EMBRYOMAP SNP SAMPLE PREP GUIDE

Vitrolife

PART OF VITROLIFE GROUP

Vitrolife Sweden AB
Box 9080
SE-400 92 Göteborg
Sweden
Tel: +46-31-721 80 00

Vitrolife Inc.
3601 South Inca Street
Englewood
Colorado 80110
USA
Tel: +1-866-VITRO US (866-848-7687)

Vitrolife Pty Ltd
Level 10, 68 Pitt Street
Sydney NSW 2000
Australia

Revision history

Version	Date	Description of Change
v1.0	21-Jul-2025	New document

TABLE OF CONTENTS

1 INTRODUCTION

The EmbryoMap SNP Sample Prep workflow simultaneously performs amplification of the DNA backbone and targeted Single Nucleotide Polymorphism (SNP) markers from multi-cell samples (e.g. trophectoderm biopsy) and prepares sequencing-ready libraries for Illumina sequencing systems. Sample assessment is enhanced by Copy Number variation analysis and the addition of SNP analysis allowing identification of external DNA contamination, and the differentiation between haploid, diploid and triploid samples.

This protocol explains how to:

- Prepare up to 96 unique dual index libraries for CNV and SNP analysis from multi-cell in vitro biopsies.
- Prepare multiplexed library pools of up to 48 samples for cluster generation and paired-end sequencing on the Illumina MiSeq® System.

The EmbryoMap SNP Solution protocol offers:

- Rapid and easy whole-genome amplification and library preparation in a single workflow.
- Low DNA input from 3-10 cells.
- Ready-to-use master-mixed reagents to reduce reagent containers, pipetting, and hands-on time.
- Safe stopping points, and sample retention points for repeat testing.

Embryo Biopsy Collection, Storage and Shipping Recommendations

Collect biopsy samples into 0.2 mL, thin-walled PCR tubes. Biopsy samples should be suspended in a total volume of 2.5 µL of molecular grade 1× PBS (Phosphate Buffered Saline) and, if used, a maximum concentration of 0.5% PVP (Polyvinylpyrrolidone). Validate before using any alternative sample collection volume, wash, and collection buffers.

Validate sample storage and shipment conditions before use, particularly if different from the following recommendations. These factors may impact the quality and integrity of the samples. For best results, store embryo biopsies at -65°C to -85°C and keep frozen during shipping. Perform the DNA Extraction and Template Preparation steps within 14 days of sample collection.

Workflow Recommendations

Protocol Compliance

- Always use the latest version of the EmbryoMap SNP Sample Prep Guide, found on the Vitrolife website.
- Avoid contamination of samples and reagents during sample preparation. Contamination of reagents will compromise your test results.
- Incubation times, incubation temperatures, 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 centrifugation and mixing steps in the protocol. Deviation from the recommended 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 of kit components, intermediate mixtures, or wastes.
- Shipping conditions might differ from storage conditions. Some kit components must be brought to room temperature before use.
- All components have an expiration date. Do not use kit components beyond the expiration date printed on component labels.
- The EmbryoMap Sample Prep kits and EmbryoMap SNP Reagent kits are manufactured in batches, called Lots, in accordance with strict quality standards. Each component or reagent in a single lot is tested to work with other reagents in the same Lot. To ensure proper performance, avoid interchanging, mixing, or combining reagents from different kits and lots.
- Do not exceed the specified maximum multiplexing capacity of pooled libraries (with positive amplification), per sequencing instrument. Higher levels of multiplexing might affect the data quality, resulting in reduced sample performance.

Best practices

- Conduct the DNA Extraction, Template Preparation and assembly of PCR reactions in a dedicated Pre-PCR environment, physically separated from exponentially-amplified genetic material.
- Use a sterile containment cabinet (vertical laminar flow cabinet) when handling embryo biopsies, during Template Preparation and Library Amplification 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 or consumables' 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 EmbryoMap protocol.
- Always use a heated lid on the PCR machine. Do not carry out PCR reactions under oil.

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 EmbryoMap reactions.
- 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.
- Always use a fresh pipette tip for each sample transfer and for adding reagents to each sample.
- Label and use separate reservoirs for each reagent.
- Seal any pierced wells of the Library Index Plate with an adhesive PCR plate seal after use to avoid contamination of work area. Change gloves if they come in contact with indexes.

Sealing the Plate

- Always seal 96-well plates before mixing, vortexing, centrifugation and thermal cycling steps.
- Apply the adhesive seal to cover the plate and seal by applying pressure around the edges of every well, e.g. with a rubber roller or applicator.

Handling SPRIselect beads

- Store the SPRIselect beads at room temperature, protected from light.
- Vortex mix the SPRIselect beads thoroughly before use.
- Pipette the SPRIselect bead suspension slowly as it is highly viscous.
- Make sure no SPRIselect bead suspension remains in the pipette after dispensing the beads into the plate wells.
- While aspirating the supernatant always place the pipette tips opposite to the bead pellet.
- If beads are accidentally aspirated with supernatant, dispense the beads back into the plate well and leave on the magnetic stand until liquid is clear.
- During the ethanol washes, dispense 80% ethanol to the opposite side to the bead pellet and do not disturb the pellet.
- Always prepare fresh 80% ethanol for use in the wash steps.

Plate transfers

- When transferring volumes between plates, transfer the specified volume from each well of a plate to the corresponding well of the other plate.
- The Library Index Plate has been designed so that each Index Name corresponds to the source plate well. For example, the unique dual index reagent in Library Index Plate well “A1” has the Index Name “UDI-A01”. To ensure sample tracking, always perform Library Amplification reactions and store final libraries in the plate well that corresponds to the index name and source well.

Additional Resources

The following documentation is available to download from the Vitrolife website.

Resource	Description
EmbryoMap eMap Software Guide (v4.0 or later)	Provides detailed instructions on run planning, data analysis and reporting with the eMap software.
eMap_Accessioning_A	Template for importing sample data for a single Subject.
eMap_Accessioning_B	Template for importing sample data for multiple Subjects.
eMap_SNP_FastTrack	Template for importing sample data, plate definition and index assignment for multiple Subjects.
eMap_QuantData	Template for uploading final library quantification data.
EmbryoMap SNP Workflow QC Guidance	Provides guidance on acceptance criteria for the data generated through the EmbryoMap SNP workflow.
EmbryoMap Calling Guide (v2.0 or later)	Provides detailed instructions on data analysis with the eMap software.

Please contact support.genomics@vitrolife.com for requirements and compatibility, additional documentation, software downloads, online training, frequently asked questions, and best practices.

For more information about updates to the MiSeq system guide and MiSeq Control Software (MCS), visit the MiSeq support pages on the Illumina website.

EmbryoMap Sample Prep Kit Components

Reagents for 96 reactions

Reagent Name	Identifier	Quantity	Description
Extraction Mix	Red cap	2	Reagents for 48 samples per tube
Amplification Mix 1	White cap	2	Reagents for 48 samples per tube
Amplification Mix 2	Blue cap	4	Reagents for 24 samples per tube
Elution Buffer	Clear cap	4	Reagents for 24 samples per tube
Library Index Plate A	96-well plate	1	Reagents for 96 samples per plate

EmbryoMap SNP Reagent Kit Components

Reagents for 96 reactions

Reagent Name	Identifier	Quantity	Description
SNP Panel 1	Yellow cap	1	Reagents for 96 samples per tube

2 PROTOCOL

Tips and Techniques

- Before beginning sample preparation, generate a Laboratory Planner using the eMap Software and generate the PDF to aid sample tracking.
- The entire workflow can be comfortably completed within the working day (approx. 4.5 hours). If the protocol must be stopped, observe the safe stopping points specified in the protocol.
- Remove cold-racks from frozen storage 10 minutes before use, to prevent samples from re-freezing during use.
- While preparing the control samples, do not mix positive control dilutions containing genomic DNA by vortexing. Use pipette-mixing or by flicking the capped tube to mix the contents. Pulse centrifuge the tubes to collect contents.

Preparation of Control Samples

Consumables

Item	Quantity
1× PBS	1.0 mL
Genomic DNA (100 ng/µL)	5 µL

Preparation

- Prepare the sterile Pre-PCR containment cabinet and associated consumables by cleaning with an appropriate DNA decontamination solution.
- If recommended by cabinet or consumables' manufacturer, incubate tips, tubes, and pipettes with UV irradiation in the containment cabinet before starting the protocol.
- Prepare a fresh aliquot of sterile 1× PBS, filtered at 0.22 µm.
- A higher input concentration of genomic DNA is required when running controls for SNP analysis. This is necessary to preserve the true allele balance ratio when diluting genomic DNA to picogram concentrations. A total input mass of 180 pg is recommended for SNP controls, whereas 62.5 pg is recommended for copy-number variation analysis controls.
  Alternatively, cell line samples (4-6 cells) of known karyotype may be used as a suitable control for SNP analysis, as sampling complete genomes does not perturb the allele balance at low input DNA mass.

Steps

1. In the Pre-PCR cabinet, label three sterile Lo-Bind 1.5 mL microcentrifuge tubes and prepare their contents, as in Table 1.

Table 1 Preparation of control samples

Control Tube	Description	Volume 1× PBS	Volume of genomic DNA / stock solution
Tube 1	1× PBS negative	100 µL	None
Tube 2	2.5 ng/µL positive	195 µL	5 µL of 100 ng/µL stock
Tube 3	72 pg/µL positive (SNP control)	236 µL	7 µL of Tube 2
Tube 4	25 pg/µL positive (CNV control)	62 µL	33 µL of Tube 3

2. Add 1×PBS into each tube, adding volumes shown in Table 1.
3. Add 5 µL of the 100 ng/µL genomic DNA to tube 2 containing 195 µL of 1× PBS and mix.
4. Add 7 µL of the diluted 2.5 ng/µL of genomic DNA from Tube 2 into Tube 3 containing 236 µL of 1×PBS and mix.
5. Add 33 µL of the diluted 72 pg/µL of genomic DNA from Tube 3 into Tube 4 containing 62 µL of 1×PBS and mix.
6. In the Pre-PCR cabinet label three sterile 0.2 ml flat-capped PCR tubes as in Table 2.

Table 2 PCR Tubes for Control Samples

PCR Tube	Description	Contents
1	Negative control	2.5 µL of control tube 1 (1× PBS negative)
2	Positive control (SNP)	2.5 µL of control tube 3 (72 pg/µL positive)
3	Positive control (CNV)	2.5 µL of control tube 4 (25 pg/µL positive)

7. Pipette 2.5 µL of each positive (SNP and CNV) and negative (1× PBS) control sample into the PCR tubes according to the contents listed in Table 2.

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

8. Store the control PCR tubes in a 96-well cold-rack until required.
9. Proceed immediately to the DNA Extraction step.

DNA Extraction

Consumables

Item	Identifier	Quantity
Extraction Mix	Red cap	7.5 µL per sample

Preparation

- Perform these steps in a Pre-PCR work area.
- Remove the cold-rack(s) from frozen storage 10 minutes before use.
- Thaw the Extraction Mix, invert to mix and pulse centrifuge. Keep on a cold-rack until use.

Steps

1. Centrifuge the embryo biopsy sample tubes and control sample tubes at 200 × g for 3 minutes (at 4°C if possible), then line up sample tubes in a 96-well cold-rack.
2. Add 7.5 µL of Extraction Mix to each sample tube, including controls. Dispense against the side of the tube above the sample droplet. Open one sample tube at a time.

ATTENTION
Do not pipette mix, flick mix or vortex the sample tubes. Use a fresh pipette tip for each sample.

Table 3 Components of the DNA Extraction reaction

Item	Cap colour	Volume (µL)
Sample	-	2.5
Extraction Mix	Red cap	7.5
Total		**10.0**

3. Centrifuge sample tubes at 280 × g for 20 seconds to collect all contents to the bottom of the tubes.
4. Incubate samples in a designated Pre-PCR thermal cycler as in Table 4, with a heated lid set at 101°C – 105°C.

Table 4 Thermal cycler program for DNA Extraction

Number of cycles	Temperature of cycle	Incubation time
1	75°C	10 min
1	95°C	4 min
1	4°C	Hold

5. After DNA Extraction, remove the sample tubes and centrifuge at 280×g for 20 seconds.
6. Place the sample tubes on a cold-rack and proceed immediately to the Template Preparation step.

Template Preparation

Consumables

Item	Identifier	Quantity
Amplification Mix 1	**White cap**	10 µL per sample
SNP Panel 1	**Yellow cap**	1 µL per sample

Preparation

- Perform these steps in a Pre-PCR work area.
- Thaw the Amplification Mix 1 and the SNP Panel 1, invert to mix and pulse centrifuge. Keep on a cold-rack until use.

Steps

1. Create a new "SNP Mix" by combining Amplification Mix 1 and EmbryoMap SNP Reagents according to the guidance in the table below, in a new 1.5 mL LoBind microcentrifuge tube.

Table 5 Components of the SNP Mix mastermix

Item	Volume per reaction	24 reactions*	48 reactions*	96 reactions*
Amplification Mix 1	10 µL	264 µL	528 µL	1056 µL
SNP Panel v1.0	1 µL	26.4 µL	52.8 µL	105.6 µL
Total	**11 µL**	**290.4. µL**	**580.8 µL**	**1161.6 µL**

*Includes 10% overage

2. Invert the SNP Mix to mix and pulse centrifuge to collect contents. Keep on a cold-rack until use. Do not vortex.

3. Add **11 µL** of **SNP Mix** to each sample tube including controls as in Table 6. Dispense against the side of the tube above the sample droplet. Open one sample tube at a time.

ATTENTION
Do not pipette mix, flick mix or vortex the sample tubes. Use a fresh pipette tip for each sample.

Table 6 Components of the Template Preparation reaction

Item	Volume
DNA Extraction product	10 µL
SNP Mix	11 µL per sample
Total	**21 µL**

4. Centrifuge sample tubes at 280 × g for 20 seconds to collect all contents to the bottom of the tubes.
5. Incubate samples in a designated Pre-PCR thermal cycler as in Table 7, with a heated lid set at 101°C – 105°C.

ATTENTION
Ensure the thermal cycler program for Template preparation with SNP Enrichment is labelled differently to the standard EmbryoMap Template Preparation Program.

Table 6 Thermal cycler program for Template Preparation with SNP Reagent enrichment

Number of cycles	Temperature of cycle	Incubation time
1	95°C	3 min
3	95°C	15 sec
	60°C	15 sec
	75°C	15 sec
1	75°C	3 min
16	95°C	15 sec
	15°C	50 sec
	25°C	40 sec
	35°C	30 sec
	65°C	40 sec
	75°C	40 sec
1	4°C	Hold

6. After completion of the Template Preparation program, remove the sample tubes and **mix well by inversion and/or flicking**, then centrifuge at 280 × g for 1 minute.
7. Return the amplified Template Preparation products to a cold-rack and proceed immediately to the Library Amplification step.

SAFE STOPPING POINT
Template Preparation products may be stored at -25°C to -15°C for up to 1 month. For long term storage, -65°C to -85°C storage is recommended.

Library Amplification

Consumables

Item	Identifier	Quantity
Amplification Mix 2	Blue cap	40 µL per sample
Library Index Plate A (96 UDIs)	96-well plate	5 µL unique index per sample

Preparation

- Assemble and perform the Library Amplification reactions in a Post-PCR work area.
- Use the eMap Laboratory Planner to aid sample-to-index tracking.
- Remove the cold-rack(s) from frozen storage 10 minutes before use.
- Thaw Library Index Plate A at room temperature for 10 minutes and centrifuge at 280 × g for 1 minute.
- Gently wipe the foil seal of the Library Index Plate A with 70% (v/v) ethanol and allow to dry.
- Thaw the Amplification Mix 2, invert to mix and pulse centrifuge. Keep on a cold-rack until use.
- If Template Preparation products were stored frozen, thaw to room temperature, mix well by inversion and flick mixing, then centrifuge at 280×g for 1 minute. Retain on a cold-rack until use.

Steps

1. Label a new 0.2 mL PCR plate as ”Library Amplification Plate” and the Plate ID that corresponds with the eMap Laboratory Planner, and place in a 96-well PCR cold-rack.
2. Dispense 40 µL of Amplification Mix 2 into each well of the Library Amplification Plate, according to the eMap Laboratory Planner.
3. Place the Library index plate A next to the Library Amplification Plate with the same orientation. Use the notch at the lower-left corner of the Library Index Plate A as a reference for well positions as illustrated below.

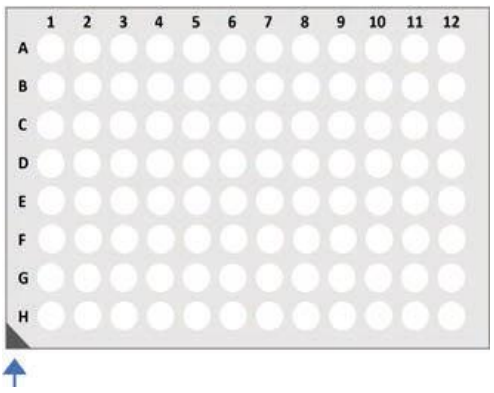

Figure 1 Orientation of the Library Index Plate A.

4. The Library Index Plate has a pierceable foil seal. For each sample, use a fresh pipette tip to pierce the foil seal and transfer 5 µL of Index to the Library Amplification Plate, according to the eMap Laboratory Planner. Dispense directly into the 40 µL of Amplification Mix 2. Do not pipette mix.
5. Thoroughly vortex the Template preparation products and pulse centrifuge.
6. Add 5 µL of Template Preparation product to the corresponding Library Amplification Plate well, according to the eMap Laboratory Planner. Dispense each sample directly into the 45 µL of amplification mixture. Do not pipette mix.
7. Store the remaining 15 µL of Template Preparation product at -20 °C for up to one month.

ATTENTION
Follow the eMap Laboratory Planner and use additional witnessing steps to ensure accurate sample-to-index tracking.

Table 9 Components of Library Amplification reaction

Item	Cap colour	Volume (µL)
Amplification Mix 2	Blue cap	40
Library Index	96-well plate	5
Template Preparation product	-	5
Total		**50**

8. Seal the Library Amplification Plate with a PCR seal and vortex mix at 1800 rpm for 1 minute.
9. Centrifuge the Library Amplification Plate at 280 x g for 20 seconds to collect contents.
10. Incubate samples in a designated Post-PCR thermal cycler as in Table 10, with a heated lid set at 101°C – 105°C.

Table 10 Thermal cycler program for Library Amplification

Number of cycles	Temperature of cycle	Incubation time
1	95°C	3 min
4	95°C	30 sec
	63°C	25 sec
	68°C	1 min
9	95°C	30 sec
	68°C	1 min
1	4°C	Hold

11. After completion of the Library Amplification program, centrifuge the Library Amplification Plate at 280 × g for 1 minute before proceeding to Library Pooling and Size Selection.

Size Selection

This step uses SPRIselect Size Selection beads to purify the library DNA by solid phase reversible immobilization (SPRI). The Size Selection step optimises the library fragment size distribution and purifies libraries for sequencing. The median fragment size following Size Selection is approximately 550 bp.

Consumables

Item	Identifier	Quantity
Elution Buffer	Clear cap	47.5 µL per sample/pool
SPRIselect Beads (Beckman Coulter; Cat# 23318)	-	45 µL per sample/pool
80% ethanol, freshly prepared (User-supplied)	-	400 µL per sample/pool

ATTENTION
Ethanol is highly flammable, keep away from all sources of ignition at all times.

Tips and Techniques

- Resuspend the SPRISelect beads completely before beginning. This step is essential for accurate quantitation and seeding of the sequencing flow cell.
- Do not aspirate beads when removing the supernatant. Loss of beads will result in reduced DNA yield. If any beads are inadvertently aspirated into the pipette tips, dispense the beads back into the plate and incubate on the magnet stand for 2 minutes or until the liquid is clear. Resume the removal of the supernatant.
- During washing, do not incubate the beads in 80% ethanol for more than 30 seconds. Longer incubation times may result in reduced yield and suboptimal size selection that may affect sequencing performance. Adjust your sample batch size accordingly for optimal performance.
- After removing excess ethanol, do not over-dry the beads before adding Elution Buffer. Adjust the drying time as appropriate for your laboratory conditions. Over-drying may result in reduced yield and suboptimal Size Selection. Beads that are over-dried appear cracked.
- During elution, if the supernatant is not clear after the 2-minute incubation on the magnet stand, gently pipette mix the supernatant whilst holding the pipette tip opposite the bead pellet. This will encourage residual beads to migrate towards the magnet. The clear supernatant can then be transferred to the Final Library Plate.

Preparation

- Make a new preparation of 80% ethanol (molecular biology grade) and maintain at room temperature.
- Thaw the Elution Buffer, invert to mix and pulse centrifuge to collect contents. Keep at room temperature.
- Vortex the SPRIselect beads thoroughly, immediately before use. Invert the tube to ensure that no bead pellet remains at the bottom of the storage container.
- For higher sample throughput, use new reagent reservoirs or troughs to facilitate multi-channel pipetting of SPRIselect beads, 80% ethanol and Elution Buffer.

Steps

1. Label two new 0.8 mL deep-well plates as "SS1" and "SS2", respectively. Label a new PCR plate as "Final Library Plate" and the Plate ID that corresponds with the eMap Laboratory Planner.
2. Add 25 µL thoroughly mixed SPRIselect beads to each well of the SS1 plate, according to the eMap Laboratory Planner.
3. Transfer 45 µL of Library Amplification product from the Library Amplification Plate to the corresponding well of the SS1 plate containing SPRIselect beads.
4. Seal the SS1 plate and shake at 1800 rpm for 1 minute.
5. Incubate the SS1 plate at room temperature for 5 minutes.
6. During the 5-minute incubation, add 20 µL thoroughly mixed SPRIselect beads to each well of the SS2 plate, according to the eMap Laboratory Planner.
7. Pulse centrifuge the SS1 plate at 280 x g for 5 seconds to collect droplets.
8. Place the SS1 plate on a magnetic stand and wait until the liquid is clear (~2 minutes). Keep the plate on the stand during the following step.
9. Transfer 65 µL of supernatant from each well of the SS1 plate to the corresponding well of the SS2 plate containing SPRIselect beads, then discard the SS1 plate.
10. Seal the SS2 plate and shake at 1800 rpm for 1 minute.
11. Incubate the SS2 plate at room temperature for 5 minutes.
12. Pulse centrifuge the SS2 plate at 280 x g for 5 seconds to collect droplets.
13. Place the SS2 plate on a magnetic stand and wait until the liquid is clear (~2 minutes).
14. With the SS2 plate on the magnetic stand, remove and discard all supernatant from each well of the SS2 plate.
15. Keeping the SS2 plate on the magnetic stand, wash two times as follows:
    a. Add 200 µL freshly prepared 80% ethanol to each well, opposite the aggregated beads, being careful not to disturb the pellet at the bottom of the well.
    b. Incubate on the magnetic stand for 30 seconds. Start the timer after dispensing 80% ethanol into the first well.
    c. Remove and discard all supernatant from each well.
16. Using fine pipette tips (10/20 µL), remove residual 80% ethanol from each well.

17. Air-dry the SS2 plate on the magnetic stand for 3 minutes, or until the beads are visibly dry but not cracked.
18. Remove the SS2 plate from the magnetic stand and add 47.5 µL of Elution Buffer to each sample well.

ATTENTION
Retain residual Elution Buffer for use during Library Dilution and Denaturation. Elution Buffer can be stored at room temperature.

19. Seal the SS2 plate and shake at 1800 rpm for 1 minute.
20. Pulse centrifuge the SS2 plate at 280 x g for 5 seconds to collect droplets.
21. Place the SS2 plate on a magnetic stand and wait until the liquid is clear (~2 minutes).
22. Transfer 45 µL of each sample supernatant to the corresponding well of the Final Library Plate according to the eMap Laboratory Planner, then proceed immediately to Library Quantification.

SAFE STOPPING POINT
Libraries may be stored pre-size selection at -25°C to -15°C for up to 1 month. For long term storage, -65°C to -85°C storage is recommended.

Library Quantification

Accurate quantification of the final Library Pool is essential for successful seeding of the sequencing instrument. Quantify the library pool using a fluorometric-based method such as the Quant-iT™ or Qubit™ dsDNA, high sensitivity, assay kits. Follow the manufacturer's instructions and safety recommendations while using the quantification kits and validate for use with your specific plate reader or Qubit™ fluorometer. Refer to the **EmbryoMap SNP Workflow QC guidance** for expected ranges.

Qubit Method

The Qubit method is a single-tube workflow that is suited to lower sample throughput, or measuring a single sample tube such as the Final Library Pool. This method uses a 2-point standard curve.

Consumables

Item	Part#
Qubit™ dsDNA HS Assay Kit	(ThermoFisher Scientific, Cat# Q32851/Q33230).
Qubit™ Assay Tubes or Qubit™ Flex Assay Tube Strips	(ThermoFisher Scientific; Cat# Q32856) or (ThermoFisher Scientific; Cat# Q33252)

Preparation

- If supplied, aliquot the Qubit dsDNA HS reagent into single-use aliquots and store at 2– 8°C to prevent freeze-thaw cycles. Protect the Qubit dsDNA HS reagent from light.
- Bring all reagents to room temperature for 30 minutes before use.
- Prepare and label the required number of 0.5 mL Qubit Assay Tubes or Assay Tube Strips for the samples and both high and low standards.

Steps

1. If not supplied as a 1× solution, prepare the Working Solution by mixing Qubit dsDNA HS reagent 1:200 with the Qubit dsDNA HS buffer.
2. Add 190 µL of the Working Solution to each Assay Tube/Strip used for the two standards.
3. Add 10 µL of standard to the respective Assay Tube/Strip.
4. Add 198 µL of the Working Solution to each Assay Tube/Strip used for the Final Library Pool.
5. Add 2 µL of sample Final Library Pool to each respective Assay Tube/Strip and mix by vortexing for 2-3 seconds.
6. Incubate the Assay Tubes/Strips for 2 minutes at room temperature whilst protected from light.

7. Measure the fluorescence and calculate the concentration of the Final Library Pool using a Qubit Fluorometer or a Qubit Flex Fluorometer as per the manufacturer's instructions.

ATTENTION
If the sample concentration falls outside the standard curve, adjust the ratio of sample to standard as necessary. Adjust for the dilution factor when calculating the final library concentration.

Quant-iT Method

The Quant-iT method is a plate-format workflow that is suited to higher sample throughput of >24 samples, however it can still be used to quantify a single sample. This method uses an 8-point standard curve.

Consumables

Item	Part#
Quant-iT™ dsDNA Assay Kit, high sensitivity	(ThermoFisher Scientific; Cat# Q33120 or Q33232)
96-well Microplates, F-Bottom (Chimney Well), Black, Non-Binding.	(e.g. Greiner BioOne; Cat# 655900)

Preparation

- If supplied, aliquot the Quant-iT dsDNA HS reagent into single-use aliquots and store at 2– 8°C to prevent freeze-thaw cycles. Protect the Quant-iT dsDNA HS reagent from light.
- Bring all reagents to room temperature for 30 minutes before use.
- The required reagents and microplates will be number of sample libraries + 16 standard curve reactions, where the 8-point standard curve should be run in duplicate.
- For microplate readers with mixing capabilities, setup for orbital shaking at ~300 rpm for 20 seconds prior to measuring. Otherwise, ensure reagents are mixed well by pipetting.

Steps

1. If not supplied as a 1× solution, prepare the Working Solution by mixing Quant-iT dsDNA HS reagent 1:200 with the Quant-iT dsDNA HS buffer.
2. Add 200 µL of the Working Solution to the required wells of the assay microplate.
3. Add 2 µL of the Final Library Pool to the sample well(s) and mix well by pipetting.
4. Add 10 µL of each Quant-iT Standard to standard wells and mix well by pipetting. Measure the standards in duplicate.
5. Incubate the assay microplate for 2 minutes at room temperature whilst protected from light. The fluorescence signal is stable for 3 hours at room temperature when kept in the dark.
6. Measure the fluorescence using a microplate reader using excitation/emission fluorescein wavelengths at ~480/530 nm.

7. Calculate the concentration of the Final Library Pool using the standard curve as directed by the microplate reader manufacturer and/or instrument-specific software tools.

ATTENTION
If the sample concentration falls outside the standard curve, adjust the ratio of sample to standard as necessary. Adjust for the dilution factor when calculating the final library concentration.

SAFE STOPPING POINT
The Final Libraries may be stored at -25°C to -15°C for up to 1 year. For long term storage, -65°C to -85°C storage is recommended.

Library Pooling

Consumables

Item	Identifier	Quantity
Final Library Plate	**Plate ID**	Variable µL

Preparation

- Generate a Pooling Guide using the eMap Software.
    - Enter the corresponding Final Library concentrations for the Plate ID.
    - Select 8–48 samples to be included in the pool.
    - Up to two additional negative amplification controls may be included in the library pool for optional sequencing QC assessment. Negative amplification samples will not consume reads on the flow cell but must have a unique index assignment.
    - Select a pooling mass to ensure a pooling volume of 2–20 µL for each positive sample. This will help to minimise variation when pipetting small volumes and ensure that sufficient material remains to allow re-pooling if required.
    - Name the pool with a unique Pool ID and click Save. Thaw the MiSeq reagent cartridge in a room temperature water bath for 45 minutes immediately before use, or at 2°C to 8°C overnight.

Steps

1. Centrifuge the Final Library Plate at 280 x g for 1 minute.
2. Label a new 1.5 mL, low DNA-retention microcentrifuge tube as “Library Pool” with the Pool ID and date, and Pool Concentration (nM), and keep on ice.
3. Combine the required volume of each sample library according to the eMap Pooling Guide. Use a fresh tip for each sample library.
4. Vortex and centrifuge the stock Library Pool.
5. [Optional] Quantify the Library Pool as described previously and calculate the nM concentration for duplex DNA with an average fragment length of 550 bp. This step is advised for improved accuracy when seeding the MiSeq instrument.

SAFE STOPPING POINT
The Final Library Pool may be stored at -25°C to -15°C for up to 1 year. For long term storage, -65°C to -85°C storage is recommended.

Library Dilution, Denaturation and MiSeq Sequencing

Consumables

Item	Identifier	Quantity
Elution Buffer	Clear cap	200 µL per pool
MiSeq® Reagent Kit v3 - Box 1 of 2	Part # 15043893	1 per run
MiSeq® Reagent Kit v3 - Box 2 of 2	Part# 15043894	1 per run

Preparation

- Complete run definition in the eMap Software by entering the Run Description and serial numbers for the flow cell, reagent cartridge and MiSeq instrument for the selected Pool ID.
- Generate the run-specific SampleSheet.csv and save in a location that is accessible by the MiSeq instrument computer.
- Thaw the MiSeq reagent cartridge in a room temperature water bath for 45 minutes immediately before use, or at 2°C to 8°C overnight.

ATTENTION
Certain sequencing components contain formamide, a probable reproductive toxin. See the SDS at support.illumina.com/sds.html.

- Ensure the cartridge is fully thawed, then invert the cartridge 10 times to mix contents. Gently tap the cartridge on the benchtop to remove bubbles from the reagent reservoirs. Dry the cartridge with a paper towel.
- Thaw the Illumina HT1 (Hyb Buffer) tube on ice and mix by vortexing. Ensure no precipitates are visible in the solution and keep on ice.
- Bring Elution Buffer to room temperature before use.
- If the stock Library Pool was stored frozen, thaw, briefly vortex to mix, pulse centrifuge to collect contents and keep on ice.
- The dilution instructions for preparing the 1 nM Pool can be found in the eMap Pooling Guide PDF.

Steps

1. Label a new 1.5 mL, low DNA-retention microcentrifuge tube as “1 nM Pool” with the Pool ID and date and keep on ice.
2. Prepare a 1 nM dilution of the stock Library Pool in a final volume of 200 µl Elution Buffer using the following formula:

   E.g. for a library pool with a Pool Concentration of 16 nM:
   Volume of Final Library Pool = 200/16 = 12.5 µL
   Volume of Elution Buffer = 200-12.5 = 187.5 µL
3. Transfer 10 µl of the 1 nM Pool to a new 0.2 mL PCR tube or PCR 8-tube strip.

4. Add 90 µl Illumina HT1 (Hyb Buffer).

ATTENTION
The optimal cluster density range for a MiSeq is from 1200–1400K/mm^2.
If necessary, adjust the proportion of 1 nM Pool in a final volume of 100 µL HT1 to optimise MiSeq seeding. Under- and over-clustering may impact the quality of the results.

5. Gently vortex and centrifuge the library pool/HT1 mixture.
6. Immediately incubate samples in a designated Post-PCR thermal cycler as in Table 12, with a heated lid set at 101°C – 105°C.

Table 12 Thermal cycler program for Denaturation

Number of cycles	Temperature of cycle	Incubation time
1	96°C	5 min
1	4°C	1 min
1	4°C	Hold

7. Aliquot 600 µL of HT1 (Hyb Buffer) into a new 1.5 mL, low DNA-retention microcentrifuge tube and place on ice.
8. During the Denature incubation, prepare and load a MiSeq flow cell into the MiSeq instrument according to the manufacturer's instructions.
9. After denaturation, remove the sample tube from the thermal cycler and centrifuge briefly to collect contents.

ATTENTION
HT1 diluted/denatured library pools are not stable for storage. Perform heat denaturation immediately before loading the library into the MiSeq reagent cartridge.

10. Immediately transfer 100 µL of denatured pool/HT1 mixture to the 600 µL pre-cooled aliquot of HT1, pulse vortex and centrifuge, then keep on ice.
11. Transfer 600 µL of the final denatured and diluted library pool to position 17 of the MiSeq reagent cartridge. Tap the cartridge on the benchtop to remove bubbles.
12. Load the remaining reagents onto the MiSeq and complete run setup, according to the manufacturer's instructions. Press Start Run to begin sequencing on the MiSeq.

ATTENTION
The EmbryoMap SNP Workflow requires a 2 x 63 bp sequencing run. Before starting the sequencing run, ensure the correct read length is displayed.

3 SUPPORTING INFORMATION

EmbryoMap™ Sample Prep (96 rxns) REF 13301

Kit Name	Identifier	REF	Quantity	Storage temp
Extraction Mix	Red cap	BE00759	2	-25°C to -15°C
Amplification Mix 1	White cap	BE00760	2	-25°C to -15°C
Amplification Mix 2	Blue cap	BE00761	4	-25°C to -15°C
Elution Buffer	Clear cap	BF00762	4	-25°C to -15°C
Library Index Plate A	96-well plate	DX00749	1	-25°C to -15°C

EmbryoMap™ SNP Reagent (96 rxns) REF 13302

Kit Name	Identifier	REF	Quantity	Storage temp
SNP Panel 1	**Yellow cap**	13302	1	-25°C to -15°C

ATTENTION
Each reagent tube in the EmbryoMap Sample Prep and SNP kits are validated for 6 freeze-thaw cycles.

MiSeq Reagent Kit v3 - Box 1 of 2 REF 15043893

Kit Name	REF	Quantity	Storage temp
Reagent Cartridge	15043963	1	-25°C to -15°C
HT1	15070155	1	-25°C to -15°C

MiSeq Reagent Kit v3 - Box 2 of 2 REF 15043894

Kit Name	REF	Quantity	Storage temp
PR2 Bottle	15041807	1	2°C to 8°C
MiSeq Flow Cell	15070155	1	2°C to 8°C

Consumables and Equipment

Make sure that you have the required consumables and equipment before starting the protocol. Some items are required only for specific workflows, e.g. quantification method. The protocol has been optimised and validated using the items listed. Comparable performance is not guaranteed when using alternate consumables and equipment.

General laboratory consumables (User Supplied)

Consumable	Supplier
10 µL filtered pipette tips	General Lab supplier
10 µL single and/or multi-channel pipettes	General Lab supplier
50 µL filtered pipette tips	General Lab supplier
50 µL single and/or multi-channel pipettes	General Lab supplier
200 µL filtered pipette tips	General Lab supplier
200 µL single and/or multi-channel pipettes	General Lab supplier
1000 µL filtered pipette tips	General Lab supplier
1000 µL single channel pipettes	General Lab supplier
Conical centrifuge tubes, 15 mL	General Lab supplier
Conical centrifuge tubes, 50 mL	General Lab supplier
Adhesive seal roller	General Lab supplier
Reagent reservoirs, multi-channel, disposable	General Lab supplier
Ethanol absolute ≥99.8%, Certified AR for Analysis	General Lab supplier
1× PBS, pH 7.4, DNase/RNase-free	General Lab supplier
Molecular Biology PCR-grade water	General Lab supplier
Thin-walled, PCR Tubes with Flat Cap, 0.2 mL	Axygen, Cat# PCR-02-L-C
PCR Tubes, 8-tube strips, 0.2 mL	Eppendorf; Cat# 0030124359
96-well PCR plates or Hard-Shell 96-well PCR plates (HSP plate)	ThermoFisher Scientific; Cat# AB0600; or Bio-Rad; Cat# HSP9601
96-well storage plates, round well, 0.8 mL	ThermoFisher Scientific; Cat# AB0859
Adhesive PCR plate seals	ThermoFisher Scientific; Cat# AB0558
DNA LoBind® Tubes,1.5 mL	Eppendorf; Cat# 0030108051

MiSeq maintenance consumables (User Supplied)

Consumable	Supplier
Sodium hypochlorite solution	Sigma-Aldrich; Cat # 239305
Tween 20	Sigma-Aldrich; Cat # P7949
MiSeq disposable wash tubes	Illumina; Cat# MS-102-9999
Whatman™ Lens Cleaning Tissue	Fisher Scientific; Cat# 11360654

Required reagent consumables (User Supplied)

Consumable	Supplier
SPRIselect, 60 mL	Beckman Coulter; Cat# B23318
One of the following quantification solutions:	
Qubit™ dsDNA HS Assay Kit; and Qubit™ Assay Tubes (for Qubit™ Fluorometer); or Qubit™ Flex Assay Tube Strips (for Qubit™ Flex Fluorometer)	ThermoFisher Scientific; Cat# Q32851 or Q33230 Cat# Q32856 Cat# Q33252
Quant-iT™ dsDNA Assay Kit, high sensitivity; and 96-well Microplates, F-Bottom (Chimney Well), Black, Non-Binding	ThermoFisher Scientific; Cat# Q33120 or Q33232 Greiner BioOne; Cat# 655900

Equipment (User Supplied)

Equipment	Supplier
General lab personal protective equipment including, safety glasses, lab coats, powder-free protective gloves, stopwatch or timer, cold-rack	General Lab supplier
Microcentrifuge	General Lab supplier
Microplate centrifuge	General Lab supplier
Vortexer, with speed and time control.	General Lab supplier
Thermal cyclers; dedicated for Pre-PCR and Post-PCR applications.	General Lab supplier
High-Speed Microplate Shaker (BioShake IQ) or High-Speed Microplate Shaker (BioShake XP) or Advanced High Speed Microplate Shaker (110/120V) or Advanced High Speed Microplate Shaker (230V) or	Qinstruments, Cat# 1808-0506 or Qinstruments, Cat# 1808-0505 or VWR, Cat# 13500-890 or VWR, Cat# 14216-214
Magnetic stand – 1.5 mL tubes	ThermoFisher Scientific, Cat# 15275126
[Optional] Magnetic stand-96	ThermoFisher Scientific, Cat# AM10027
Microplate reader, 96-well plate fluorometer (or equivalent) or Qubit 4 Fluorometer or Qubit Flex Fluorometer	BMG LabTech Cat# FLUOstar Omega or ThermoFisher Scientific, Cat# Q33238 or ThermoFisher Scientific, Cat# Q33327
MiSeq System or MiSeq Dx System (RUO Mode)	Illumina, Cat# SY-410-1003 or Illumina, Cat# DX-410-1001

4 TECHNICAL SUPPORT

For technical assistance, contact Vitrolife Genomics Support:

Email support.genomics@vitrolife.com

Website www.vitrolife.com

Safety Data Sheets

Safety data sheets (SDSs) are available Online at
https://www.vitrolife.com/support/support-material/support-documents?type=safetydatasheet

TOGETHER. ALL THE WAY™

Americas
Vitrolife Inc., 3601 South Inca Street
Englewood, Colorado 80110, USA Tel
866-848-7687 (866-VITRO US)
Fax 866-848-7632 (866-VITROFAX)

Europe, Middle East, Asia/Pacific
Vitrolife Sweden AB, Box 9080
SE-400 92 Göteborg, Sweden
Tel +46-31-721 80 20, Fax +46-31-721 80 99

Global Technical Support
support.genomics@vitrolife.com

Internet
http://www.vitrolife.com

Vitrolife
PART OF VITROLIFE GROUP