Ampli1 whole genome amplification protocol

Optimized for amplification of single cells isolated with the puncher device

Version 1.4

Version 1.4

Reagents

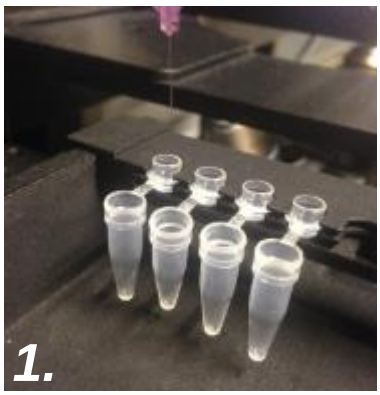
All reagents must only be opened in a PCR oriented cabinet/room to prevent contamination. Filter all reagents before use, except for the *Ampli*1 Single Cell WGA kit.

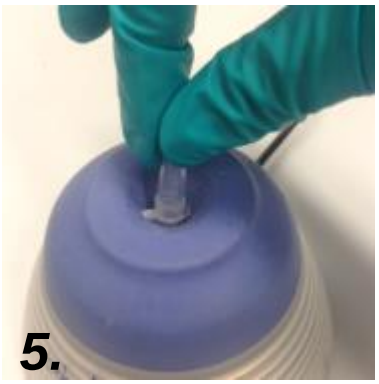
- *Ampli*1 Single Cell WGA kit (Silicon BioSystems, #WG 000 050 R01)
- Electran molecular biology grade ethyl alcohol absolute (VWR chemicals, #437433T)
- 1x PBS, sterile

Devices and consumables

- Sterile 0.2mL thin-walled tube, classic semi-domed cap, regular profile (BIOplastics, C78401)
- PCR Machine with a 96-wells plate module
- Centrifuge capable of spinning down 0.2ml PCR tubes

Obtaining the single cell for whole genome amplification





1. Pipet 30µl absolute EtOH in the cap of the 0.2ml tube and place it in the sample holder on the puncher device, as shown in image to the left.
2. Bend the cap so that the top of cap is at the same height as the tube.
3. Select the tube collection format and align the needle to punch into the cap of the tube(s).
4. Punch the desired cell into the cap of the PCR tube. **Ensure that the next steps are performed within 10 minutes after this step!**
5. Close the tube upside down, with the sample liquid still the cap, and vortex the tube upside down for 5 seconds (shown in image to the left).
6. Immediately spin down at 10,000g for 1 minute.
7. To dry the samples, open the PCR tube and spin off at 5,000g for 1 hour, without the inner lid of the centrifuge attached to the rotor, see image. Spin off longer if any EtOH still is present.
8. Add 1µl PBS (sterile) to every sample, as stated as starting point according to the Ampli1 WGA protocol.

VyCAP

Whole genome amplification with the Ampli1 kit

Because this whole genome amplification is such an extremely sensitive method, all of the following steps must be performed in a flowhood, along with wearing gloves at any given time. All mixtures must be prepared in a PCR oriented cabinet/room.

Step 1: Cell lysis

Follow steps 1.1 and 1.2 from the Ampli1 whole genome amplification procedure, see appendix I. Continue with the following step:

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	Overnight (16 hours)	3µl
65	30 minutes	
80	15 minutes	
4	∞	

Step 2A: DNA digestion

Follow steps 2A.1 and 2A.2 of the Ampli1 whole genome amplification procedure, see appendix I. Continue with the following step:

2A.3 Incubate all samples according to Table 2A.2
Put all the samples in a 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	5µl
65	5 minutes	
4	∞	

Step 2B: Preannealing

Follow all steps of the preannealing according to the Ampli1 whole genome amplification procedure, see appendix I.

Step 3: Ligation

Follow steps 3.1 and 3.2 of the Ampli1 whole genome amplification procedure, see appendix I. Continue with the following step:

3.3 Incubate all samples according to Table 2A.2

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

Tab. 3.2: Thermal incubation profile of Ligation Reaction

Temperature [°C]	Hold	Volume [µl]
15	Overnight (16 hours)	10µl
15	∞	

Step 4: Primary PCR

Follow all steps of the primary PCR according to the Ampli1 whole genome amplification procedure, see appendix I.

Appendix I: Ampli1 whole genome amplification procedure

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

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*Ampli*1™ WGA Kit

Whole Genome Amplification for Single Cells

USER MANUAL

Version 02

Content version: May 2014

REF WG 001 050 R02

50 reactions

Store the kit at −20°C

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Appendix I: Ampli1 whole genome amplification procedure

Appendix I: Ampli1 whole genome amplification procedure

1. Kit Contents

	Vial	Label	Cap Color	Contents
Reagents	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

Appendix I: Ampli1 whole genome amplification procedure

2. Storage and Handling

Store the *Ampli*1™ WGA Kit at –20°C; ship at –20°C. Transfer Enzyme 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 set up 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 (MSDS).
MSDS of all Silicon Biosystems kits and components are available online at http://www.siliconbiousa.com.

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

Appendix I: Ampli1 whole genome amplification procedure

6. Additional Required Materials

- Thermal Cycler (e.g. Applied Biosystems®, GeneAmp® 9700 or superior)
- 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 an *Ampli*1™ WGA procedure is a library of highly concentrated DNA, which can be employed for further targeted genetic research analysis. The main features 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 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
- Optimized amplification of all adapter-ligated sequences.

Appendix I: Ampli1 whole genome amplification procedure

8. *Ampli*1™ WGA Kit Application

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

- Mutation detection by sequencing
- Mutation detection by pyrosequencing
- SNP detection
- Microsatellite or other PCR-based genotyping analysis
- Metaphase CGH
- ArrayCGH
- Next Generation Sequencing

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 contact Silicon Biosystems Technical Support to check for compatibility with your research protocol. An example is provided in Appendix A for illustration purposes only.

*Ampli*1™ WGA uses a polymerase with proofreading activity, with lower error rate (4.8X10-6) with respect to standard Taq DNA polymerases.

9. How to use the *Ampli*1™ WGA product

- Quality Control: to evaluate the quality of the *Ampli*1™ WGA output product it is strongly advisable to run the *Ampli*1™ QC Kit (Silicon Biosystems, Product code: WG-QC4-200 RWB). This assay is a multiplex PCR of four markers indicative of the quality of the DNA library obtained, and predictive of the success rate of further downstream assays. Please refer to the *Ampli*1™ QC Kit user manual for further specification.
- Purification: *Ampli*1™ WGA output product does not require purification for most downstream applications (*e.g.* PCR, sequencing). If purification is needed, it is advisable to use Agencourt® AMPure® XP kit (Part. Number: A63880, Beckman Coulter), which minimizes template loss. Purification with this kit further eliminates residual unligated primers left in solution at the end of the WGA reaction.

Appendix I: Ampli1 whole genome amplification procedure

- Quantitation: for *Ampli*1™ WGA output product quantification, Qubit® Fluorometer (Life Technologies™) with the corresponding Qubit® dsDNA BR Assay Kit (ref: Q32850), is recommended. This method quantifies all the double stranded DNA produced and present in the *Ampli*1™ WGA output products.
  NOTE: WGA primers and adaptors, unligated to a fragment but left in the reaction after the WGA Primary PCR, are also double stranded and they will be included in the overall quantitation of the sample. In order to have a more reliable quantitation of the *Ampli*1™ WGA target DNA it is suggested to perform a purification step prior to the quantitation.
- Reamplification protocol:1 ul of Ampli1TM WGA product can be reamplified to obtain 50 ul of reamplified WGA DNA for further downstream analysis, if required.
  Please contact ampli1.support@siliconbiosystems.com for *Ampli*1™ WGA Reamplification protocol.
- No-cell Control: the no-cell control each from *Ampli*1™ WGA run should be analyzed in downstream assays such as *Ampli*1™ QC or *Ampli*1™ Gene-specific Kits. A negative assay result from the no-cell control confirms the absence of contamination in the WGA workflow. Other evaluation methods may not be suitable for the *Ampli*1™ WGA no-cell control: some amplification of non-human DNA background, originating from the bacterial DNA present in the enzymes (see chapter 3 of this manual), may occur. This will negatively affect quantitation methodologies and result in a smear on an agarose gel, similar to what is typically obtained for the samples with cells. For this reason, running agarose gel to evaluate *Ampli*1™ WGA yield is NOT recommended.
- Use in PCR: the *Ampli*1™ WGA products can be directly used in PCR without dilution and/or purification. In standard amplification conditions (20 µl of reaction volume, with 0.5 µM final concentration of each primer) 2 µl of *Ampli*1™ WGA product shall be used. Lower or higher input could generate amplification drop-out and/or unspecific amplification.
  Use of kits from the *Ampli*1™ product line (Silicon Biosystems®) for the amplification of cancer related mutations (such as KRAS, BRAF, PIK3CA, EGFR, ALK) is strongly recommended to take advantage of the pre-validated and optimized reagents and conditions.
- Use in Next Generation Sequencing:
  1-Amplicon based methods (AmpliSeq or similar) can be directly applied to the WGA DNA obtained from the procedure, provided no *Ampli*1™ restriction site (TTAA) is present in the amplicon (Appendix A).
  2-Capture based methods: digestion of WGA adaptors is recommended before sequencing library preparation, in order to avoid unwanted unspecific product capture. Please contact ampli1.support@siliconbiosystems.com to obtain latest version of the removal protocol.
  3- Whole Genome sequencing: a sonication of the WGA product, after WGA adaptor removal and before sequencing is advised to reduce size of larger WGA fragments .

Appendix I: Ampli1 whole genome amplification procedure

10. 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 (up to 1000) or DNA (*e.g.* from tissue, or FFPE samples) resuspended in 1 µl of PBS1X. Although best results are obtained with live cells, the *Ampli*1™ WGA Kit allows whole genome amplification 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 the yield: staining with Hoechst 33342 (Sigma-Aldrich cat. B2261), final staining concentration 1 µg/ml, 5-10' at RT is a suitable working condition.
- *Ampli*1™ Whole Genome Amplification Kit is also suitable for the amplification of DNA from single sperm cells. In this case it is mandatory to add 1 µl of DTT 100 mM in the lysis reaction calculated for 10 samples; the volume of water should be decreased from 12.8 to 11.8 µl to reach a final concentration of 5 mM DTT in 20 µl. For more than 10 samples, adjust all reagent volumes.

Appendix I: Ampli1 whole genome amplification procedure

11. Things to Do Before Starting

1. Working Area Organization

The *Ampli*1™ WGA Kit has been optimized to enable amplification of DNA content from one single cell; as such, it is a powerful tool to amplify nucleic acid. In order to prevent any contamination due to carryover of amplified DNA, 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, pipettes, 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, remove the tubes from the thermal cycler and store them in a -20°C freezer in a separate lab space dedicated to downstream analysis of amplified products.
- Perform each type of downstream analysis (*e.g.*, PCR, sequencing, mCGH, etc) in a separate lab with dedicated equipment and materials: this step is the most important aspect in order to avoid carryover of amplified DNA.

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: prepare reaction mix for 10 samples to accomodate the no-cell and positive controls.

3. Pipetting Tips

All pipetting must be 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, in order to avoid inadvertently removing the cell.
- Add the required volume by pipetting the fluid directly onto the wall of the tube, without disturbing the fluid already present in the tube.
- Always collect all the liquid by a short centrifuge spin after adding reaction mix and before putting the samples in the thermal cycler.

Appendix I: Ampli1 whole genome amplification procedure

12. *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.

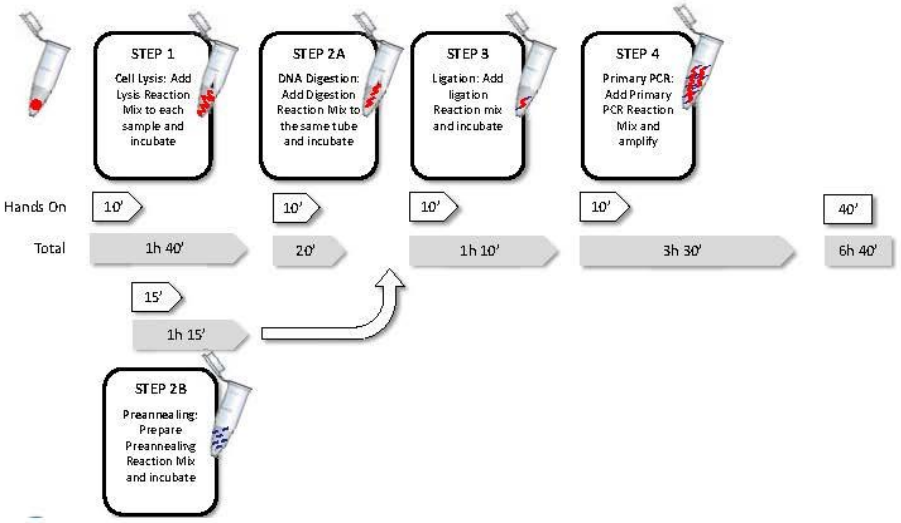

Fig. 1: *Ampli*1™ Whole Genome Amplification Procedure

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.

Appendix I: Ampli1 whole genome amplification procedure

Step 1: Cell Lysis

1.1. Prepare Lysis Reaction Mix according to Table 1.1.

⚠ It is recommended to prepare a Lysis Reaction Mix for at least 10 reactions even if fewer than 10 samples will be run (excess reagents for the Lysis Reaction Mix have been included in the *Ampli*1™ WGA Kit). To prepare a larger amount of Lysis Reaction Mix, increase all reagent volumes proportionally.

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 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 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.

Appendix I: Ampli1 whole genome amplification procedure

Tab. 1.2: Thermal incubation profile of Lysis Reaction

Temperature [°C]	Hold	Volume [µl]
42	45 minutes	3
65	30 minutes	
80	15 minutes	
4	∞	

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.

Optional: for user convenience the lysis step (incubation at 42°C) can be extended up to an overnight incubation.

For the success of the entire procedure, it is crucial to run the enzyme inactivation steps (30 minutes at 65°C and 15 minutes at 80°C). Those steps must be kept also if an overnight lysis step at 42°C is performed.

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

Step 2A: DNA Digestion

2A.1 Prepare Digestion Reaction Mix according to 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 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.

Appendix I: Ampli1 whole genome amplification procedure

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	5 minutes	5
65	5 minutes	
4	∞	

Once the thermal cycler has reached 4°C, remove all the reaction tubes; place 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 Table 2B.1

This step could be done in parallel with step 1 (advisable) and/or 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 a component for the reaction mix of step 3: for each different reagent, calculate the volume required by multiplying the overall number of samples times the required volume for one sample.

Optional: Preannealing Reaction Mix can be pre-made and stored at -20°C. However, Preannealing Reaction Mix for all 50 samples cannot be made in a single 0.2 ml PCR tube, as the total volume will exceed the limit of volume under temperature control in the thermal cycler.

Therefore, it is advisable to divide Preannealing Reaction Mix into aliquots corresponding to the planned use, *e.g.* if each *Ampli*1™ WGA run tipically includes for 10 samples, it is advisable to divide the Reaction Mix into 5 aliquots for 10 samples each.

Appendix I: Ampli1 whole genome amplification procedure

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 down in order to collect all 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.

🔍 it is crucial to follow exactly the thermal amplification profile for the Preaannealing reaction. Please refer to the Thermal Cycler User Manual to set the correct parameters.

Appendix I: Ampli1 whole genome amplification procedure

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 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 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	1 hour	10
15	∞	

Optional: Ligation Reaction may be extended to an overnight 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

Appendix I: Ampli1 whole genome amplification procedure

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 40 µl of Primary PCR Reaction Mix 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 all the sample tubes.

Put 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 57 68	40 sec 30 sec 1:30* min:sec	* = +1 sec/cycle	
8	94 57** 68	40 sec 30 sec 1:45* min:sec	** = +1°C/cycle * = +1 sec/cycle	50
22	94 65 68	40 sec 30 sec 1:53* min:sec	* = +1 sec/cycle	
1	68 4	3:40 min:sec ∞		

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

⚠ Store the samples at –20°C.

It is crucial to follow the thermal amplification profile indicated for the Primary PCR, as it guarantees a good amplification yield for longer DNA fragments. Please refer to the Thermal Cycler User Manual to correctly set the amplification parameters.

13. 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.

14. 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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Appendix I: Ampli1 whole genome amplification procedure

15. 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↓TAA ... 3'

3'... AAT↑T ... 5'

The specific action of the Digestion Enzyme makes 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 in the *Ampli*1™ WGA amplification product, requires specific considerations:

1. Identify the target of the downstream assay: sequence, mutation, micro-satellite, 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 considering the WGA-Amplicon generated.
7. Do not design primers that overlap digestion sites.

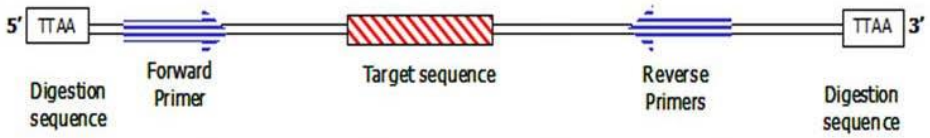

Fig. 2: Primers design for PCR as downstream analysis

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 possible degenerate base variants, if present.
3. Do not design primers that overlap digestion sites.