

USER GUIDE

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by *life* technologies™

Ion Xpress™ Template Kit v2.0

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technologies™

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About this guide



CAUTION! ABBREVIATED SAFETY ALERTS. Hazard symbols and hazard types specified in procedures may be abbreviated in this document. For the complete safety information, see the “Safety” appendix in this document.

IMPORTANT! Before using this product, read and understand the information the “Safety appendix” in this document.

IMPORTANT! If you use and/or install the Personal Genome Machine™ (PGM™) in an unspecified manner, you may impair the protection provided by the equipment.

Equipment intended use

The PGM™ System is intended for performing genomic sequencing of amplified DNA, and should only be used for life science research applications. The PGM™ sequencer should only be used by professionals trained in laboratory techniques and who have studied the instrument instructions. The Ion Personal Genome Machine™ Sequencer is For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.

Purpose

The *Ion Xpress™ Template Kit User Guide v2.0* provides reference information for template preparation for the Ion Personal Genome Machine™ (PGM) system workflow and is to be used in conjunction with the Ion Xpress™ Template Kit (Part no. 4469001).

IMPORTANT! This user guide should not be used with the Ion Xpress Template Kit (Part no. 4466457).

Prerequisites

The manufacturer is not liable for damage or injury that results from use of this manual by unauthorized or untrained parties. This guide uses conventions and terminology that assume a working knowledge of the Microsoft® Windows® operating system, the Internet, and Internet-based browsers.

User attention words

Five user attention words may appear in this document. Each word implies a particular level of observation or action as described below:

Note: Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! Provides information that is necessary for proper instrument operation or accurate chemistry kit use.



CAUTION! Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.



WARNING! Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.



DANGER! Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

Except for IMPORTANTs, the safety alert words in user documentation appear with an open triangle figure that contains a hazard symbol. These hazard symbols are identical to the hazard symbols that are affixed to the instrument. See the “Safety appendix” for descriptions of the symbols.

Product information

Purpose of the product

The Personal Genome Machine™ (PGM™) System sequences DNA templates that have been clonally amplified on Ion Sphere™ Particles (ISPs). Thus, the Ion Template Preparation protocol has been designed to achieve amplification of a template onto ISPs.

This protocol describes how to use emulsion PCR to generate ISPs with amplified templates that are suitable for use with the Ion Sequencing Kit (Part no. 4468997). Following amplification, the template-positive ISPs must be enriched to maximize the number of sequence reads produced by the PGM™ System.

Kit contents and storage conditions

The components of the Ion Xpress™ Template Kit (Part no. 4469001) are shipped in the three boxes, as detailed in the following table.

Box	Components	Cap color	Part Number	Quantity	Shipping	Storage
Ion Template Preparation Kit (Part no. 4469000)	Emulsion Oil	—	603-1040-01	90 mL	Ambient	4°C
	Nuclease-Free Water	—	603-1061-01	15 mL		
Ion Template Reagents Kit (Part no. 4468999)	10X PCR Enzyme Mix	Brown	602-1089-01	1 mL	-20°C	-20°C
	5X PCR Reagent Mix	Violet	602-1090-01	2X 1 mL	-20°C	4°C
	Ion Sphere™ Particles	Black	602-1051-04	1 mL	-20°C	-20°C
Ion Template Solutions Kit (Part no. 4468998)	Recovery Solution	—	603-1042-02	100 mL	Ambient	4°C
	Wash Solution	—	603-1043-01	60 mL		

Materials and equipment required

Note: Ion Torrent has validated this protocol using the specific materials listed in the following table. Substitution may adversely affect system performance.

Note: Where noted, supplies are available from Major Laboratory Suppliers (MLS).

Description	Supplier	Part Number	Quantity
Ion Template Preparation Tubes	Ion Torrent	4467226	10/pack
IKA® Ultra-Turrax® Tube Drive for the PGM™ System	Ion Torrent	4464747	1
Equipment/supplies¹			
1-butanol (molecular biology grade, ≥99%)	Sigma	B7906	—
DynaMag™-2 magnet (microcentrifuge tube magnet)	Invitrogen	123-21D	1
Dynabeads® MyOne™ Streptavidin C1 Magnetic Beads	Invitrogen	650.01	—
Eppendorf® CombiTips® Plus, 10-mL, sterile	Eppendorf	022496123	—
<ul style="list-style-type: none"> • GeneAmp® 96-well PCR System 9700 or • DNA Engine® Tetrad® 2 or Dyad®² 	Applied Biosystems	N8050200 (Base) 4314443 (Block)	1
<ul style="list-style-type: none"> • MicroAmp® Optical 96-Well Reaction Plates or • Eppendorf® twin.tec real-time PCR plates, 96-well, semi-skirted 	Applied Biosystems	N8010560	10/box
<ul style="list-style-type: none"> • MicroAmp® Optical 8-Cap Strips or • Bio-Rad Optical Flat 8-Cap Strips 	Eppendorf	951020303	25/box
<ul style="list-style-type: none"> • MicroAmp® Optical 8-Cap Strips or • Bio-Rad Optical Flat 8-Cap Strips 	Applied Biosystems	N8010535	300
<ul style="list-style-type: none"> • Bio-Rad Optical Flat 8-Cap Strips 	Bio-Rad	TCS0803	50/box
DNA LoBind Tubes, 1.5 mL	Eppendorf	022431021	250/box
Nuclease-free water	Applied Biosystems	AM9932	1 L
PCR workstation	ISC BioExpress (or comparable)	H-6590-3B	1
Repeater® Plus Pipette	Eppendorf	022260201	—
SOLiD® Emulsion Collection Tray Kit ³ or	Applied Biosystems	4415129	10 each
<ul style="list-style-type: none"> • PVC basin 	VWR	21007-970	—
<ul style="list-style-type: none"> • Pipette, 8-channel 	MLS	—	—

¹ Using the indicated equipment creates an efficient workflow.

² Ion Torrent validated this protocol using this specific material. Substitution may affect system performance.

³ Ion Sphere™ Particles Recovery Collection Tray Protocol requires a large swing-bucket centrifuge. If this type of centrifuge is not available, then order the materials required for the Ion Sphere™ Particles Recovery PVC Basin Protocol.

Description	Supplier	Part Number	Quantity
Standard lab equipment/supplies			
Barnstead/Thermolyne Labquake Tube Shaker/Rotator	MLS (such as VWR)	400110Q or 56264-302	1
Externally vented chemical hood ³	—	—	1
(<i>Recommended</i>) Eppendorf Microcentrifuges, Model 5424 ⁴	Eppendorf	022620401	1
NaOH (10 M)	MLS	—	—
Pipettes (P2, P20, P200, P1000) and appropriate low-retention tips (including a wide-bore tip for a P200 pipette)	Ranin or MLS	—	1 each
Tween [®] 20 Molecular Grade	MLS	—	—
Vortex with a rubber platform	MLS	—	1

³ Any model capable of negative pressure at 100 linear feet per minute.

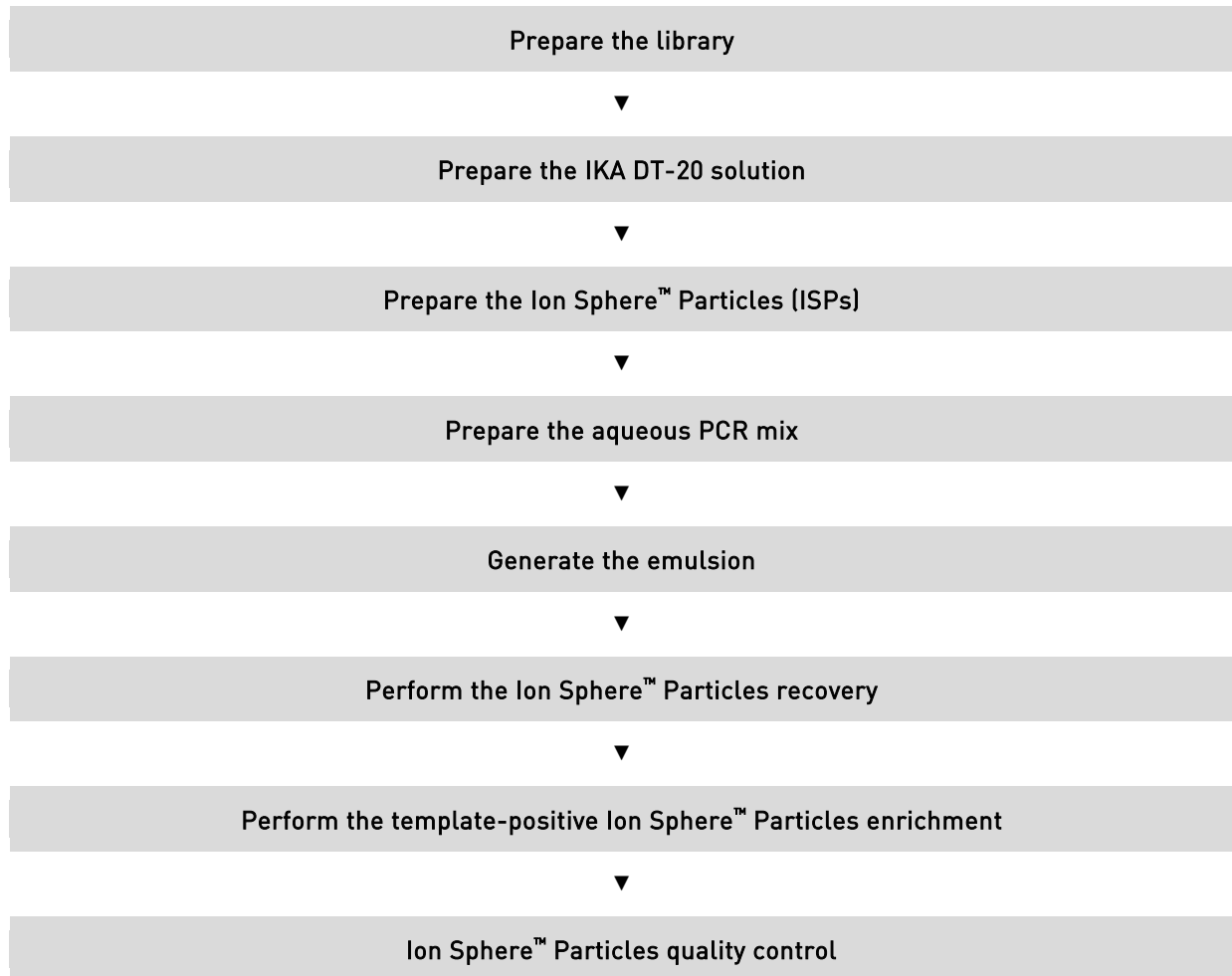
⁴ Must fit standard 1.5- and 0.2-mL microcentrifuge tubes; must generate 21,130 × g.

Workflow

This protocol describes how to process one sample at a time. If you are processing multiple samples, either perform the protocol in parallel or repeat it for each sample.

IMPORTANT! Multiple samples can be processed in parallel, but every step must be performed individually for each sample. Pooling reactions and processes will result in suboptimal performance.

The Ion Xpress™ Template kit preparation protocol consists of the following steps:



Procedure

Prepare the library

The optimal number of library molecules to achieve 10–25% positive Ion Sphere™ Particles might vary between libraries. Generally, approximately 280×10^6 molecules/reaction is recommended, but the optimal library concentration may range from 140×10^6 to 560×10^6 molecules/reaction.

- If you have previously determined the optimal library concentration, and have a dilution of the library at the optimized concentration, go to “Prepare the IKA DT-20 solution” below.
- If you are sequencing a library for the first time, follow the appropriate procedure below for preparing one or more library dilutions, based on the library quantitation method used.

IMPORTANT! You must determine the optimal input concentration for each DNA library before proceeding. The quality of your sequencing data relies greatly upon achieving the correct concentration of starting library.

Prepare library dilutions

The library dilutions described in this section assume that 18 μ L of the library dilution will be used.

Libraries quantitated using the Ion Library Quantitation Kit

Prepare a single dilution of the library at the Template Dilution Factor determined with the Ion Library Quantitation Kit (Part no. 4468802). The Template Dilution Factor targets a library concentration resulting in $\sim 280 \times 10^6$ molecules/reaction.

Example: The Template Dilution Factor is 100. Mix 1 μ L of library with 99 μ L of Nuclease-free Water to prepare a 1:100 dilution.

(Optional) You may choose to prepare additional library dilutions 2-fold higher and 2-fold lower than the Template Dilution Factor (that is, $\frac{1}{2} \times$ Template Dilution Factor and $2 \times$ Template Dilution Factor). Follow the instructions provided below for libraries quantitated by Bioanalyzer™ analysis to prepare three serial dilutions.

Libraries quantitated by Bioanalyzer™ analysis

Because Bioanalyzer™ quantitation may not be as precise as qPCR, we recommend that you prepare three serial dilutions of the library at $\frac{1}{2} \times$ Template Dilution Factor, Template Dilution Factor, and $2 \times$ Template Dilution Factor, to ensure that one or more dilutions are in the optimized concentration range.

The Template Dilution Factor is calculated from the molar concentration determined in Bioanalyzer™ analysis during the library preparation procedure. Refer to the user guide for your library preparation procedure.

Example: The Template Dilution Factor is 385. To prepare library Dilution 1 at 1:192.5, mix 1 μ L of library with 191.5 μ L of water.

Dilution	Dilution factor	Target number of molecules per reaction*	Library volume	Water
1	$\frac{1}{2} \times$ Template Dilution Factor	560×10^6	1 μ L	$[(\frac{1}{2} \times \text{Template Dilution Factor}) - 1] \mu\text{L}$
2	Template Dilution Factor	280×10^6	50 μ L Dilution 1	50 μ L
3	$2 \times$ Template Dilution Factor	140×10^6	50 μ L Dilution 2	50 μ L

* Molecules/18 μ L input in the aqueous master mix.

Prepare the IKA DT-20 solution

1. Remove the Emulsion Oil from the refrigerator, then gently invert the bottle several times to mix.

Note: Mix the Emulsion Oil immediately after removing the bottle from the refrigerator.

2. While the mixed Emulsion Oil is cool (approximately 4°C), add 9 mL of Emulsion Oil to a IKA® DT-20 tube using an Eppendorf® Repeater® Pipettor fitted with a 10-mL Eppendorf® Combitips® Plus tip.

Note: Dispense the first 1 mL of oil back into the oil bottle, then proceed dispensing 9 mL of oil into an IKA® DT-20 tube.

IMPORTANT! Do not use a standard pipette to transfer the mixture. You must use a 10-mL Eppendorf® Combitips Plus® pipette to ensure the complete transfer of the oil to the IKA® DT-20 tube.

3. Place the filled IKA® DT-20 tube on ice until ready for use.

Prepare the Ion Sphere™ Particles (ISPs)

Note: The Ion Sphere™ Particles are stored at –20°C in glycerol.

To resuspend the particles you need to vortex the ISPs for 1 minute at maximum speed before use.

IMPORTANT! The ISPs must be thoroughly resuspended before use.

Prepare the aqueous PCR mix

Prepare the aqueous PCR mix for each library dilution. After determining the library concentration, dilute the library in water accordingly such that the volume you will use for the emulsion PCR is equivalent to 18 µL. If you are troubleshooting the emulsion PCR process, use the *E. coli* DH10B control library (Ion Control Material Kit, Part no. 4466465) as a control. In the DNA LoBind Tube, dilute 1 µL of control library into 260 µL of nuclease-free water. Mix by vortexing then add 18 µL of the diluted library as described in the table below.

Note: Store all reagents on ice during the PCR mix preparation.

1. Thaw the 5X PCR Reagent Mix.

Note: The 5X PCR Reagent Mix is shipped at -20°C and should be thawed before the first use and stored at 4°C. The same process must be repeated for each subsequent use.

2. In a 1.5-mL tube, assemble the amplification solution on ice:

Note: Vortex the tube for 5 seconds at maximum speed, and briefly centrifuge the tube contents after adding each component.

IMPORTANT! Add the reagents in order specified in the following table.

Order	Reagent	Cap color	Volume (µL)
1	Nuclease-free water	—	582 µL
2	5X PCR Reagent Mix	Violet	200 µL
3	10X PCR Enzyme Mix	Brown	100 µL
4	Ion Sphere™ Particles	Black	100 µL
	Total	—	982 µL

3. Add 18 µL of diluted library (not the stock material) to the amplification solution, vortex at maximum speed for 5 seconds, then proceed immediately to “Generate the emulsion”.

Generate the emulsion

Note: If this is the first use of the IKA® Ultra-Turrax® Tube Drive, go to step 1; otherwise, proceed directly to step 2.

1. Set the IKA® Ultra-Turrax® Tube Drive settings:
 - a. Press and hold **Start/Stop**, then press **Power** so that the unit displays “-1” indicating that Minutes Mode is engaged.
 - b. Press the **1** button so that the unit displays “05” indicating that the unit is set for 5 minutes.

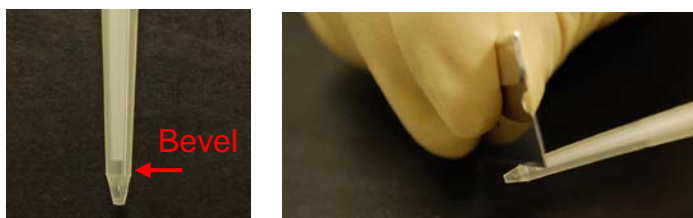
Note: The IKA® Ultra-Turrax® Tube Drive for PGM™ System is specifically calibrated for use with the Ion PGM™ System.
2. Remove the IKA® DT-20 tube containing 9 mL oil from ice and position it on the IKA® Ultra-Turrax® Tube Drive for the PGM™ System. Once the tube is in position, rotate the tube to lock it in place.
3. Load the IKA® DT-20 tube:
 - a. Remove the adhesive label from the cap of the IKA® DT-20 tube to expose the sample loading port. Do not discard the adhesive label.
 - b. Vortex the aqueous PCR mix (from “Prepare the aqueous PCR mix”), then pulse centrifuge for 3 seconds.
 - c. Press **Start** to power on the IKA® Ultra-Turrax® Tube Drive, then wait 2 seconds for the instrument reach a constant speed.
 - d. While the tube is in motion, use a P1000 pipette to steadily dispense (over the course of 3 seconds) the entire volume of aqueous PCR mix through the opening in the blue cap of the IKA® DT-20 tube, then replace the adhesive label on the tube to seal the opening.

IMPORTANT! When dispensing the aqueous PCR mix, place the pipette tip completely into the hole in the cap of the IKA® DT-20 tube.

Note: For a video demonstration of the transfer technique, visit the Ion Community website (<http://ioncommunity.iontorrent.com>).



4. After mixing for 5 minutes on the IKA® Ultra-Turrax® Tube Drive, place the emulsion on ice for 5 minutes.
5. Create a wide-bore tip for the emulsion transfer by cutting approximately 4–5 mm from a pipette tip for a 10-mL Eppendorf® Combitips Plus® pipette.



6. Transfer the emulsion to a 96-well PCR plate:
 - a. Using an Eppendorf® Repeater® Pipettor fitted with the wide-bore tip from step 5, draw up the emulsion from the IKA® DT-20 tube and dispense the first 100 μ L (or until there is no air remaining in the tip) back into the same vial.
 - b. Transfer 100 μ L of the emulsion to each well of a 96-well PCR plate. The emulsion should fill approximately 90 wells.

IMPORTANT! Do not rest the tip against the side of the plate while dispensing.

IMPORTANT! Do not use a standard pipette to transfer the emulsion.

- c. Use a P200 pipette with a wide-bore tip to transfer the residual emulsion from the IKA® DT-20 tube to the 96-well plate. If a wide-bore tip is unavailable, create one by cutting approximately 4–5 mm from a standard pipette tip.
7. Cap the 96-well plate using the cap strips recommended by the manufacturer.

Note: Use Applied Biosystems MicroAmp® Optical Caps Strips to seal Applied Biosystems MicroAmp® 96-Well Reaction Plates; use Bio-Rad Optical Flat 8-Cap Strips to seal Eppendorf® twin.tec real-time PCR plates.

Note: Applied Biosystems MicroAmp® 96-Well Reaction Plates are compatible only with Applied Biosystems thermal cyclers; Eppendorf® twin.tec real-time PCR plates are compatible with both Applied Biosystems and Bio-Rad thermal cyclers.

8. Load the PCR plate into a thermal cycler according to the manufacturer's instructions, then perform the following protocol:
 - **Ramp setting:** Maximum cycle
 - **Cover temperature:** 100°C
 - **Thermal cycling protocol:** (see table)

Note: The duration of the thermal cycling program is approximately 3 hours.

Stage	Step	Temperature	Time
Hold	Denature	94°C	6 min
Cycle (40 cycles)	Denature	94°C	30 sec
	Anneal	58°C	30 sec
	Extend	72°C	90 sec
Cycle (5 cycles)	Denature	94°C	30 sec
	Extend	68°C	6 min
Hold ⁵	—	10°C	∞

STOPPING POINT The reactions can be stored for up to 2 days at 10°C in the thermal cycler, or at 4°C in the refrigerator.

Note: If you are using a GeneAmp[®] 96-Well PCR System 9700, attach a compression pad to the plate before loading it into the thermal cycler.

⁵ The plate can be stored overnight at 10°C in the thermal cycler or on ice before continuing to ISP recovery.

Perform the Ion Sphere™ Particles recovery

Note: If you do *not* have a tabletop centrifuge with deep-well plate bucket, follow the procedures included in the Ion Sphere™ Particles Recovery PVC Basin Protocol section of the Appendix: Supplementary Procedures. If you have a tabletop centrifuge with deep-well plate bucket, follow the procedures included in the Ion Sphere™ Particles Recovery Collection Tray Protocol section.

For this method you must have:

- One tabletop centrifuge with deep-well plate bucket capable of 140 X g
- SOLiD® Emulsion Collection Tray
- Applied Biosystems MicroAmp® 96-Well Reaction Plates

Note: If you use the AB MicroAmp® 96-Well Reaction Plates, you do *not* need to secure the tray and plate together.

or

- Eppendorf® twin.tec real-time PCR plates, 96-well, semi-skirted

Note: If you use the Eppendorf® twin.tec real-time PCR plates, you do *need* to secure the SOLiD® Emulsion Collection Tray to the plate.

IMPORTANT! Prior to performing the following procedures, test the protocol with PCR plate filled with water to ensure that no liquid is lost during centrifugation. Conduct the actual protocol only after ensuring that the plate can be sealed securely to prevent the loss of actual samples due to poor seal.

Ion Sphere™ Particles Recovery Collection Tray Protocol

1. Collect and transfer the Ion Sphere™ Particles (ISPs) to six 1.5-mL tubes by performing the following:
 - a. Review the PCR plate for broken wells. A broken emulsion has a distinct separation between oil and aqueous phases such that the top phase is clear instead of appearing cloudy. A clear layer may also be visible at the bottom of the tube depending on the PCR plate or tubes used for amplification. If more than 15% of the wells display these characteristics, discard the reactions and start again.

Normal Well



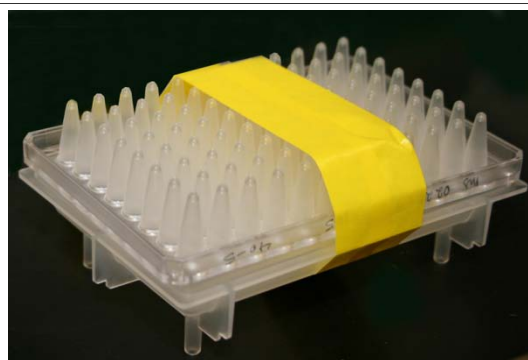
- b. Label a SOLiD® Emulsion Collection Tray with the sample ID.
- c. Carefully remove the caps from the 96-well plate containing the ISPs, then place the labeled SOLiD® Emulsion Collection Tray upside-down on top of the 96-well plate.
- d. If you are using a 96-well Eppendorf® twin.tec real-time PCR plate, bind the 96-well plate and the SOLiD® tray together using adhesive tape or an elastic band. See the following picture.

Note: Applied Biosystems MicroAmp® 96-Well Reaction Plates do not require binding.

MicroAmp® 96-Well Reaction Plates



Eppendorf® twin.tec real-time PCR plate with the SOLiD® Emulsion Collection Tray



- e. Invert the coupled plates so that the 96-well plate is upside-down over the SOLiD® Emulsion Collection Tray.
 - f. Using a large-format centrifuge, centrifuge the coupled plates at $130 \times g$ for 4 minutes.

Note: If processing a single plate, construct a balance by coupling an empty collection tray and an empty 96-well PCR plate. Use the same PCR plate type as used for the emulsion to be collected.
 - g. During centrifugation, label *six* 1.5-mL DNA LoBind Tubes with the sample ID.
 - h. Remove the plates from the centrifuge, then carefully detach the 96-well plate from the SOLiD® Emulsion Collection Tray.
 - i. Using a P1000 pipette, pipet the emulsion in the SOLiD® Emulsion Collection Tray up and down to mix, then transfer the emulsion to the *six* 1.5-mL DNA LoBind Tubes (approximately 1.2 mL per tube).
2. Centrifuge the *six* DNA LoBind Tubes (1.5-mL), in the Eppendorf microcentrifuge, at $15,500 \times g$ for 2 minutes to collect the emulsion.

Note: We recommend that you use the Eppendorf microcentrifuge for this procedure.
 3. During centrifugation, prepare the Breaking Solution:
 - a. If one plate of material needs to be broken, mix 2 mL of Recovery Solution with 6 mL of 1-butanol in a 15-mL tube.

Note: Use 50-mL tube if more than one plate needs to be broken and scale the volumes accordingly.
 - b. Vortex for 1 minute or until the mixture forms a fine white emulsified material. If a phase separation is visible, vortex until the mixture is uniform.

IMPORTANT! Prepare fresh Breaking Solution daily.

 4. Wash the samples with Breaking Solution:
 - a. After centrifugation, carefully remove the clear top fraction of oil from each 1.5-mL tube.

IMPORTANT! Do not disturb the white emulsion phase (approximately 200 μ L) at the bottom of each tube.

 - b. Vortex the Breaking Solution again, then pipet 1 mL of Breaking Solution into each of the 1.5-mL tubes containing the white emulsion.
 - c. Vortex the 1.5-mL tubes on maximum setting for 30 seconds to solubilize the bottom phase containing the emulsion. After vortexing, invert each tube to confirm that the emulsion has been disrupted. If the emulsion is still pelleted, vortex the tube again until disrupted.
 - d. Centrifuge the tubes at $15,500 \times g$ for 2 minutes. After centrifugation, the tubes should contain two distinct phases separated by a white interface. See the following picture.



- e. Without disturbing the interface, carefully remove the top organic phase from each 1.5-mL tube by pipetting from the top down to the white interface.
5. Wash the samples with Recovery Solution:
 - a. Add 1 mL of Recovery Solution to each 1.5-mL tube.
 - b. Vortex the tubes for 30 seconds, then centrifuge at 15,500 X g for 3 minutes.
 - c. Remove the supernatant from each tube by pipetting from the top. Remove as much of the white emulsified oil layer as possible, leaving behind approximately 100 μ L in bottom of each tube.
6. Pool the material from the *six* 1.5-mL tubes into a new 1.5-mL tube:
 - a. With the same pipette tip, resuspend each pellet by pipetting up and down.

IMPORTANT! Do not change the pipette tips during resuspension.

 - b. Transfer all six resuspended solutions into a single new 1.5-mL tube.

Note: White residue may be left behind inside the old tubes.
 - c. Using a P200 pipette, rinse three of the *six* 1.5-mL tubes with a single 200- μ L aliquot of Recovery Solution. Pipet the solution up and down in the bottom of the first tube, transfer the solution to the next tube and repeat the rinse, then repeat for the third tube. After rinsing the third tube, transfer the solution to the tube that contains the combined, resuspended pellets.
 - d. Repeat step 6c to perform a single rinse of the three remaining tubes.
7. Wash the samples once more with Recovery Solution:
 - a. Add Recovery Solution until the total volume is level with the 1.5-mL mark on the tube.
 - b. Vortex the 1.5-mL tube for 30 seconds, then centrifuge at 15,500 X g for 3 minutes.
 - c. Remove the supernatant by pipetting from top. Remove as much of the white emulsified oil layer as possible, leaving behind approximately 100 μ L in the bottom of the tube.
8. Resuspend the material by pipetting up and down, then transfer the sample to a new 1.5-mL DNA LoBind Tube and retain the pipette tip. Add 100 μ L of Wash Solution to the original tube. Using the retained tip, pipet up and down 3 times to rinse the tip and then transfer the solution to the new tube containing the sample.
9. Wash the sample twice (2 \times) with Wash Solution:
 - a. Add 1 mL of Wash Solution to the new 1.5-mL DNA LoBind Tube.
 - b. Vortex the 1.5-mL DNA LoBind Tube for 30 seconds, then centrifuge the tube at 15,500 X g for 3 minutes.
 - c. Remove the supernatant by pipetting from top. Remove as much of the white emulsified oil layer as possible, leaving behind approximately 100 μ L in the bottom of the tube.
 - d. Repeat steps 9a–9c.

10. You may perform a quality control step to assess which library dilution will result in optimal sequencing results. Choose from *one* of the following options:

Next action	Then...
(Optional) Quality assessment using a Qubit® 2.0 Fluorometer	Go to the “Quality Control of Ion Sphere™ Particles using a Qubit® 2.0 Fluorometer” section.
Quality assessment by sequencing	Place the sample on ice, then proceed to go to the “Determine recovery and percentage template-positive Ion Sphere™ Particles” section.

Note: If you do not have a Qubit® 2.0 Fluorometer to analyze the pre-enriched sample quality, we recommend that you transfer approximately 10% of the collected material to a separate 1.5-mL DNA LoBind Tube and store it for later use. If enrichment fails, you can sequence the sample to perform an unenriched sequencing run, which can be used to troubleshoot the experiment. See “Determine recovery and percentage pre-enriched template-positive Ion Sphere™ Particles by sequencing”.

STOPPING POINT The sample can be stored at 4°C for up to 3 days.

11. Place the sample on ice, then proceed to “Perform the template-positive Ion Sphere™ Particles enrichment”.

Perform the template-positive Ion Sphere™ Particles enrichment

IMPORTANT! Adhere to the recommended vortexing and centrifugation speeds in the following section.

1. Prepare for the enrichment:

- a. Confirm that the sample to be enriched is in a total volume of 100 µL of Wash Solution.

If the volume is...	Then...
< 100 µL	Add Wash Solution to increase the total volume to 100 µL.
> 100 µL	Centrifuge the sample at 15,500 X g for 3 min, then remove the excess supernatant leaving behind approximately 100 µL in the tube.

- b. Set aside *three* 1.5-mL DNA LoBind Tubes and label them:

1. Unbound
2. Enriched-1
3. Enriched-2

- c. Prepare fresh Melt-Off Solution by combining:

- 200 µL of 1M NaOH
- 16 µL of 10% Tween® 20 in molecular biology grade water
- 1.38 mL molecular biology grade water

IMPORTANT! Prepare Melt-Off Solution daily.

Note: The final composition of the Melt-Off Solution is 125 mM NaOH and 0.1% Tween® 20.

2. Wash the Dynabeads® MyOne™ Streptavidin C1 beads with Wash Solution:

- a. Vortex the bottle containing the Dynabeads® MyOne™ Streptavidin C1 beads.
- b. Transfer 10 µL of MyOne™ beads to a 1.5-mL tube.
- c. Add 70 µL of Wash Solution to the MyOne™ beads, then vortex to mix.

Note: If you are enriching more than one sample, you can wash the MyOne™ beads in a single 1.5-mL tube. Ensure that you scale the volumes accordingly.

- d. Place the tube on a magnet for 2 minutes, then discard the supernatant.

- e. Add 10 μL of Wash Solution to the MyOne™ beads to resuspend them.
Note: If washing MyOne™ beads for more than 1 sample, add the original volume to the beads to resuspend them.

3. Perform the capture:

- a. Add 10 μL of Dynabeads® MyOne™ Streptavidin C1 beads to the sample tube containing the ISPs, then pipet up and down and vortex for several seconds to mix.
- b. Add 100 μL Annealing Buffer to the sample tube, then pipet up and down and vortex for 2 seconds to mix. The Annealing Buffer is provided within the PGM™ Reagent Kit (Part no. 4468994) of the Ion Sequencing Kit (Part no. 4468997).
- c. Rotate the sample tube end-over-end for 10 minutes at room temperature using a tube rotator.
Note: Clumping or granularity may be observed at this point. Proceed to the next step.
- d. Centrifuge the sample tube for 2 seconds to remove sample from the cap and sides of the tube.
- e. Place the sample tube on the magnet for 2 minutes, then transfer the supernatant to the “Unbound” tube.

4. Wash the beads twice (2 \times) with Wash Solution:

- a. Add 200 μL of Wash Solution to the sample tube, then pipet up and down 15 times to mix.
- b. Place the sample tube on a magnet for 2 minutes, then transfer the supernatant to the “Unbound” tube.
- c. Repeat steps 4a and 4b.

5. Elute the ISPs from the Dynabeads® MyOne™ Streptavidin C1 beads:

- a. Add 400 μL of Melt-Off Solution (from step 1 of this procedure) to the sample tube, then vortex to mix.
- b. Rotate the sample tube end-over-end for 7 minutes at room temperature using a tube rotator.
- c. While the tube is rotating, add 400 μL of Wash Solution to the “Enriched-1” tube.
- d. After the sample tube has completed rotating, vortex, then centrifuge the sample tube to remove sample from the cap and sides of the tube.
- e. Place the sample tube on a magnet for 2 minutes, then transfer the supernatant to the “Enriched-1” tube.

IMPORTANT! Be careful not to transfer any Dynabeads® MyOne™ beads to the “Enriched-1” tube.

- f. Vortex the combined “Enriched-1” tube and centrifuge the sample at 15,500 X g for 4 minutes.
- g. Remove all but 100 μL of the supernatant from the “Enriched-1” tube.

IMPORTANT! Do not remove the remaining 100 μL of the supernatant because it contains enriched ISPs.

6. Treat the ISPs with Wash Solution:

- a. Add 1 mL of Wash Solution to the “Enriched-1” tube.
- b. Vortex, then centrifuge the “Enriched-1” tube at 15,500 X g for 4 minutes.
- c. Remove and discard all but 100 μL of the supernatant from the “Enriched-1” tube.

7. Remove the last traces of the Dynabeads® MyOne™ beads:

- a. Mix the solution in the “Enriched-1” tube by pipetting up and down. Ensure that bubbles are not generated during this step.
- b. Vortex, then centrifuge the “Enriched-1” tube for 2 seconds to remove sample from the cap and sides of the tube.

- c. Place the “Enriched-1” tube on a magnet for 2 minutes, then transfer the supernatant to the “Enriched-2” tube.

8. Choose from the following:

Are you sequencing the sample immediately?	Then...
Yes	Proceed immediately to the instructions listed in the <i>Ion Sequencing Kit User Guide v2.0</i> (part no. 4469714).
No	Store the material at 4°C. STOPPING POINT The sample can be stored at 4°C for up to 3 days.

Ion Sphere™ Particles quality control

You can determine the appropriate library dilution and/or the enrichment efficiency using *one* of the methods below. See the following sections for details.

- Quality Control of Ion Sphere™ Particles using a Qubit® 2.0 Fluorometer
- Determine recovery and percentage pre-enriched template-positive Ion Sphere™ Particles (ISPs) by sequencing
- *Ion Sphere™ Particles Quality Assessment Using the guava® easyCyte™ 5 Flow Cytometer User Bulletin* (Part no. 4470082). To access this protocol, visit the Ion Community website (<http://ioncommunity.iontorrent.com>).

Appendix: Supplementary Procedures

This appendix includes the following topics:

- Ion Sphere™ Particles Recovery PVC Basin Protocol
- Quality Control of Ion Sphere™ Particles using a Qubit® 2.0 Fluorometer
 - Section 1: Upgrade the Qubit® 2.0 Fluorometer Firmware and Software
 - Section 2: Calculate the Qubit® 2.0 Fluorometer Calibration Factor
 - Section 3: Measure the Ion Sphere™ Particles Sample
- Determine recovery and percentage pre-enriched template-positive Ion Sphere™ Particles (ISPs) by sequencing

Ion Sphere™ Particles Recovery PVC Basin Protocol

The following procedure is an alternative to the “Ion Sphere™ Particles Recovery Collection Tray Protocol” procedure.

Materials

The following materials are required in addition to the materials listed in “Materials and equipment required”.

- Pipette, 8-channel
- PVC basin

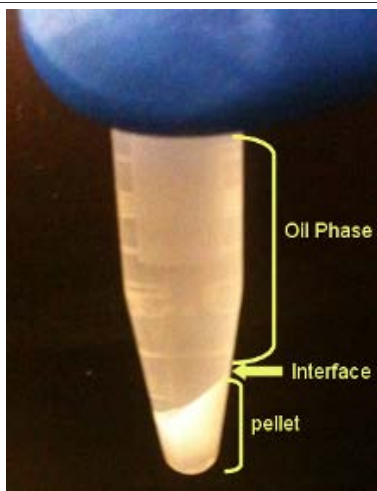
Procedure

1. Using an 8-channel pipette, transfer the emulsion from the wells of the PCR plate to a PVC basin. Before each transfer, pipet the emulsion up and down to mix.

IMPORTANT! Do not discard the pipette tips (you will use these in step 6 of this procedure).

2. Using the P1000 pipette, evenly distribute the emulsion from the PVC basin into *six* 1.5-mL DNA LoBind Tubes. The total volume of the emulsion should be approximately 9-10 mL, so each tube should contain approximately 1 mL of emulsion.
3. Centrifuge the *six* DNA LoBind Tubes (1.5-mL), in the Eppendorf microcentrifuge, at 15,500 X g for 2 minutes to collect the emulsion.
Note: We recommend that you use the Eppendorf microcentrifuge for this procedure.
4. While the 1.5-mL DNA LoBind Tubes are spinning, prepare the Breaking Solution:
 - a. To a 15-mL tube, add 2.5 mL of Recovery Solution and 7.5 mL of 1-butanol.
 - b. Vortex the solution for 1 minute until it forms a fine, white emulsified material. If a phase separation is visible, vortex until the solution is uniform.
5. After centrifugation, carefully remove and discard the clear, top layer of oil from each 1.5-mL tube without disturbing the white emulsion phase (approximately 150 µL) at the bottom. See the following picture.

Layers



6. Wash the wells of the PCR plate twice (2X) with Breaking Solution:
 - a. Vortex the Breaking Solution again.
 - b. Using an 8-channel pipette, transfer 100 μL of Breaking Solution to each of the 8 wells in the first row of the PCR plate, mix by pipetting up and down, then transfer the solution to the next row of the plate. Pipet the solution up and down in the new row, then transfer the solution to the next row, and so on until you have rinsed each row of the PCR plate.
 - c. After rinsing the last row of the plate, transfer the solution to the PVC basin.
 - d. Repeat steps 6a and 6b to wash the PCR plate again with Breaking Solution, transferring the resulting solution to the PVC basin.
7. Wash the PVC basin thoroughly to collect all of the ISPs, then transfer the solution to the six 1.5-mL tubes that contain the white emulsion pellet.
8. Wash the emulsions with Breaking Solution:
 - a. Vortex the Breaking Solution again.
 - b. To each 1.5-mL tube containing an emulsion, add enough Breaking Solution to reach the 1 mL mark on the tube.
 - c. Vortex the tubes on maximum for 1 minute to solubilize the bottom phase, which contains the emulsion. After vortexing, invert the tubes and inspect them. If you can see emulsion clumped to the walls of the tubes, vortex again for longer until the emulsion is no longer distinct.
 - d. Centrifuge the tubes at 15,500 X g for 2 minutes. After centrifugation, the tubes should contain two distinct phases with little or no material in the interface.
 - e. Without disturbing the interface, carefully remove and discard the top organic phase from each 1.5-mL tube. Get as close as possible to the white interface without disturbing it.
9. Wash the samples with Recovery Solution:
 - a. Add 1 mL of Recovery Solution to each 1.5-mL tube.
 - b. Vortex the tubes for 1 minute, then centrifuge them 15,500 X g for 3 minutes.
 - c. Remove the supernatant from each tube by pipetting from top. Remove as much of the white emulsified oil layer as possible, leaving behind approximately 50 μL in the bottom of each tube.
10. Pool the material from the six 1.5-mL tubes into a new 1.5-mL tube. See “Perform the Ion Sphere™ Particles recovery” section for details.

Quality Control of Ion Sphere™ Particles using a Qubit® 2.0 Fluorometer

IMPORTANT! This protocol is only for use with the Qubit® 2.0 Fluorometer and not for use with any other previous versions/models of the instrument.

The Quality Control of Ion Sphere™ Particles (ISPs) using a Qubit® 2.0 Fluorometer consists of three sections:

- Section 1: Qubit® 2.0 Fluorometer Firmware and Software Upgrade
- Section 2: Qubit® 2.0 Fluorometer Calibration Factor Calculation
- Section 3: Ion Sphere™ Particles Sample Measurement

Before using the Qubit® 2.0 Fluorometer for the first time, sections 1 and 2 must be completed.

Note: Sections 1 and 2 only need to be completed one time for each Qubit® 2.0 Fluorometer.

If sections 1 and 2 have been successfully completed for a particular Qubit® 2.0 Fluorometer already, then go directly to Section 3.

Section 1: Upgrade the Qubit® 2.0 Fluorometer Firmware and Software

Section 1 describes the procedure for upgrading the Qubit® 2.0 Fluorometer with the Ion Sphere™ Quality Control software.

Required materials

- Qubit® 2.0 Fluorometer (Invitrogen, Part no. Q32866)
- USB drive (included with Qubit® 2.0 Fluorometer)
- Program (.qbt) and upgrade (.bin) files:
 - Ion_Plugin_2.qbt
 - V3.00 Qubit_FW_MainCPU.bin
 - V3.00 Qubit_FW_UsbHost.bin

File download guidelines

- We recommend that you use a Windows® OS-based computer to download and transfer the Qubit® 2.0 files to the USB Drive. If you do not have access to a Windows-based computer, contact your Life Technologies Field Application Specialist (FAS) or Field Service Engineer (FSE) for assistance.

IMPORTANT! Do not use a Macintosh computer to transfer the files to the USB drive.

- We also recommend that you use the USB drive provided with Qubit® 2.0 for file transfer. Other, compatible USB drives may also be used. Refer to the Ion Community website (www.iontorrent.com/qubit_files/) for a list of approved USB drives.
- Files on the USB drive must be in the root directory and cannot be in a folder.

Prepare a USB drive for the upgrade or upload

1. Download the program (.qbt) file and two upgrade (.bin) files from the Ion Community website (www.iontorrent.com/qubit_files/) onto a windows PC desktop.
2. Transfer the three downloaded files to the USB drive provided with the Qubit® 2.0 (recommended), or another compatible USB drive. Files on the USB drive must be in the root directory and cannot be in a folder.

Upgrade the firmware

1. Power off the Qubit® 2.0 Fluorometer by unplugging the unit.

Note: The Qubit[®] 2.0 Fluorometer does not have a power switch. To power off the Qubit[®] 2.0, the unit must be unplugged.

2. Insert the USB drive, containing the program (.qbt) and upgrade (.bin) files, into the USB port on the instrument.
3. Power on the Qubit[®] 2.0 Fluorometer by plugging in the unit.
4. In the upper-right corner of the main menu, touch **V2.00**.

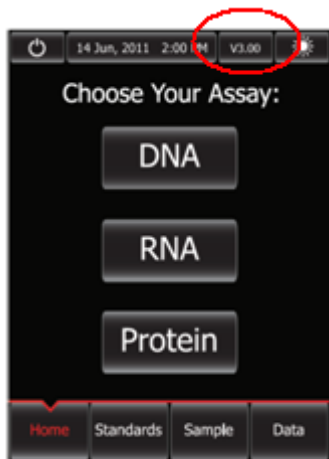


5. If the image of the USB drive in the update screen displays a green dot, touch Update to upgrade the instrument firmware. The firmware update lasts approximately 2 minutes, during which the Qubit[®] Fluorometer screen flashes.

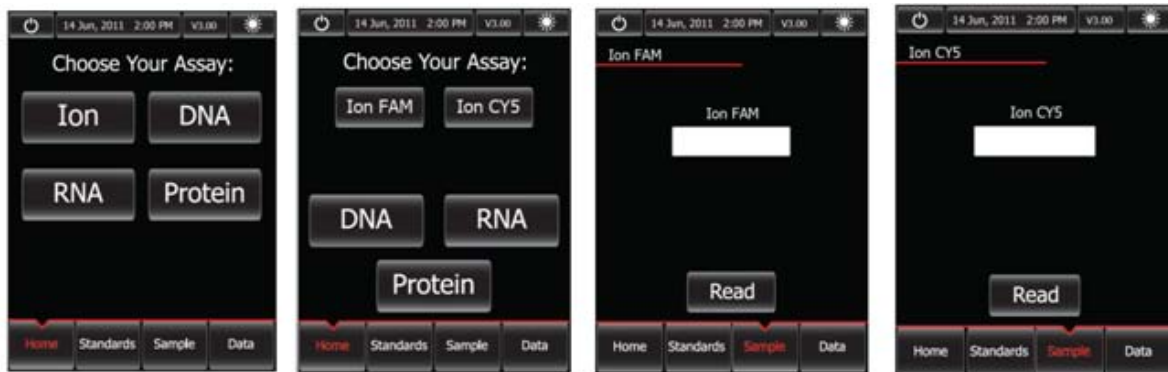
Note: If the image displays a red dot, the Qubit[®] 2.0 Fluorometer cannot detect the USB drive. Ensure the USB drive is in place securely. If the problem persists, remove the USB and reinsert.



6. When the Qubit[®] 2.0 Fluorometer displays the main screen, confirm that V3.00 is displayed in the upper-right corner, which confirms that the upgrade was successful.



7. With the USB still inserted into the Qubit[®] 2.0 Fluorometer, power-cycle the Qubit[®] 2.0 Fluorometer by unplugging and plugging it back in.
8. Confirm the Ion Sphere[™] QC program (.qbt) file is functional by checking the following screens:
 - a. In the main menu, an Ion button is present.
 - b. After pressing the Ion button, IonFAM and IonCy5 buttons are present.
 - c. Pressing **IonFAM** or **IonCy5** enters the respective measurement channels.



9. Proceed to complete the instruction in Section 2: Qubit[®] 2.0 Fluorometer Calibration Factor Calculation.

Section 2: Calculate the Qubit® 2.0 Fluorometer Calibration Factor

Section 2 describes the procedure to determine the Qubit® 2.0 Fluorometer instrument specific calibration factor.

IMPORTANT! You must upgrade the Qubit® 2.0 Fluorometer firmware and software prior to performing the following procedure. See “Section 1: Qubit® 2.0 Fluorometer Firmware and Software Upgrade” for more information.

Each Qubit® 2.0 Fluorometer has a unique Calibration Factor that must be calculated and applied to all Percent Templated ISPs calculations. It is only necessary to calculate the calibration factor once for a particular instrument, unless a problem is suspected.

Required materials

- Qubit® 2.0 Easy Calculator Microsoft® Excel® Spreadsheet (available at www.iontorrent.com/qubit_files/)
- Qubit® 2.0 Fluorometer (Invitrogen, Part no. Q32866) updated to V3.00 firmware
- USB drive containing the three upgrade files
- Qubit® Assay Tubes (Invitrogen, Part no. Q32856)
- Ion Sphere Quality Control Kit (Ion Torrent, Part no. 4468656)

Download Qubit® 2.0 Easy Calculator

Download the Qubit® 2.0 Easy Calculator Microsoft® Excel® Spreadsheet file from the Ion Community website (www.iontorrent.com/qubit_files/) and save the file to the computer used for Qubit® 2.0 data analysis.

Calibration standard preparation

1. From the Ion Sphere™ Quality Control Kit, thaw the Ion FAM™ dye and Ion Cy5® Calibration Standard reagents.
Note: Both the FAM™ dye and Cy5® molecules are photosensitive, so avoid exposure to light for long periods of time and direct sunlight.
2. Vortex well to mix and pulse-spin the tube to remove any liquid trapped in the cap.
3. Transfer 200 µL of each standard into two separate Qubit® assay tubes. Pulse-spin to bring all the liquid to the bottom of the tube.

Calibration standard measurement

IMPORTANT! Prior to powering on the Qubit® 2.0 Fluorometer, the USB drive containing the Ion_Plugin_2.qbt file must be inserted into the USB port to run the Ion Sphere™ QC program. After the initial power on, the Qubit® 2.0 retains the Ion Sphere™ QC program functionality if the USB drive is removed. If the Qubit® 2.0 is powered off for any reason, the USB drive must be re-inserted before powering the instrument back on.

1. Insert the USB drive into the USB port of the Qubit® 2.0 Fluorometer and power on the instrument.
2. Press **Ion** button to access IonFAM and IonCy5 measurement options.
3. Press **IonFAM** button, insert Ion FAM™ dye calibration standard into the Qubit® 2.0 Fluorometer, close the lid, and press **Read**.
Note: The lettering on the Read button changes from white to red when reading a sample. The lettering changes back to white when the reading of the sample is finished in approximately 5 seconds.
4. Record the RFU value and remove sample from Qubit® 2.0 Fluorometer.
5. Press **Home** button, press the **IonCy5** button, insert the Ion Cy5® calibration standard into the Qubit® 2.0 Fluorometer, close the lid, and press **Read**.

Note: The lettering on the Read button changes from white to red when reading a sample. The lettering changes back to white when the reading of the sample is finished in approximately 5 seconds.

- Record the RFU value and remove sample from Qubit[®] 2.0 Fluorometer.

Calibration Factor Calculation

- In the Qubit[®] 2.0 Easy Calculator, enter each recorded RFU value in the appropriately labeled green cell to display the Calibration Factor specific for the Qubit[®] 2.0 Fluorometer.
- Save a copy of the Qubit[®] 2.0 Easy Calculator containing the Calibration Factor for use as a template for future Percent Templated ISPs calculations.

Note: Affix a sticker with the instrument-specific Calibration Factor to the Qubit[®] 2.0 Fluorometer.

Qubit Calibration Factor Calculation						
Calibration Standard	RFU	Calibration Factor				
FAM Calibration Standard		#DIV/0!				
Cy5 Calibration Standard		#DIV/0!				
Templated Signal Ratio						
Sample ID	Raw RFU Value		Background RFU (Negative Control Tube)		Conversion Factor*	Percent Templated ISPs
	IonFAM	IonCy5	IonFAM	IonCy5		
						#DIV/0!
						#DIV/0!
						#DIV/0!
						#DIV/0!
* Conversion factor can be found on Ion Community website (www.iontorrent.com/qubit_files/) and is Template Kit lot specific.						
Green Cells = Raw RFU values of FAM and Cy5 Calibration Standards supplied in the Ion Sphere Quality Control Kit						
Red Cells = Raw RFU values measured in Section 3: Ion Sphere Particles Quality Control Sample Measurement						
Purple Cells = Raw RFU values measured for negative control in Section 3: Ion Sphere Particles Quality Control Sample Measurement						
Blue Cells = Template kit lot specific conversion factor						

IMPORTANT! For each Qubit[®] 2.0 Fluorometer used, save a separate Qubit[®] 2.0 Easy Calculator Microsoft[®] Excel[®] Spreadsheet file containing the Calibration Factor specifically calculated for that particular instrument.

Section 3: Measure the Ion Sphere™ Particles Sample

Section 3 describes the procedure for determining the Percent Templated ISPs for pre-enriched and enriched Ion Sphere™ Particles.

Required materials

- Qubit® 2.0 Fluorometer (Invitrogen, Part no. Q32866) updated with the V3.00 firmware
- USB drive containing the three upgrade files
- Qubit® Assay Tubes (Invitrogen, Part no. Q32856)
- PCR tubes, 0.2 mL (Axygen Part no. PCR-02-L-C or BioExpress Part no.T-3035-1)
- Qubit® 2.0 Easy Calculator Microsoft® Excel® Spreadsheet file containing the instrument specific Calibration Factor. See “Section 2: Qubit® 2.0 Fluorometer Calibration Factor Calculation” for details.
- Ion Sphere™ Quality Control Kit (Ion Torrent, Part no. 4468656)
- Unenriched or enriched Ion Sphere™ Particles

Sample preparation

1. From the Ion Sphere™ Quality Control Kit, thaw the Ion Probes tube, Annealing Buffer, and Quality Control Wash Buffer.
2. Unenriched and enriched ISPs samples require different volumes for ISPs quality control:
 - a. For unenriched ISPs, if not 100 µL, adjust sample volume to 100 µL in Ion Sphere™ Wash Solution (from Ion Xpress™ Template Kit, Part no. 4469001) and *transfer 2 µL* to a 0.2-mL PCR tube.
 - b. For enriched ISPs if not 100 µL, adjust sample volume to 100 µL in Ion Sphere™ Wash Solution (from Ion Xpress™ Template Kit, Part no. 4469001) and *transfer 10 µL* to a 0.2-mL PCR tube.
3. Add Ion Probes to sample(s).
 - a. If processing one sample, add 19 µL Annealing Buffer and 1 µL Ion Probes directly to the 0.2-mL PCR tube containing the ISPs and mix well by pipetting up and down.
 - b. If processing more than one sample, generate an Ion Probe Master Mix:
 1. (21 µL Annealing Buffer * # samples) + (1 µL Ion Probes * # samples) = total volume required
 2. Add 20 µL of Ion Probe Master Mix to the 0.2-mL PCR tubes containing the ISPs, then mix well by pipetting up and down.
4. Load the tube(s) into a thermal cycler, then perform the following protocol to anneal the Ion Probes:

Temperature	Time
95°C	2 min
37°C	2 min

5. Remove unbound probes by washing the sample(s) three times with 200 µL Quality Control Wash Buffer.

IMPORTANT! Do not use the Ion Sphere™ Wash Solution provided in the Ion Xpress™ Template Kit (Part no. 4469001).

- a. Add 200 µL Quality Control Wash Buffer to the 0.2-ml tube(s).
- b. Vortex properly to mix and spin down at 15,500 rcf for 1.5 minutes.
- c. Being careful not to disturb the pelleted ISPs, remove the supernatant and leave behind 10 µL.

Note: Compare to a 10 µL standard for reference.

- d. Repeat steps 5a–5c two times for a total of *three* Quality Control Wash Buffer washes.

6. After the final wash, add 190 μL of Quality Control Wash Buffer for a total volume of 200 μL , mix by pipetting up and down five times and transfer the entire sample to a Qubit assay tube.

IMPORTANT! Ensure that you measure the volumes accurately.

7. To generate a negative control, add 200 μL of Quality Control Wash Buffer to a fresh Qubit assay tube.
8. Read the sample(s) using the Qubit[®] 2.0 Fluorometer as described below.

Sample measurement

IMPORTANT! Prior to powering on the Qubit[®] 2.0 Fluorometer, the USB drive containing the Ion_Plugin_2.qbt file must be inserted into the USB port to run the Ion Sphere[™] QC program. After the initial power on, the Qubit[®] 2.0 retains the Ion Sphere[™] QC program functionality if the USB drive is removed. If the Qubit[®] 2.0 is powered off for any reason, the USB drive must be re-inserted before powering the instrument back on.

1. Insert the USB drive into the USB port of the updated Qubit[®] 2.0 Fluorometer and power on the instrument.
2. Press **Ion** button to access IonFAM and IonCy5 measurement options.
3. Press the **IonFAM** button and insert the sample into the Qubit[®] 2.0 Fluorometer, close the lid, and press **Read**.

Note: The lettering on the Read button changes from white to red when reading a sample. The lettering changes back to white when the reading of the sample is finished in approximately 5 seconds.

4. Record the value.

Note: The data retained on the Qubit[®] 2.0 Fluorometer can be transferred to a USB drive. See the “(Optional) Data Transfer to USB Drive” section for details.

Note: If more than one sample is being processed, all samples can be read with the IonFAM setting before moving on to the IonCy5 setting.

5. Press **Home** button, press **IonCy5** button, insert the sample into the Qubit[®] 2.0 Fluorometer, close the lid, and press **Read**.

Note: The lettering on the Read button changes from white to red when reading a sample. The lettering changes back to white when the reading of the sample is finished in approximately 5 seconds.

6. Record the value.

IMPORTANT! Ensure that you read the negative control (Quality Control Wash Buffer only) in both the IonFAM and IonCy5 settings and record the RFU values.

(Optional) Data Transfer to USB Drive

1. Ensure that the USB drive is inserted in the instrument.
2. In the main menu, touch Data (at the bottom right corner of the screen).
3. In the data screen, touch USB drive button, then wait for the instrument to download the data to the USB drive.

Note: The download creates a .csv file that can be opened on your computer using any spreadsheet software, such as Microsoft® Excel® software.



Templated Ion Sphere™ Particle evaluation

1. Open the saved Qubit® 2.0 Easy Calculator Microsoft® Excel® Spreadsheet file containing the Calibration Factor specifically calculated for the Qubit® 2.0 Fluorometer used.
2. Enter the raw RFU values from Cy®5 and FAM™ dye measurements in the appropriate fields for both the ISPs containing samples (red cells) and negative control sample (purple cells).
3. Enter the Ion Sphere™ Particles lot specific conversion factor found on the Ion Community website (www.iontorrent.com/qubit_files/) in the appropriate field (blue cells).
4. The Percent Templated ISPs calculates automatically and is displayed for each sample.

Qubit Calibration Factor Calculation		
Calibration Standard	RFU	Calibration Factor
FAM Calibration Standard	6548	1.72
Cy5 Calibration Standard	10265	

Templated Signal Ratio						
Sample ID	Raw RFU Value		Background RFU (Negative Control Tube)		Conversion Factor*	Percent Templated ISPs
	IonFAM	IonCy5	IonFAM	IonCy5		
						#DIV/0!
						#DIV/0!
						#DIV/0!
						#DIV/0!

* Conversion factor can be found on Ion Community website (www.iontorrent.com/qubit_files/) and is Template Kit lot specific.

Green Cells = Raw RFU values of FAM and Cy5 Calibration Standards supplied in the Ion Sphere Quality Control Kit
 Red Cells = Raw RFU values measured in Section 3: Ion Sphere Particles Quality Control Sample Measurement
 Purple Cells = Raw RFU values measured for negative control in Section 3: Ion Sphere Particles Quality Control Sample Measurement
 Blue Cells = Template kit lot specific conversion factor

Acceptance criteria for unenriched ISPs samples

The optimal amount of library corresponds to the library dilution point that gives Percent Templated ISPs between 10–30%. If the results are outside the desired Percent Templated ISPs range, then increase or decrease the library input appropriately.

Percent Templated ISPs	Description
<10%	Sample contains an insufficient number of templated ISPs to achieve optimal loading density on the Ion Chip.
10–30%	Optimal amount of library.
>30%	Sample will yield multi-templated ISPs (mixed reads).

Acceptance criteria for enriched ISPs samples

Percent Templated ISPs	Description
>50%	Satisfactory enrichment, continue to sequencing.

Determine recovery and percentage unenriched template-positive Ion Sphere™ Particles (ISPs) by sequencing

If you do not have access to a fluorometer or flow cytometer, you can perform quality control of unenriched Ion Sphere™ Particles (ISPs) by sequencing.

Note: The following protocol requires additional consumption of sequencing chips and reagents.

1. Resuspend the recovered Ion Sphere™ Particles by slowly pipetting up and down.
2. Take 10% of recovered ISPs per sample, then complete the procedures in the *Ion Sequencing Kit User Guide v2.0* (Part no. 4469714).
3. When sequencing run is complete, launch the Torrent Browser.

Note: For additional detail on how to use the Torrent Browser software, refer to the Torrent Browser documentation accessible from the Help menu of the Torrent Browser.

4. Select the **Reports** tab, then click the run of interest to start the Analysis Report.
5. While viewing the Analysis Report for the run, calculate the percentage of template-positive ISPs in the reaction using the following formula:

$$\frac{\text{Verified Library Ion Spheres}}{\text{Wells with Ion Spheres} - \text{Test Fragment Ion Spheres}} \times 100$$

If the percentage is between 10–30%: The library concentration is within the acceptable range. Beginning at “Prepare the IKA DT-20 solution”, proceed with the remainder of the sample.

If the percentage is less than 10% or greater than 30%: Adjust the input library molecules per emulsion to achieve approximately 10–30% template-positive ISPs.

For example, if the percentage is 40%, then reduce the input DNA by factor of 2–3.

Filter Metrics

Library key:	TCAG
Verified Library Ion Sphere Particles:	196,382

Ion Sphere™ Particles Identification Summary

Wells with Ion Sphere Particles:	755,129
Live Ion Sphere Particles:	650,912
Test Fragment Ion Sphere Particles:	5,375
Library Ion Sphere Particles:	645,537
Template-positive Ion Sphere Particles:	86%
Library Key:	TCAG
Verified Library Ion Sphere Particles:	522,149

Safety appendix



WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.




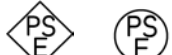
- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the “Documentation and support” section in this document.

Symbols on this instrument

Symbols may be found on the instrument to warn against potential hazards or convey important safety information. In this document, the symbol is used along with user attention words described in the “About This Guide” section to highlight important safety information. The following table gives the meaning of these symbols.

Symbol	English	Français
	Caution, risk of danger Consult the manual for further safety information.	Attention, risque de danger Consulter le manuel pour d'autres renseignements de sécurité.
	Caution, hot surface	Attention, surface chaude
	Caution, risk of electrical shock	Attention, risque de choc électrique
	On	On (marche)
	Off	Off (arrêt)
	On/Off	On/Off (marche/arrêt)
	Standby	En attente
	Terminal that can receive or supply alternating current or voltage	Borne pouvant recevoir ou envoyer une tension ou un courant de type alternatif
	Terminal that can receive or supply alternating or direct current or voltage	Borne pouvant recevoir ou envoyer une tension ou un courant continu ou alternatif
	Do not dispose of this product in unsorted municipal waste To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.	Ne pas éliminer ce produit avec les déchets usuels non soumis au tri sélectif. Pour minimiser les conséquences négatives sur l'environnement à la suite de l'élimination de déchets électroniques, ne pas éliminer ce déchet électronique avec les déchets usuels non soumis au tri sélectif. Se conformer aux ordonnances locales sur les déchets municipaux pour les dispositions d'élimination et communiquer avec le service à la clientèle pour des renseignements sur les options d'élimination responsable.

The following table shows the conformity marks and provides their descriptions.

Conformity mark	Description
	<p>Indicates conformity with safety requirements for Canada and U.S.A.</p>
	<p>Indicates conformity with European Union requirements for safety and electromagnetic compatibility.</p>
	<p>Indicates conformity with Australian standards for electromagnetic compatibility.</p>
	<p>Indicates conformity with requirements for safety with Japanese requirements.</p>

Safety information for instruments not manufactured by Life Technologies

Some of the accessories provided as part of the instrument system are not designed or built by Life Technologies. Consult the manufacturer's documentation for the information needed for the safe use of these products.

Instrument safety

General



CAUTION! Do not remove instrument protective covers. If you remove the protective instrument panels or disable interlock devices, you may be exposed to serious hazards including, but not limited to, severe electrical shock, laser exposure, crushing, or chemical exposure.



CAUTION! Solvents and Pressurized fluids. Wear eye protection when working with any pressurized fluids. Use caution when working with any polymeric tubing that is under pressure:

- Extinguish any nearby flames if you use flammable solvents.
 - Do not use polymeric tubing that has been severely stressed or kinked.
 - Do not use polymeric tubing with tetrahydrofuran or nitric and sulfuric acids.
 - Be aware that methylene chloride and dimethyl sulfoxide cause polymeric tubing to swell and greatly reduce the rupture pressure of the tubing.
 - Be aware that high solvent flow rates (~40 mL/min) may cause a static charge to build up on the surface of the tubing and electrical sparks may result.
-

Physical injury



CAUTION! Moving and Lifting Injury. The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide.

- Improper lifting can cause painful and permanent back injury.
 - Things to consider before lifting or moving the instrument or accessories:
 - Depending on the weight, moving or lifting may require two or more persons.
 - If you decide to lift or move the instrument after it has been installed, do not attempt to do so without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques.
 - Ensure you have a secure, comfortable grip on the instrument or accessory.
 - Make sure that the path from where the object is to where it is being moved is clear of obstructions.
 - Do not lift an object and twist your torso at the same time. Keep your spine in a good neutral position while lifting with your legs.
 - Participants should coordinate lift and move intentions with each other before lifting and carrying.
 - For smaller packages, rather than lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone else slides the contents out of the box.
-

Electrical



WARNING! Ensure appropriate electrical supply. For safe operation of the instrument:

- Plug the system into a properly grounded receptacle with adequate current capacity.
 - Ensure the electrical supply is of suitable voltage.
 - Never operate the instrument with the ground disconnected. Grounding continuity is required for safe operation of the instrument.
-



WARNING! Power Supply Line Cords. Use properly configured and approved line cords for the power supply in your facility.



WARNING! Disconnecting Power. To fully disconnect power either detach or unplug the power cord, positioning the instrument such that the power cord is accessible.

Cleaning and decontamination



CAUTION! Cleaning and Decontamination. Using a cleaning or decontamination method not specified by the manufacturer may result in damage to the equipment. For the protection of others, ensure the instrument is properly decontaminated prior to having the instrument serviced at your facility or before sending the instrument for repair, maintenance, trade-in, disposal, or termination of a loan. Decontamination forms may be requested from customer service.

Chemical safety



WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the “Documentation and support” section in this document.
 - Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
 - Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
 - Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
 - Handle chemical wastes in a fume hood.
 - Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
 - After emptying a waste container, seal it with the cap provided.
 - Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
 - Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
 - **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.
-

Documentation and support

Related documentation

The following related documents are shipped with the system:

Document	Part number	Description
<i>Ion Xpress™ Template Kit Quick Reference Guide v2.0</i>	4469711	Provides information on preparing, maintaining, and troubleshooting the system.
<i>Ion Sequencing Kit User Guide v2.0</i>	4469714	Provides information for performing genomic sequencing of amplified DNA.

Portable document format (PDF) versions of this guide and the Ion Xpress™ Template Kit Quick Reference Guide v2.0 are also available on the www.iontorrent.com.

To open the user documentation included on the www.iontorrent.com, use the Adobe® Reader® software available from www.adobe.com.

Obtaining SDSs

Safety Data Sheets (SDSs) are available from www.appliedbiosystems.com/sds and www.invitrogen.com/sds

For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

Obtaining Certificates of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to www.invitrogen.com/support and search for the Certificate of Analysis by product lot number, which is printed on the box.

Obtaining support

For the latest services and support information for all locations, go to:

www.iontorrent.com/support/

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches



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