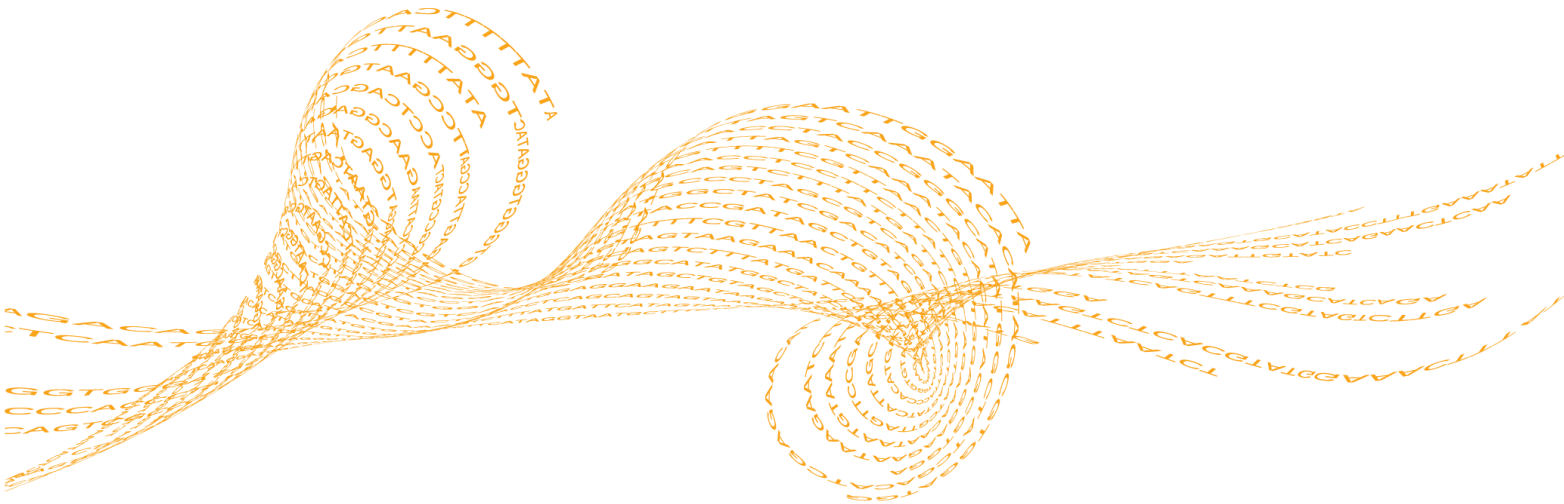
Single Cell Washing and Handling



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Introduction

This document describes the steps involved in washing single cells (polar body 1, polar body 2, blastomere, and trophectoderm biopsy) that have been dissected from an oocyte, embryo, or blastocyst. It then describes the steps for loading the single cells into PCR tubes for use with DNA amplification techniques such as SurePlex and SureMDA and then used with 24sure, VeriSeq PGS, and 24sure+ technologies.

The information supplied in this document is offered as a guide only and Illumina accepts no responsibility for carrying out the procedure. Good laboratory practice and good scientific judgment are always required. Adapt the protocol according to local laboratory rules, regulations, and the facilities available.

Appropriate witnessing may be required and proper procedures should be established in the local setting.

Precautions

DNA contamination into the PCR tubes must always be minimized. Extraneous, contaminating DNA could result in an incorrect diagnosis and lead to a serious adverse outcome.

Minimize DNA contamination by:

- Always keeping gloves sterile and clean.
- Changing gloves immediately if you suspect contamination has occurred.
- Handling PCR tubes carefully, as described; only touching the outside.
- Never touching the mouthpiece and proximal tubing of the mouth pipette.
- Never allowing the tubing of the mouth pipette to touch skin.
- Carrying out the procedure, including the setting up of culture dishes and PCR tubes, in a laminar flow hood.
- Avoiding keeping PCR tubes open for longer than necessary.
- Wearing appropriate personal protective equipment (lab coat, gloves, cap, facemask).

Equipment

1 **PBS 1% PVP**
   - Sterile PBS (20x stock supplied by Cell Signaling Technologies – Catalog No. 9808)
   - PVP (10% clinical grade liquid supplied by Origio – Catalog No. 10905000)

   NOTE
   To make PBS (1x) PVP (1%), aliquot 0.5 ml 20x PBS, 1 ml 10% PVP, and 8.5 ml sterile nuclease free water into 0.5 ml volumes. Aliquots can be stored at -25°C to -15°C.

2 **Sterile 0.2 ml PCR tubes**, stored in small sterile bags

3 **Culture medium**, containing 4mg/ml albumin

4 **Sterile, long Pasteur pipettes**, pulled and flame polished (one for each cell plus a few extra)

5 **Mouth pipette**

   NOTE
   Using a mouth pipette is highly recommended for loading small volumes with the cell.

6 **35 mm sterile culture dishes**

7 **0.2 ml PCR tube rack**

8 **Chilled 0.2 ml PCR tube rack**

9 **Dissecting microscope**

10 **Sterile forceps**

11 **Dedicated 10 µl pipette**

12 **Sterile 10 µl filter tips**

13 **Fine-tipped permanent marker pen**

Preparation

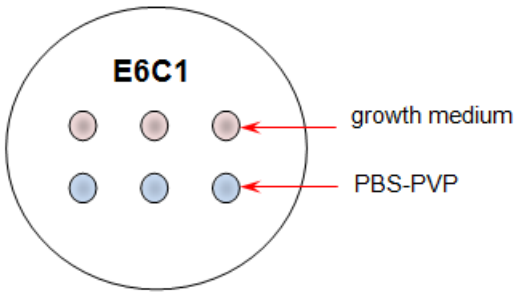
To perform this procedure, it is necessary to wear clean theater dress and a long sleeved gown. Sterile gloves must be worn, covering the cuffs of the gown, and hair must be covered with a cap. The preparation of dishes and tubes must be carried out in a clean laminar flow hood to protect the tubes from contamination.

1 In the hood, remove the same number of culture dishes as there are cells. Use one culture dish per cell.

2 Label the dishes in accordance with local procedures on both the lid and the base. For example, label the embryo number and cell number i.e. E6C1 would be cell 1 of embryo number 6.

3 Using a clean, sterile Pasteur pipette, add three small drops (~15–20 µl) of growth medium and three small drops (~15–20 µl) of PBS-PVP arranged, as shown in Figure 1.

Figure 1 Growth medium and PBS-PVP arrangement in culture dish



4 When the dishes are prepared, place dishes to the side, within reach of the microscope.

5 In the hood, open the sterile autoclave bag containing the PCR tubes. Remove the tubes one by one from the bag, using the clean forceps, and place them in the room temperature tube rack. Close the lids immediately, taking care not touch the inside of the lid.

6 Each cell requires one 0.2 ml sterile tube. Using a fine tipped marker pen, label the tubes in a similar way to the dishes, i.e. E6C1. For each patient, it is necessary to collect a small volume of PBS-PVP from one of the last cell washes to provide a negative control in the SurePlex reaction. It is necessary to prepare one extra tube per patient and label accordingly. Label the tubes on the top and sides, as shown in Figure 2.

Figure 2 Example of 0.2 ml sterile tube

7 Using a 10 µl pipette and sterile filter tips, pipette 2 µl of PBS-PVP to each tube. Be careful not to touch the tip anywhere other than inside the tube. Change to a clean sterile tip every three or four tubes, or whenever contamination is suspected.

8 When all the tubes are prepared, place tubes in a rack in the order that they are required.

NOTE
For the SureMDA protocol, Illumina recommends collecting samples in 4 µl PBS-PVP.

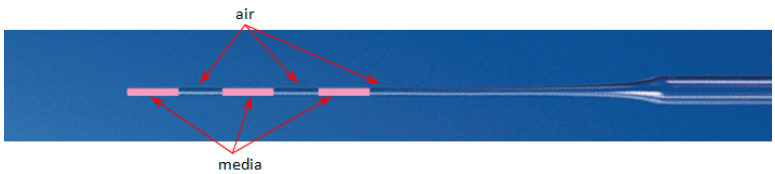
Cell Wash Protocol

1 Put the mouthpiece in the mouth pipette, being careful not to allow gloves to touch the mouth or skin.

2 Place the dish containing biopsied cells on to the microscope stage. Make sure that the patient ID and the embryo number on the dish containing the cells match the labeling on the culture dishes and PCR tubes.

3 Put a pulled, flame-polished Pasteur pipette into the mouth pipette. Load the pipette with clean culture medium (plus albumin) so that there are three sections of medium separated by air, as shown in Figure 3.

Figure 3 Pipette with clean culture medium, loaded as three sections separated by air



4 Looking into the microscope, pick up a cell from the dish on the microscope stage in a minimal volume of media. Refer to Figure 4 for placement of the cell.

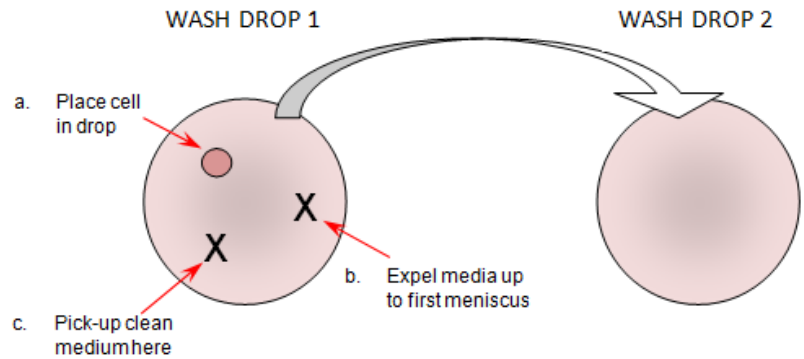
a Place the cell in the top left of wash drop 1 in the labeled wash dish.

b Move the pipette to another location in wash drop 1 and expel any remaining media up to the first meniscus of the pipette.

c Move the pipette to another location still in the same drop and pick up a small volume of media.

d Pick up the cell in minimal volume and move it to the second drop (wash drop 2) of the same dish

Figure 4 Wash drop 1 and Wash drop 2



5 Repeat steps a–c in wash drop 2 and 3, and through the PBS-PVP wash drops, 4–6.

6 At wash drop 6, pick up the cell in a small volume of PBS-PVP. Make sure that the cell is not too close to the tip of the pipette or too close to the meniscus. While holding the pipette, pick up the correctly labeled PCR tube. Open the lid using only the lip and hold the tube sideways on the stage of the microscope so the PBS-PVP can be visualized at the bottom of the tube.

7 Carefully insert the pipette tip into the tube. Take care to avoid the pipette touching the outside of the PCR tube, and to avoid breaking the pipette into the tube.

8 Use the microscope to observe the pipette tip as it goes into the PBS-PVP, trying to avoid touching the pipette on the side of the tube.

9 Gently expel the cell into the PBS-PVP in a minimal volume so that when combined with the 2 µl in the tube, the volume is ~2.5µl. Remove the pipette from the tube, close the lid of the tube, and place it in the chilled PCR tube block.

10 To collect PBS-PVP wash for use as a negative control, use the same pipette. Go back to wash drop 6 and pick up a small volume of PBS-PVP (the equivalent to the amount transferred with the cell). Carefully add the PBS-PVP to the appropriate PCR wash tube.

11 Discard the pipette. If the cells are going to be processed within 24 hours, store at 2°C to 8°C until used. Alternatively, store the tubes at -25°C to -15°C, while retaining the PBS-PVP, including cells, at the bottom of the tubes.

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 1 Illumina General Contact Information

Illumina Website	www.illumina.com
Email	techsupport@illumina.com

Table 2 Illumina Customer Support Telephone Numbers

Region	Contact Number	Region	Contact Number
North America	1.800.809.4566	Italy	800.874909
Austria	0800.296575	Netherlands	0800.0223859
Belgium	0800.81102	Norway	800.16836
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	United Kingdom	0800.917.0041
Ireland	1.800.812949	Other countries	+44.1799.534000

Safety Data Sheets

Safety data sheets (SDSs) are available on www.cambridgebluegnome.com.

Product Documentation

Product documentation in PDF is available for download from the Illumina website. Go to www.illumina.com/support, select a product, then click **Documentation & Literature**.

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