SurePlex Summary Protocol

Reference Guide

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Document # 15053626 v02

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September 2020

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Introduction

The SurePlex DNA Amplification System amplifies DNA from embryo biopsy samples (blastomere and trophectoderm biopsied cells) to quantities suitable for the VeriSeq PGS assay. This process creates highly representative samples that are ideal for pre-implantation genetic screening (PGS) during an *in vitro* fertilization (IVF) cycle.

SurePlex follows an easy-to-use single tube protocol. It is possible to complete the SurePlex protocol within a two and a half hour period.

Recommendations

For quality results, adhere to the following recommendations.

Sample Input Recommendations

All biopsy samples for VeriSeq PGS processing must be suspended in molecular grade 1x PBS (Phosphate Buffered Saline) and, if used, with a maximum concentration of 0.5% PVP (Polyvinylpyrrolidone) in a volume of 2.5 µl. Validate before using any alternative sample collection volume, wash, and collection buffers.

Validate biopsy sample storage and shipment conditions before use. These factors may impact the quality and integrity of the samples. To maintain the integrity of the biopsy samples, store the biopsies at -65°C to -85°C and ship the biopsy tubes frozen. Store the biopsy tubes at -65°C to -85°C after collection. Complete the cell lysis to the PCR amplification steps within 14 days as described in the library preparation sections of this guide. Biopsy stability has not been evaluated under storage and shipping conditions other than these recommendations.

Protocol Compliance

- Always use the current version of the SurePlex Summary Protocol Reference Guide, found on the Illumina Support website.
- Avoid contamination of samples and reagents during library preparation. Contamination of reagents will compromise your test results.
- Incubation times, incubation temperature, and pipetting volumes that differ from the specifications in this protocol will lead to suboptimal end products and compromise your test results.
- Adhere to all centrifuge and mixing steps in the protocol. Deviation from the recommended centrifugation and vortexing steps will lead to suboptimal end products and will compromise your test result.

Warnings and Precautions

- Always use Personal Protective Equipment (PPE) during the laboratory steps.
- This kit is intended for use by qualified laboratory staff only. All laboratories are expected to follow Good Laboratory Practices (GLP) and have appropriate safety control measures in place.
- Check the documentation and safety data sheets (SDS) for guidelines on handling, preparing, and disposing kit components, intermediate mixtures, or wastes. For more information, see the SDS for this kit at support.illumina.com/sds.html.
- All components have an expiration date. Do not use kit components beyond the expiration date printed on component labels.

Best Practices

- Conduct all pre-amplification activities (cell lysis, pre-amplification, and amplification set-up procedures) in a dedicated environment (Pre-PCR) physically separated from amplified genetic material.
- Use a sterile containment cabinet (vertical laminar flow cabinet) when handling embryo biopsies, during pre-amplification and amplification set up steps of the protocol. Follow applicable safety practices when handling embryo biopsies.
- Thoroughly clean the containment cabinet before starting the protocol.
- If recommended by the cabinet manufacturer, incubate tips, tubes, and pipettes with UV irradiation in the containment cabinet before starting the protocol.
- Make sure that you have a clean set of calibrated pipettes that are suitable for Pre-PCR activities, preferably exclusively dedicated to the SurePlex amplification protocol.

Avoiding Contamination

- Pre-PCR environmental and sampling control measures (i.e., single-use of molecular grade reagents and consumables) are required to reduce the risk of sample contamination prior to input into the SurePlex reaction.
- Failure to follow Pre-PCR best practices during the reaction set-up may lead to contamination of the PCR products and may affect subsequent analysis and/or compromise your test results.

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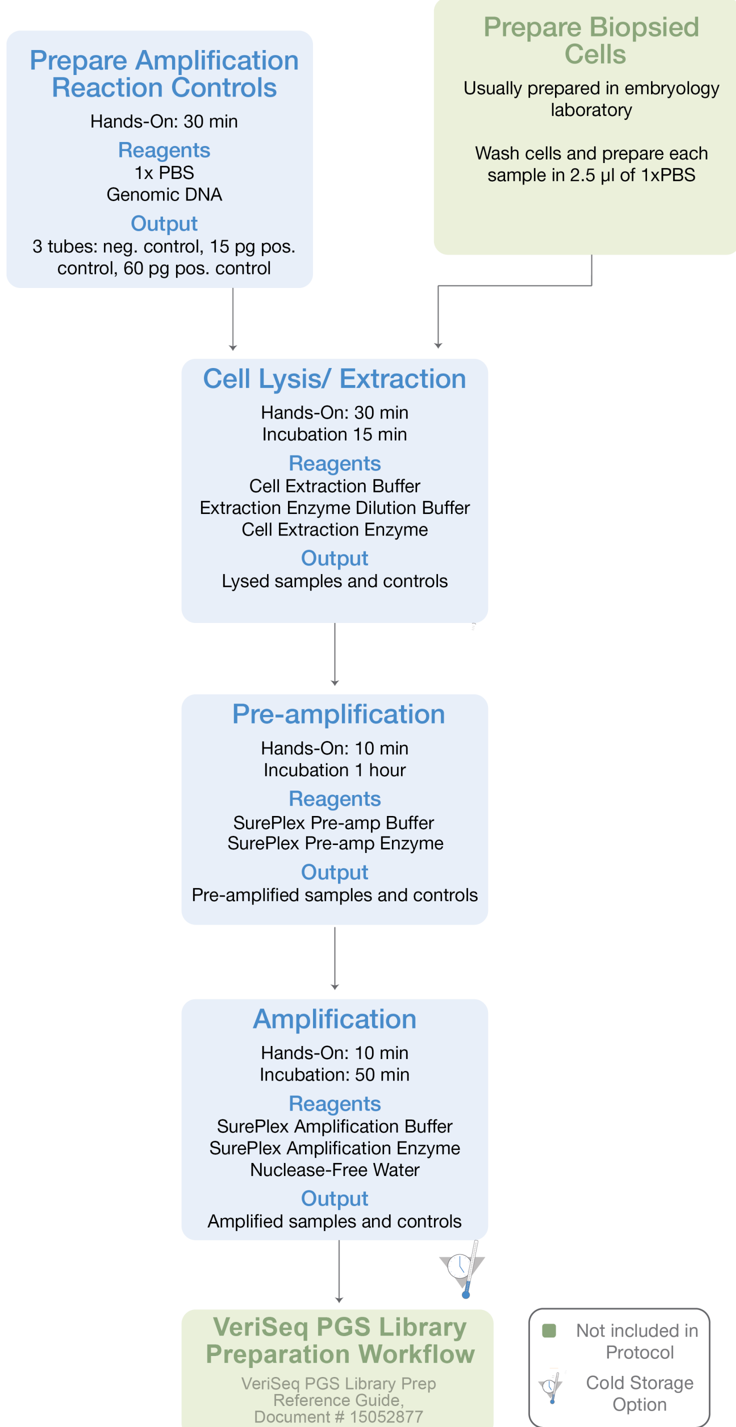
Workflow Diagram

Figure 1 SurePlex Protocol Workflow Diagram



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Preparation and Materials

Table 1 Materials Required for Sample Preparation

Materials Required	Ordering Information
SurePlex DNA Amplification System	Illumina PR-40-415101-00
20x PBS (dilute to 1x with nuclease-free water)	Cell Signaling Technologies 9808
Genomic control DNA	Promega G1521
PCR tubes (0.2 ml, thin walled, flip cap) OR 96-well PCR plate and adhesive plate seals	Thermo Scientific AB-0620 Thermo Scientific AB-0600 (plate) AB-0558 (seals)
Microcentrifuge tube (1.5 ml, flip cap)	Sarstedt 72.690.001

Table 2 Starting Materials for Sample Preparation

Starting Materials
Single cell in 2.5 µl 1x PBS in 0.2 ml tubes or PCR plates
Positive control 1: 15 pg genomic DNA in 2.5 µl 1x PBS
Positive control 2: 60 pg genomic DNA in 2.5 µl 1x PBS
Negative control: 2.5 µl of 1x PBS (0.2 ml tube/PCR plate)
Negative control: 2.5 µl of collection buffer control (0.2 ml tube/PCR plate)

SurePlex DNA Amplification System (50 reactions) - Kit Components

Table 3 SurePlex DNA Amplification System Kit Components

Component Name	Cap Color	Volume	Quantity	Prepare
Cell Extraction Buffer		300 µl	1	Thaw on ice
Extraction Enzyme Dilution Buffer		300 µl	1	Thaw on ice
Cell Extraction Enzyme		15 µl	1	Transfer to ice before use
SurePlex Pre-amp Buffer		300 µl	1	Thaw on ice
SurePlex Pre-amp Enzyme		15 µl	1	Transfer to ice before use
SurePlex Amplification Buffer		1.4 ml	1	Thaw on ice
SurePlex Amplification Enzyme		50 µl	1	Transfer to ice before use
Nuclease-free water	Clear	1 ml	2	Thaw on ice

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Preparation of Amplification Reaction Controls

Estimated Time

Hands-on time: 30 minutes

Consumables

Item	Quantity
1x PBS	805 µl
Genomic DNA (100ng/µl)	5 µl

Steps

1 In a sterile containment cabinet (vertical laminar flow cabinet), label four sterile 1.5 ml microcentrifuge tubes and prepare their contents, as in Table 4.

Table 4 Control Tubes for Sample Preparation

Control Tube	Description	Volume of 1x PBS	Volume of Female Genomic DNA /Stock Solution
1	1x PBS negative	100 µl	None
2	2.5 ng/µl positive	195 µl	5 µl of 100 ng/µl stock
3	25 pg/µl positive	495 µl	5 µl of 2.5 ng/µl stock (from tube 2)
4	6.25 pg/µl positive	15 µl	5 µl of 25 pg/µl stock (from tube 3)

2 Add 1x PBS into each tube, adding the volumes shown in Table 4.

3 Add 5 µl of female genomic DNA to tube 2 and mix.

4 Dilute 5 µl of tube 2 into tube 3 and mix.

5 Dilute 5 µl of tube 3 into tube 4 and mix.

6 In the sterile containment cabinet, label three sterile 0.2 ml flat-capped PCR tubes as in Table 5.

Table 5 PCR Tubes for Sample Preparation

PCR tube	Label on Tube Cap	Contents
1	15.6 pos	2.5 µl of control tube 4 (6.25 pg/µl positive)
2	62.5 pos	2.5 µl of control tube 3 (25 pg/µl positive)
3	Neg	2.5 µl of control tube 1 (1x PBS)

7 Pipette 2.5 µl of the control tubes into the PCR tubes, according to the contents listed in Table 5.

NOTE

Use a clean tip for each tube and cap each tube immediately after transfer.

8 Store the control PCR tubes in a 96-well rack on ice until required.

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Cell Lysis/Extraction

Estimated Time

Hands-on time: 30 minutes

Incubation time: 15 minutes

Consumables

Item	Quantity
1x PBS	2.5 µl per sample
Cell Extraction Buffer (green cap)	2.5 µl per sample
Extraction Enzyme Dilution Buffer (purple cap)	4.8 µl per sample
Cell Extraction Enzyme (yellow cap)	0.2 µl per sample

Steps

1 Collect samples in 2.5 µl of 1x PBS and 2.5 µl of collection buffer control. Line up sample tubes and control tubes in a 96-well rack on ice until required.

2 Centrifuge the tubes/plate at 200 × g for 3 minutes (at 4°C if possible).

3 Add 2.5 µl of Cell Extraction Buffer (green cap) to each sample (including controls) and store at 2°C to 8°C.

4 Following the specifications in Table 6, prepare the Extraction Cocktail master mix on ice and mix well.

Table 6 Extraction Cocktail Master Mix

Extraction Cocktail	Cap Color	Volume per Single Sample	Volume per 5 Samples
Extraction Enzyme Dilution Buffer		4.8 µl	24 µl
Cell Extraction Enzyme		0.2 µl	1 µl
Total Volume		5 µl	25 µl

5 For every sample or control, add 5 µl of the freshly prepared Extraction Cocktail.

6 Briefly centrifuge samples to get all contents to the bottom of the tube.

7 Incubate samples in a PCR thermal cycler as in Table 7.

Table 7 Thermal Cycler Program for Cell Lysis/Extraction

Number of Cycles	Temperature of Cycle	Incubation Time
1	75°C	10 min
1	95°C	4 min
1	22°C	Hold

CAUTION

Always use a heated lid on a PCR machine. Do not carry out PCR reactions under oil.

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Pre-amplification

Estimated Time

Hands-on time: 10 minutes

Incubation time: 1 hour

Consumables

Item	Quantity
SurePlex Pre-amp Buffer (red cap)	4.8 µl per sample
SurePlex Pre-amp Enzyme (white cap)	0.2 µl per sample

Steps

1 Combine the Pre-amp Cocktail components as in Table 8 and mix well.

Table 8 Pre-amp Cocktail

Pre-amp Cocktail	Cap Color	Volume per Single Sample	Volume per 5 Samples
SurePlex Pre-amp Buffer		4.8 µl	24 µl
SurePlex Pre-amp Enzyme		0.2 µl	1 µl
Total Volume		5 µl	25 µl

2 For each 10 µl sample prepared in *Cell Lysis/Extraction*, add 5 µl of SurePlex Pre-amp Cocktail. Briefly centrifuge.

3 Incubate samples according to the thermal cycler program in Table 9.

Table 9 Thermal Cycler Program for Pre-amplification Reaction Products

Number of cycles	Temperature of cycle	Incubation Time
1	95°C	2 min
12	95°C 15°C 25°C 35°C 65°C 75°C	15 sec 50 sec 40 sec 30 sec 40 sec 40 sec
1	4°C	Hold

4 Place the pre-amplification reaction products on ice.

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Amplification

Estimated Time

Hands-on time: 10 minutes

Incubation time: 50 minutes

Consumables

Item	Quantity
SurePlex Amplification Buffer (orange cap)	25 µl per sample
SurePlex Amplification Enzyme (blue cap)	0.8 µl per sample
Nuclease-free water (clear cap)	34.2 µl per sample

Steps

1 Combine the Amplification Cocktail components in Table 10 and mix.

Table 10 Amplification Cocktail components

Amplification Cocktail	Cap Color	Volume per Single Sample	Volume per 5 Samples
SurePlex Amplification Buffer		25 µl	125 µl
SurePlex Amplification Enzyme		0.8 µl	4 µl
Nuclease-free water	Clear	34.2 µl	171 µl
Total Volume		***60 µl***	***300 µl***

NOTE

Sample amplification efficiency may be analyzed by using a real-time thermal cycler and adding SYBR Green I dye (Invitrogen, Catalog # S7563) at 0.125x final concentration in the Amplification Cocktail.

2 Add 60 µl of the freshly prepared Amplification Cocktail to the 15 µl pre-amplification reaction product, cap the tube, and invert to mix. Centrifuge briefly.

3 Amplify samples according to the thermal cycler program in Table 11.

Table 11 Thermal Cycler Program for Amplification of Samples

Number of Cycles	Temperature of Cycle	Incubation Time
1	95°C	2 min
14	95°C 65°C 75°C	15 sec 1 min 1 min
1	4°C	Hold

4 Keep amplified SurePlex products on ice before proceeding with the VeriSeq PGS assay protocol.

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SAFE STOPPING POINT

If you are stopping, leave the tubes on the thermal cycler overnight at 4°C or store at -25°C to -15°C for up to one month. The integrity of the PCR amplification products is affected by suboptimal storage conditions, the quality and grade of the plastic consumable used, and the nuclease-free control measured implemented..

Revision History

Document	Date	Description of Change
Document # 15053626 v02	September 2020	• Added Avoiding Contamination sub-section to Best Practices. • Updated workflow diagram. • Updated the lysis hold temperature program. • Updated the description of the safe stopping point after the amplification step. • Added the amplification hold temperature program.
Document # 15053626 v01	February 2018	• Converted document to new style and reference guide format. • Removed references to 24sure arrays. • Updated volumes in Kit Components table; added a Quantity column. • Revised Introduction and added Recommendations section. • Corrected minor errors and text edits.
Document # 15053626 v00	July 2015	Initial release.

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Technical Assistance

For technical assistance, contact Illumina Technical Support.

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Safety data sheets (SDSs)—Available on the Illumina website at support.illumina.com/sds.html.

Product documentation—Available for download from support.illumina.com.

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Document # 15053626 v02

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