a site specific DNA digestion.

The output of a *Ampli*1™ WGA procedure is a library of highly concentrated DNA, which can be employed for further targeted genetic research analysis. The main features are of the *Ampli*1™ Whole Genome Amplification kit are:

- Comprehensive and homogenous amplification of the whole genome isolated from a single cell
- Robust and reproducible reaction results
- No need for precipitation steps: all preparatory steps are performed in one tube to avoid template loss
- Complete representation of the genome with fragments of about 0.2-2 kb
- Sequence complexities of multiple primer binding sites are avoided resulting in optimal amplification conditions for all adapter-ligated sequences used.

8. *Ampli*1™ WGA Kit Application

The *Ampli*1™ WGA Kit and procedure allows many different types of downstream research procedures, such as:

- Mutation detection by sequencing
- Mutation detection by pyrosequencing
- SNPs detection
- Microsatellite or other PCR based genotyping Analysis
- Metaphase CGH

Changes might be needed to adapt research protocols for the above techniques to be compatible with the *Ampli*1™ Whole Genome Amplification product output. Please enquire with Silicon Biosystems Technical Support to check for compatibility with your research protocol. An example is provided in Appendix A for illustration purposes only.

9. Sample Specifications

- The *Ampli*1™ WGA procedure is designed to work with an input sample of one single cell in 1 µl of PBS1X. The kit also can be used to amplify the DNA content from samples containing higher number of cells or DNA, in 1 µl of PBS1X
- Although best results are obtained with live cells,the *Ampli*1™ WGA Kit allows the whole genome amplification also from single fixed (or fixed and permeabilized) cells. As an example good results have been obtained with the following:
  - Paraformaldehyde (1%-2% PFA, 10'-20' at RT)
  - Single cells isolated from blood samples collected in CellSave tubes and processed with Veridex CellSearch®
  - Samples processed with Inside Stain (Inside Fix / Inside Perm) from Miltenyi Biotec GmbH;
- Cell staining with antibodies conjugated with fluorophores does not affect the yield of an *Ampli*1™ WGA amplification procedure.
- Nuclei staining might negatively impact yield: staining with Hoechst 33342 (Sigma-Aldrich cat. B2261), final staining concentration 1 µg/ml, 5-10' at RT is a suitable working condition.

10. Things to Do Before Starting

1. Working Area Organization

The *Ampli*1™ WGA Kit is a powerful tool to amplify nucleic acid since it enables the amplification of the DNA content from one single cell. In order to prevent any contamination due to amplified DNA carryover, it is strongly recommended to:

- Dedicate a separate laboratory (or at least a separate working space) to single cell amplification and organize it with dedicated materials such as laminar flow hood, thermal cycler, pipette, pipette tips, PCR 0.2 ml micro centrifuge tubes, 1,5 ml micro centrifuge tubes, tube racks, 0.2 ml PCR tubes compatible centrifuge, vortex, lab coats, –20°C freezer, etc.
- Use barrier tips: Eppendorf Dualfilter PCR clean/sterile are suggested.
- Once Primary PCR Reaction thermal cycling program has finished, take the tube off the thermal cycler and store them in a -20°C dedicated to the downstream analysis in a separate lab.
- Perform each type of downstream analysis (*e.g.*, PCR, sequencing, mCGH, etc) in a separate lab with separate materials: this step is the most important aspect to care for in order to avoid carryover of amplified DNA to single cells samples.

2. Control Samples

It is recommended to process the following controls along with samples in each run of an *Ampli*1™ Whole Genome Amplification procedure:

- No-cell control: 1 µl of *Ampli*1™ Water.
- Positive Control: prepare a positive control by adding in the positive control tube 1 µl of DNA 1ng/µl concentrated. In order to avoid cross contaminations, it is suggested to process the positive control as last sample of each step.

Remember to take into account the no-cell and positive control samples when setting up the correct volume of each reaction mix.

For example, to amplify 8 samples consider also 1 no-cell control, 1 positive control: prepare reaction mix for 10 samples.

3. Pipetting Tips

All pipetting must the carried out under the dedicated laminar-flow hood. The *Ampli*1™ WGA procedure requires working with very small volumes: to avoid loss of materials, it is recommended to proceed as follow:

- All the reactions described in the *Ampli*1™ WGA procedure take place in the same tube in which the single cell has been originally isolated: for that reason it is important to carefully dispense the appropriate volume for each reaction without disturbing the liquid already present in the tube with the pipette tip, in order to avoid to inadvertently removing the cell.
- Just add the required volume by pipetting the fluid directly onto the wall of the tube, without disturbing the fluid.
- Always collect all the liquid by a short centrifuge spin after adding reaction mix and before putting the samples in the thermal cycler.

11. *Ampli*1™ Whole Genome Amplification Procedure

1. *Ampli*1™ WGA procedure overview

All the reactions required for *Ampli*1™ Whole Genome Amplification procedure take place in the same tube in which the single cell has been isolated, starting from 1 µl of PBS 1X. Therefore all the reaction mixes will be subsequently added to that same tube, as shown in Fig. 1.

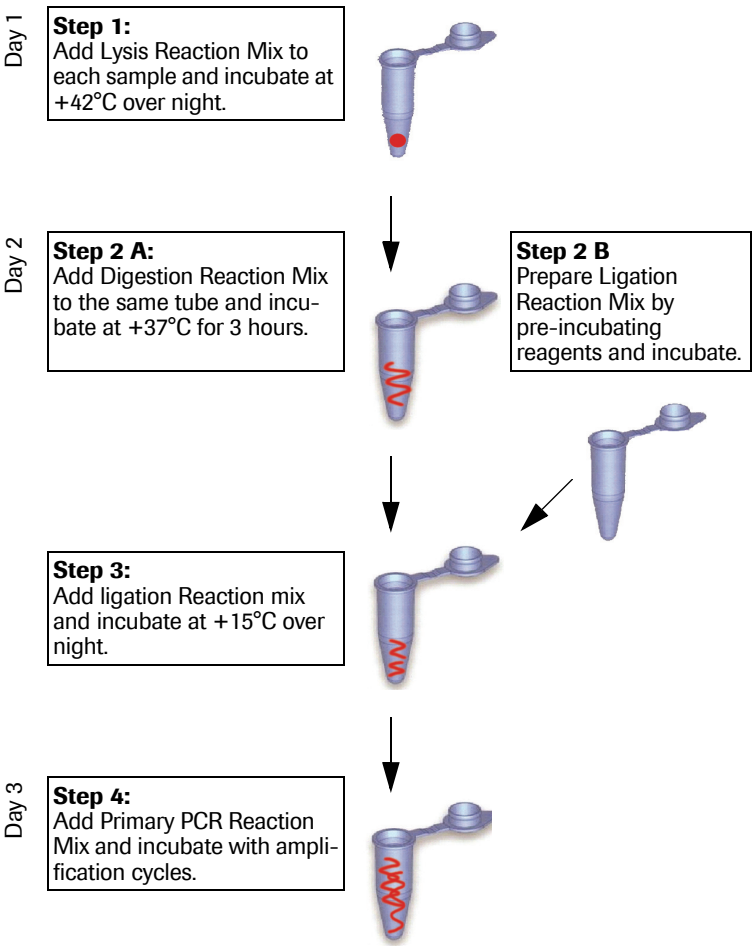

Fig. 1: *Ampli*1™ Whole Genome Amplification Procedure

2. *Ampli*1™ WGA procedure and reaction

Before starting make sure that all the samples meet the requirements described in section 9 "Sample Specification", and that the working area is properly equipped.

Step 1: Cell Lysis

1.1. Prepare Lysis Reaction Mix according to the protocol in Table 1.1.

⚠ It is recommended to prepare a Lysis Reaction Mix for at least 10 reactions even if less than 10 samples will be run; preparation of a larger amount of Lysis Reaction Mix needs to proportionally increase all other *Ampli*1™ WGA reaction volumes needed in this step.

Tab. 1.1: Preparation of Lysis Reaction Mix

Vial	Label	Cap Color	Volume per 10 reactions (µl)
R1	*Ampli*1™ Reaction Buffer 1	white	2.0
R2	*Ampli*1™ Reagent 2	blue	1.3
R3	*Ampli*1™ Reagent 3	blue	1.3
E1	*Ampli*1™ Enzyme 1	blue	2.6
H_2O	*Ampli*1™ Water	colorless	12.8
			20.0
		per reaction	2.0

Once the Lysis Reaction Mix has been prepared, briefly vortex and spin it down in order to collect all the reaction mix at the bottom of the tube.

1.2. Add 2 µl of Lysis Reaction Mix to each sample

⚠ Pipette 2 µl of Lysis Reaction Mix delivering it onto the wall of the tube above the other liquid already present (1 µl) but without touching it. Final volume at this point = 3 µl.

1.3. Incubate all samples according to Table 1.2

⚠ Briefly spin down all the sample tubes prior to placing them in the thermal cycler.

Tab. 1.2: Thermal incubation profile of Lysis Reaction

Temperature (°C)	Hold	Volume (µl)
42	15 hours	3
80	10 minutes	
4	∞	

The Lysis Reaction requires an over night incubation; once the thermal cycler has reached 4°C, remove all the reaction tubes: put them in a microtube rack and store the samples at 4°C while preparing mix for next step.

⚠ Do Not Freeze the samples! Directly proceed with Step 2a.

Step 2A: DNA Digestion

2A.1 Prepare Digestion Reaction Mix according to the protocol in Table 2A.1.

⚠ Prepare the Digestion Reaction Mix as follows for the overall number of samples, by calculating the total volume needed as described: 10 reactions is an example.

Tab. 2A.1: Preparation of Digestion Reaction Mix

Vial	Label	Cap Color	Volume per 1 reactions (µl)	Volume per 10 reactions (µl)
R1	*Ampli*1™ Reaction Buffer 1	white	0.2	2.0
E2	*Ampli*1™ Enzyme 2	black	0.2	2.0
H_2O	*Ampli*1™ Water	colorless	1.6	16.0
		per reaction	2.0	20.0

Once the mix has been prepared, briefly vortex and spin it down in order to collect all the reaction mix.

2A.2 Add 2 µl of Digestion Reaction Mix to each sample

⚠ Pipette 2 µl of Digestion Reaction Mix delivering it onto the wall of the tube above the other liquid already present (3 µl) but without touching it. Final volume at this point = 5 µl.

⚠ Briefly spin down the samples tube and put them back in a microtube rack.

2A.3 Incubate all samples according to Table 2A.2

Put all the samples in the thermal cycler and start the run as follows.

Tab. 2A.2: Thermal incubation profile of Digestion Reaction

Temperature (°C)	Hold	Volume (µl)
37	3 hours	5
65	5 minutes	
4	∞	

Once the thermal cycler has reached 4°C, remove all the reaction tubes: placing them in a microtube rack and store the samples at 4°C while preparing the mix for next step.

⚠ Do Not Freeze the samples! Directly proceed with Step 2B, or if this step has already been done in a separate thermal cycler, proceed with step 3.

Step 2B: Preannealing

2B.1 Prepare Preannealing Reaction Mix according to the protocol in Table 2B.1

This step could be done in parallel with step 2A by using a different thermal cycler or a different plate in the same thermal cycler.

Otherwise the two steps will be done subsequently in the same thermal cycler, storing the samples at 4°C

⚠ This step is a pre-preparation of the reaction mix of the step 3: calculate the needed volume by calculating the overall number of samples multiplied by the required volume for one sample for each different reagent.

Tab. 2B.1: Preparation of Preannealing Reaction Mix

Vial	Label	Cap Color	Volume per 1 reactions (µl)	Volume per 10 reactions (µl)
R1	*Ampli*1™ Reaction Buffer 1	white	0.5	5.0
R4	*Ampli*1™ Reagent 4	yellow	0.5	5.0
R5	*Ampli*1™ Reagent 5	yellow	0.5	5.0
H_2O	*Ampli*1™ Water	colorless	1.5	15.0
		per reaction	3.0	30.0

Once the mix has been prepared briefly vortex and spin it down in order to collect al the reaction mix.

⚠ Do not add Preannealing Reaction Mix to the samples!

2B.2 Incubate the preannealing reaction mix according to Table 2B.2

Put the Preannealing Reaction Mix in the thermal cycler and start the run as follows:

Tab. 2B.2: Thermal incubation profile of Preannealing Reaction

Cycle Numbers	Temperature (°C)	Hold	Volume (µl)
1	65*	1 minutes	# samples × 3 µl
	↓	1 minutes	
	15	1 minutes	
1	15	∞	

*= start at 65°C for 1 min incubation and then reduce for 1°C per cycle incubating for 1 minute for each temperature until +15°C.

Step 3: Ligation

3.1. Prepare Ligation Reaction Mix according to the protocol in Table 3.1.

Tab. 3.1: Preparation of Ligation Reaction Mix

Vial	Label	Cap Color	Volume per 1 reactions (µl)	Volume per 10 reactions (µl)
	Preannealing reaction		3.0	30.0
R6	*Ampli*1™ Reagent 6	green	1.0	10.0
E3	*Ampli*1™ Enzyme 3	green	1.0	10.0
		per reaction	5.0	50.0

3.2 Add 5 µl of Ligation Reaction Mix to each sample.

⚠ Pipette 5 µl of Ligation Reaction Mix delivering it onto the wall of the tube above the other liquid already present (5 µl) but without touching it. Final volume at this point = 10 µl.

⚠ Briefly spin down the samples tube and put them back in a microtube rack.

3.3 Incubate the Ligation reaction mix according to Table 3.2.

Put all the samples in the thermal cycler and start the run.

Tab. 3.2: Thermal incubation profile of Ligation Reaction

Temperature (°C)	Hold	Volume (µl)
15	over night (≥12 h)	10

Ligation Reaction requires over night incubation. Remove all the reaction tubes: put them in a microtube rack and store the samples at 4°C while preparing mix for next step.

⚠ Do Not Freeze the samples! Directly proceed with Step 4

Step 4: Primary PCR

4.1 Prepare Primary PCR Reaction Mix according to the protocol in Table 4.1

Tab. 4.1: Preparation of Primary PCR Reaction Mix

Vial	Label	Cap Color	Volume per 1 reactions (µl)	Volume per 10 reactions (µl)
R7	*Ampli*1™ Reaction Buffer 7	purple	3.0	30.0
R8	*Ampli*1™ Reagent 8	purple	2.0	20.0
E4	*Ampli*1™ Enzyme 4	purple	1.0	10.0
H_2O	*Ampli*1™ Water	colorless	34.0	340.0
		per reaction	40.0	400.0

4.2 Add 40 µl of Primary PCR Reaction Mix to each sample

⚠ Pipette 40µl of Primary PCR Reaction Mix delivering it onto the wall of the tube above the other liquid already present (10 µl) but without touching it. Final volume = 50 µl.

4.3 Incubate the Primary PCR Reaction Mix according to Table 4.2

Briefly spin down all the sample tubes.

Put all the samples in the thermal cycler and start the run as described in table 4.2.

Tab. 4.2: Thermal incubation profile of Primary PCR Reaction

Cycle Numbers	Temperature (°C)	Hold	Additional time and temperature	Volume (µl)
	68	3 minutes		
14	94	40 sec		
	57	30 sec		
	68	1:30* min:sec	* = +1 sec/cycle	
8	94	40 sec		
	57**	30 sec	** = +1°C/cycle	50
	68	1:45* min:sec	* = +1 sec/cycle	
22	94	40 sec		
	65	30 sec		
	68	1:53* min:sec	* = +1 sec/cycle	
1	68	3:40 min:sec		

Notes: * = +1 sec/cycle
** = +1°C/cycle

⚠ Store the samples at –20°C.

12. Patent & Trademark Information

Use of this product is covered by US patent No. 6,673,541 and corresponding patent claims outside the US. 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 for the purchaser's own internal research. Silicon Biosystems SpA products may not be transferred to third parties, resold, modified for resale, used to manufacture commercial products without written approval of Silicon Biosystems SpA.

*Ampli*1™ is a trademark of 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. 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. Silicon Biosystems SpA's liability is limited to either replacement of the products or refund of the purchase price. Silicon Biosystems SpA is not liable for any property damage, personal injury or economic loss caused by the product.

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14. Appendix A

An example of use of *Ampli*1™ WGA output in PCR downstream research assays.

The library of fragments generated through the *Ampli*1™ WGA procedures originates from DNA digested as follows:

5'… T↓T A A … 3'

3'… A A T↑ T … 5'

The specific action of the Digestion Enzyme make it possible to determine the exact sequence of *Ampli*1™ amplification products around any target region.

Primer design

Designing target specific PCR to amplify and analyze one target sequence of an the *Ampli*1™ WGA amplification product, requires specific considerations:

1. Identify the target of the PCR downstream assay: sequence, mutation, microsatellite, etc.
2. Download the DNA sequence containing the target.
3. Determine where the flanking restriction sites are.
4. Do not use mRNA sequence data as the flanking restriction sites could reside in introns.
5. Extract the sequence of the WGA-Amplicon that will contain the target.
6. Design the downstream assay just considering the WGA-Amplicon generated.
7. Do not design primers that overlap digestion sites.

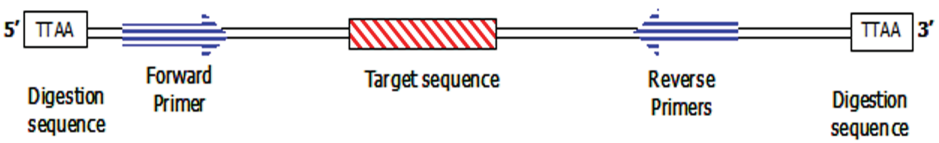

Fig. 2: Primers design for PCR as downstream analysis

Verification of primer pairs

1. Download from the database the sequence target encompassed by the primer pairs: it is necessary to work on the DNA sequence as in the mRNA sequence some digestion sites could be hidden due to splicing.
2. Verify that the target sequence of the primers does not include the digestion motif, taking into account also the possible degenerate base variants, if present.
3. Do not design primers that overlap digestion sites.

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