iontorrent

Ion PGM™ Template IA 500 Kit

USER GUIDE

for use with: the Ion PGM™ System

Catalog Number A24622
Publication Number MAN0009347
Revision C.0

For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

The information in this guide is subject to change without notice.

DISCLAIMER

TO THE EXTENT ALLOWED BY LAW, LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. MAN0009347

Revision	Date	Description of Change
C.0	20 May 2016	• Added Chapter 4 "Create a Planned Run".
B.0	4 April 2016	• Reagent cap colors in Ion PGM™ Template IA Reagents 500 and Reactions 500 updated for ease of use. • Updated with support for Ion PGM™ Template IA Primer Mix L and Primer Mix S. • Updated with improved workflow and enhanced graphics.
A.0 (Tech Access)	6 December 2013	Provides detailed, step-by-step instructions on use of a new non-emulsion PCR template preparation kit with the Ion PGM™ System: the Ion PGM™ Template IA 500 Kit.

Corporate entity: Life Technologies Corporation | Carlsbad, CA 92008 USA | Toll Free in USA 1 800 955 6288

Contents

About this guide

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

Purpose

This user guide describes how to use the Ion PGM™ Template IA 500 Kit to prepare enriched, template-positive Ion PGM™ Template IA Ion Sphere™ Particles (ISPs) with 500 base-pair average insert libraries for sequencing on the Ion Personal Genome Machine™ (PGM™) System. The Ion PGM™ Template IA 500 System includes reagents for template preparation, the Ion OneTouch™ ES Instrument and Ion OneTouch™ ES reagents and supplies.

The user guide is organized as follows:

- Prepare template-positive ISPs containing clonally amplified DNA, using the Ion PGM™ Template IA 500 Kit for up to 500 base-read libraries (see Chapter 2, "Prepare template-positive Ion PGM™ Template IA ISPs").
- Enrich the template-positive ISPs with the Ion OneTouch™ ES Instrument (see Chapter 3, "Enrich the template-positive Ion PGM™ Template IA ISPs").
- Create a Planned Run in the Torrent Browser on the Torrent Server connected to your Ion PGM™ Sequencer (see Chapter 4, "Create a Planned Run").

1 Product information

Product description

The Ion PGM™ Template IA 500 Kit includes reagents for preparing 4 reactions of template-positive Ion PGM™ Template IA Ion Sphere™ Particles (ISPs) for sequencing with the Ion PGM™ System. Template preparation is carried out using Ion IA technology, which clonally amplifies DNA up to 500 base-pairs in length onto an ISP surface through a non-emulsion, isothermal reaction.

IMPORTANT! Use only the Ion PGM™ Template IA 500 Kit (Cat. No. A24622) with this user guide. Do not use the kit with the Ion OneTouch™ or Ion OneTouch™ 2 Instrument. Do not mix reactions or disposables including plates, solutions, and kit reagents from other template preparation kits.

Kit contents and storage

Kit summary

Component	Part number	Quantity per kit
Ion PGM™ Template IA Supplies 500	A24618	1 box
Ion PGM™ Template IA Reagents 500	A24619	1 box
Ion PGM™ Template IA Reactions 500	A24620	1 box
Ion PGM™ Template IA Solutions 500	A24621	1 box

IMPORTANT! Ion PGM™ Template IA Reactions 500 is shipped at 4°C to 8°C. Upon receipt, store at –30°C to –10°C. Immediately before use, thaw tubes on ice as needed.

Kit contents

Contents[1]	Cap color	Amount	Storage
Ion PGM™ Template IA Supplies 500 (Part No. A24618)			
Ion OneTouch™ ES Supplies, including: • Eppendorf™ LoRetention Dualfilter, 300 µL PCR pipette tips (5) • 8-well strips (12)	—	1 bag	15°C to 30°C
Ion PGM™ Template IA Reagents 500 (Part No. A24619)			
Ion PGM™ Template IA Pellets 500	—	4 foil pouches [2]	4°C to 8°C
Ion PGM™ Template IA ISP Dilution Buffer	Yellow	1 mL	
Ion PGM™ Template IA Start Solution	Purple	2 × 1200 µL	
Ion PGM™ Template IA Reactions 500 (Part No. A24620)			
Ion PGM™ Template IA Ion Sphere™ Particles	Orange	86 µL	–30°C to –10°C
Ion PGM™ Template IA Primer Mix S	Black	40 µL	
Ion PGM™ Template IA Primer Mix L	Blue	40 µL	
Ion PGM™ Template IA Rehydration Buffer	White	2 × 1.5 mL	
Ion PGM™ Template IA Solutions 500 (Part No. A24621)			
Ion PGM™ Template IA Stop Solution	—	2.8 mL	15°C to 30°C
Ion PGM™ Template IA Recovery Solution	—	7 mL	
Ion PGM™ Template IA Wash Solution	—	4 mL	
MyOne™ Beads Wash Solution	Green	1.04 mL	
Neutralization Solution	Red	40 µL	
Tween™ Solution	—	3.5 mL	

[1] We have verifed this protocol using this specific material. Substitution may adversely affect performance.

[2] One Ion PGM™ Template IA Pellet 500 per tube per pouch. One pellet used per IA 500 amplification reaction.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**www.fisherscientific.com**) or other major laboratory supplier.

✓	Item	Source
	Materials required for preparation of template-positive ISPs	
	2.0-mL Eppendorf DNA LoBind™ Tubes	Fisher Scientific 13-698-792
	Microcentrifuge	MLS
	Pipettors (P2, P20, P200, P1000) and appropriate low retention barrier tips	MLS
	Nuclease-free Water	AM9939
	Vortexer	MLS
	Heat block set to 40ºC	MLS
	0.2-mL PCR tubes	Fisher Scientific 14-222-283 or MLS
	GeneAmp™ PCR System 9700 thermal cycler or equivalent	N8050200 (Base) 4314443 (Block)
	Additional materials required for ISP enrichment	
	Ion OneTouch™ ES Instrument The system includes: • Ion OneTouch™ ES • AC Power Supply Cables • Bag containing accessories and spare parts: – Tray – Tip Arm – Tip Loaded – Corning™ Brand 96-well Strip Ejector (Fisher Part No. 07-200-22) – Spare fuses – AC Line Voltage Fuse Module • Elbow fitting for Ion OneTouch™ ES	4473574
	Ion PGM™ Enrichment Beads (Dynabeads™ MyOne™ Streptavidin C1 Beads)	4478525
	DynaMag™-2 magnet	12321D

✓	Item	Source
	Xiameter™ PMX-200 Silicone Fluid[1]	Neely Industries PMX200- 12500PT
	1M NaOH	MLS

[1] Material required for periodic maintenance of the Ion OneTouch™ ES.

Instrument clearances

Position the Ion OneTouch™ ES instrument so that the front is a minimum of 12 in. (30.5 cm) from the front of the laboratory bench. Place the instrument at least 40 in. (1 meter) away from major sources of electronic noise such as refrigerators or microwaves.

Contamination

CAUTION! A primary source of contamination is spurious DNA fragments from previous sample processing steps. Do not introduce amplified DNA into library preparation laboratory or work area. If feasible, set up a pre-PCR clean room for Ion PGM™ Template IA amplification reaction assembly. Use of a laminar flow hood is recommended but not required if a separate post-PCR room is used for the amplification reaction and ISP recovery.

Workflow

Prepare template-positive ISPs	**"Perform the IA reaction" on page 15** ▼ **"Recover the template-positive ISPs" on page 17** ▼
Enrich template-positive ISPs	**"Prepare reagents then fill the 8-well strip" on page 19** ▼ **"Prepare the Ion OneTouch™ ES" on page 22** ▼ **"Perform the Ion OneTouch™ ES run" on page 24** ▼
Create a Planned Run	**"Create a Planned Run in Torrent Suite™ Software" on page 27**

2 Prepare template-positive Ion PGM™ Template IA ISPs

Procedural guidelines

Guidelines for removing adapter dimers from library

Ion PGM™ Template IA ISP preparation is very sensitive to low concentrations of adapter dimers generated during library preparation. The following recommendations are designed to improve purification efficiency and reduce the levels of adapter dimers present in library preparations.

- For Ion Xpress™ Plus gDNA fragment libraries, refer to the current revision of the *Ion Xpress™ Plus gDNA Fragment Library Preparation User Guide* for recommended methods to purify your library after adapter ligation, size selection, and library amplification.
- For Ion AmpliSeq™ DNA libraries, additional library purification is required if the Ion Library Equalizer™ Kit is not used to normalize the library:
  - If using "Option 2: Quantify the unamplified library by qPCR" of the *Ion AmpliSeq™ DNA and RNA Library Preparation User Guide* (Pub. No. MAN0006735), perform a second round of purification with the Agencourt™ AMPure™ XP reagent. See "Purify Ion AmpliSeq™ libraries before templating with the Ion PGM™ Template IA 500 Kit" on page 36 for the full purification procedure.
  - If using "Option 3: Quantify the amplified library with the Qubit™ Fluorometer or Agilent™ 2100 Bioanalyzer™ instrument", perform a third round of purification with the Agencourt™ AMPure™ XP reagent. See "Purify Ion AmpliSeq™ libraries before templating with the Ion PGM™ Template IA 500 Kit" on page 36 for the full purification procedure.
- We recommend the use of library kits and protocols supplied by Thermo Fisher Scientific for all library preparation. If third-party reagents or protocols are used, library preparations may contain high levels of adapter dimers, which are not easily detected by Agilent™ 2100 Bioanalyzer™-based quantification methods. To reduce the amount of residual adapter dimers in the library, an additional round of purification with the AMPure™ XP reagent is required.

Additional guidelines for library preparation

- If you are using Ion SingleSeq™ libraries, refer to the *Ion ReproSeq™ PGS Kits User Guide* (Pub. No. MAN0013762) for the template preparation procedure appropriate for Ion SingleSeq™ libraries.
- Ion PGM™ Template IA ISP preparation requires a library prepared with full-length P1 primer/adapter, or P1 primer/adapters nearly full-length at the 5′ end. Amplicon libraries prepared with 5′-truncated P1 primer/adapter sequences are not compatible with the Ion PGM™ Template IA templating method.

Guidelines for library quantification and titration

Follow these recommendations for quantifying and titrating your libraries:

- We recommend quantifying the library by qPCR with the Ion Library TaqMan™ Quantitation Kit (Cat. No. 4468802). The concentration of the *E. coli* DH10B Ion Control Library in the Ion Library TaqMan™ Quantitation Kit is ~68 pM. This information is required to calculate your library concentration.
- If you use the Agilent™ 2100 Bioanalyzer™ instrument or the Qubit™ 2.0 or 3.0 Fluorometer to quantify your library, the library concentration derived using these methods is usually higher than by qPCR measurement of the same library. Our recommendation on the amount of library used in an Ion PGM™ Template IA reaction is based on qPCR-quantified libraries. You may need to use a higher than recommended library concentration when using the Bioanalyzer™ instrument or Qubit™ Fluorometer to quantify your library.
- To perform a sequencing-based library titration, use 48×10^6, 95×10^6 and 190×10^6 copies of library molecules per reaction. Polyclonal read percentage from an Ion PGM™ Template IA sequencing run is typically between 30-40%. If the percentage of polyclonal reads is greater than 50% for a library, fewer library molecules should be used in an Ion PGM™ Template IA amplification reaction.

Guidelines for preventing contamination

The IA reaction is highly sensitive to contaminating DNA. Follow the guidelines below to prevent introduction and carryover of contaminating DNA sequences in the work area.

- A primary source of contamination is spurious DNA fragments from previous sample processing steps. Do not introduce amplified DNA into the work area.
- To prevent carryover contamination, wipe down pipettors, hood and other work surfaces with a 70% ethanol-moistened Kimwipes™ disposable wipe before each experiment.
- After performing the reaction, rinse tube racks with Milli-Q™-quality water and dry.
- If possible, perform Ion PGM™ Template IA ISP preparation/reaction assembly and amplification/ISP recovery in two rooms: a pre-PCR clean room for ISP preparation/reaction assembly and a “dirty” post-PCR room for amplification and ISP recovery.
- If two rooms are used, ISP preparation and reaction assembly in a laminar flow hood is recommended but not required.
- If the entire procedure needs to be performed in one room, prepare the Ion PGM™ Template IA ISPs in a clean pre-PCR laminar flow hood.

Guidelines for performing the IA reaction

- The Ion PGM™ Template IA 500 Kit includes two primer mixes optimized for library length. Choose the primer mix based on the insert length of your library:
  - If the average length of inserts in your library is ≤350 bp, use Ion PGM™ Template IA Primer Mix S (black cap).
  - If the average length of inserts in your library is >350 bp, use Ion PGM™ Template IA Primer Mix L (white cap).
- Adjust the library input to the IA reaction according to whether or not your library is amplified. If you are using an amplified library, use 3.2 µL of a 50 pM library solution in the IA reaction. If you are using a non-amplified library (for example, an Ion AmpliSeq™ library or fragment library quantified by qPCR), use 4.8 µL of a 50 pM library solution in the IA reaction.
- To achieve optimal results, do not agitate tubes after the pulse-centrifuge step that follows addition of the Ion PGM™ Template IA Start Solution to the IA reactions.
- The length of the IA reaction (25 minutes at 40°C) is important. Follow these guidelines if you are performing multiple reactions:
  - Limit the number of reactions you perform at the same time to four.
  - If you need to perform more than four reactions, stagger the start and termination of each additional reaction by 5 minutes.

Materials required

Provided in the Ion PGM™ Template IA Reagents 500 Kit (Part. No. A24619):

- Ion PGM™ Template IA ISP Dilution Buffer
- Ion PGM™ Template IA Pellet 500
- Ion PGM™ Template IA Start Solution

Provided in the Ion PGM™ Template IA Reactions 500 Kit (Part No. A24620):

- Ion PGM™ Template IA Ion Sphere™ Particles
- Ion PGM™ Template IA Primer Mix S or L
- Ion PGM™ Template IA Rehydration Buffer

Provided in the Ion PGM™ Template IA Solutions 500 Kit (Part No. A24621):

- Ion PGM™ Template IA Stop Solution
- Ion PGM™ Template IA Recovery Solution
- Ion PGM™ Template IA Wash Solution

Other Materials and Equipment:

- 2-mL Eppendorf LoBind™ Tubes
- Nuclease-Free Water
- Vortexer
- Microcentrifuge
- Low-retention barrier pipette tips
- Pipettors
- Kimwipes™ disposable wipes
- Heat block set to 40°C (must accommodate 2-mL tubes)
- Ice

Before you begin

1. Dilute your library to 50 pM (30×10^6 copies/µL) in Nuclease-free Water in the clean room.
2. Preheat a heat block to 40°C in a post-PCR room. Add water to the wells to accelerate equilibration of the reaction tube.
3. Thaw the Ion PGM™ Template IA Primer Mix S or L, and keep it and the Ion PGM™ Template IA Start Solution on ice while setting up the reaction.

   Note: Use Primer Mix S if your library insert length is ≤350 bp. Use Primer Mix L if your library insert length is >350 bp.

Perform the IA reaction

1. Prepare Templating Solution in a 2-mL Eppendorf LoBind™ Tube on ice (or a cold block) using the following table. Adjust library input according to whether your library is amplified or non-amplified.

Order of addition	Component	Volume per reaction	
		Amplified library	Non-amplified library
1	Ion PGM™ Template IA ISP Dilution Buffer (yellow cap)	130 µL	128 µL
2	Ion PGM™ Template IA Primer Mix S or Primer Mix L[1]	8 µL	8 µL
3	Ion PGM™ Template IA ISPs[2] (orange cap)	21 µL	21 µL
4	Library (50 pM)	3.2 µL	4.8 µL
—	**Total**	**≈162 µL**	**≈162 µL**

[1] Use Primer Mix S if your library insert length is ≤350 bp. Use Primer Mix L if your library insert length is >350 bp.

[2] Vortex 30 seconds at maximum speed to resuspend immediately prior to addition.

2. Vortex the tube containing the Templating Solution for 2 seconds at the maximum setting to mix, pulse-centrifuge, then return the tube to ice.

3. Invert the Ion PGM™ Template IA Rehydration Buffer (white cap) three times to mix, then add 720 µL to the tube containing the Ion PGM™ Template IA Pellet to rehydrate the pellet. Vortex for 2 seconds at maximum setting, then pulse-centrifuge to collect the contents at the bottom of the tube. Place the rehydrated pellet on ice or in a cold block.

   Note: The rehydrated Ion PGM™ Template IA Pellet is opaque.

4. Transfer the rehydrated Ion PGM™ Template IA Pellet to the Templating Solution on ice, vortex for 2 seconds at the maximum setting, then pulse-centrifuge.

   IMPORTANT! The rehydrated pellet solution is viscous. Ensure that you transfer the entire volume by pulse-centrifuging the rehydration tube after transfer and pipetting any residual volume into the Templating Solution.

5. Invert the Ion PGM™ Template IA Start Solution (purple cap) three times to mix, then add 300 µL to the Template/IA Solution using the reverse pipetting technique.

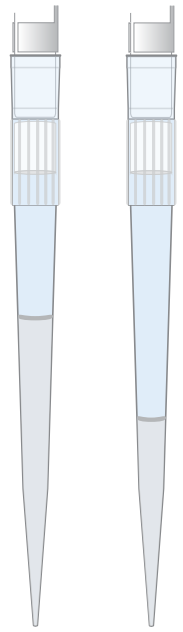
   Note: If you are setting up more than one IA reaction, follow steps 5 through 7 for each reaction before beginning the next reaction.

   To use the reverse pipetting technique, perform the following steps:

   a. Set a 1-mL pipettor to 300 µL.

   b. Press the pipette knob to the second stop and dip the tip into the Start Solution.

   c. Slowly release the pipettor knob until it returns to the starting position. Allow 10 seconds for Start Solution to be fully drawn up into the tip (Fig 5a).



   d. Dispense the solution into the Templating Solution tube by gently pressing the pipette knob to the first stop point only. Wait at least 5 seconds until the liquid in the pipette tip stops moving. Some liquid will remain in the tip (Fig. 5b).

   e. Withdraw the tip from the tube. If any liquid adheres to the outer surface of the tip, touch the tip to the inner wall of the tube to transfer the liquid to the tube.

6. Vortex the tube ten times in 1 second pulses at the maximum vortexer setting. Invert the tube and repeat the ten 1 second pulses.

7. Pulse-centrifuge the tube to collect contents, then immediately place the tube on ice.

   IMPORTANT! Handle the tube gently after centrifuging. To achieve optimum results, do not agitate the tubes from this point on.

8. Start the IA reaction by gently placing the tube in the 40°C heat block. Make sure the tube is immersed in water.

9. Incubate the IA reaction for 25 minutes at 40°C.

Recover the template-positive ISPs

1. Stop the IA reaction by removing the tube from the heat block and adding 650 uL of Ion PGM™ Template IA Stop Solution.
2. Vortex the tube well to mix contents thoroughly, then centrifuge the tube at 7,500 × *g* for 3 minutes.
3. Aspirate and discard the supernatant, being careful not to disturb the pellet. Leave ~100 µL in the tube.
4. Resuspend the pellet in 1 mL Ion PGM™ Template IA Recovery Solution.
   a. Pipette up and down to resuspend the pellet.
   b. Add an additional 700 µL Ion PGM™ Template IA Recovery Solution and vortex thoroughly.
5. Incubate for 5 minutes with vortexing 5 seconds every minute.
6. Centrifuge for 3 minutes at 12,000 × *g*.
7. Immediately remove and discard all of the supernatant without disturbing the ISP pellet. Remove any bubbles prior to removing the bulk of the liquid to avoid frothing in subsequent steps.

   Note: The ISPs will form a glassy pellet that is barely visible. Note the orientation of the tube in the centrifuge so that the position of the ISP pellet is known. The supernatant must be removed immediately to minimize the resuspension of ISPs.
8. Add 100 µL of the Ion PGM™ Template IA Wash Solution to the ISP pellet.
9. Resuspend the templated ISPs completely by vortexing for 4 seconds at maximum speed, then pipet the ISP suspension up and down four times. Proceed to Chapter 3, "Enrich the template-positive Ion PGM™ Template IA ISPs".

STOPPING POINT Store templated ISPs in Ion PGM™ Template IA Wash Solution at 4°C for up to one week.

3 Enrich the template-positive Ion PGM™ Template IA ISPs

Materials required

Provided in Ion PGM™ Template IA Solutions 500 (Part No. A24621):

- Ion OneTouch™ Template IA Wash Solution
- MyOne™ Beads Wash Solution
- Tween™ Solution
- Neutralization Solution

Provided in Ion PGM™ Template IA Supplies 500 (Part No. A24618):

- 8-well strip
- Eppendorf™ LoRetention Dualfilter Tips (P300)

Other Materials and Equipment:

- Ion OneTouch™ ES Instrument
- Ion PGM™ Enrichment Beads (Cat. No. 4478525; Dynabeads™ MyOne™ Streptavidin C1 Beads)
- 1.5-mL Eppendorf LoBind™ Tubes
- 0.2-mL PCR tubes
- Nuclease-free Water
- 1 M NaOH
- Pipettes
- Vortexer
- DynaMag™-2 magnet
- Microcentrifuge

Determine if a residual volume test is necessary

IMPORTANT! Ensure that the AC line voltage module is installed correctly into the Ion OneTouch™ ES Instrument. Refer to the *Ion OneTouch™ 2 System User Guide* (Pub. No. MAN0014388) for information about instrument setup, calibration and maintenance.

Follow these guidelines to determine if a residual volume test is necessary:

If the condition is...	Then...
First use of the instrument and during monthly maintenance	Perform a residual volume test (see "Ion OneTouch™ ES Instrument installation, setup, and maintenance" in the *Ion OneTouch™ 2 System User Guide*, Pub. No. MAN0014388).
Routine use and residual volume in Well 1 and Well 8 is >5.0 µL	
Routine use and residual volume in Well 1 and Well 8 is ≤5.0 µL	Operate the instrument without performing the residual volume test. Proceed to "Prepare reagents then fill the 8-well strip" on page 19.

Prepare reagents then fill the 8-well strip

Prepare Melt-Off Solution

Prepare fresh Melt-Off Solution by combining the components in the following order:

Order	Component	Volume
1	Tween™ Solution	280 µL
2	1 M NaOH	40 µL
—	**Total**	**320 µL**

IMPORTANT! Prepare Melt-Off Solution as needed, but appropriately dispose of the solution after 1 day.

The final composition of the Melt-Off Solution is 125 mM NaOH and 0.1% Tween™ 20 detergent.

Wash and resuspend the Dynabeads™ MyOne™ Streptavidin C1 Beads

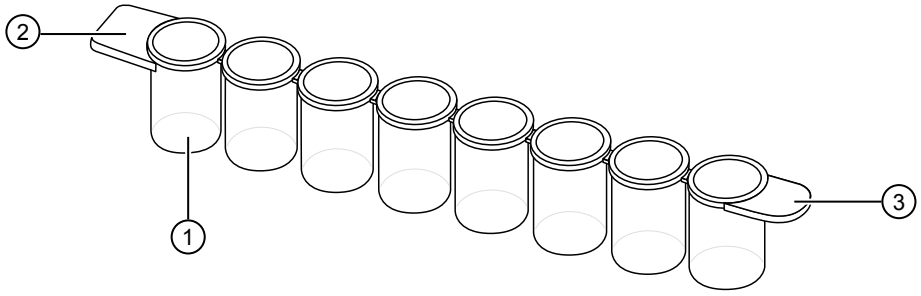
1. Vortex the tube for 30 seconds to thoroughly resuspend the beads, then centrifuge the tube of Dynabeads™ MyOne™ Streptavidin C1 Beads for 2 seconds.
2. Open the tube, then use a new tip to pipet up and down the dark pellet of beads until the pellet disperses. *Immediately* proceed to the next step.
3. Transfer 13 µL of Dynabeads™ MyOne™ Streptavidin C1 Beads to a new 1.5-mL Eppendorf LoBind™ Tube.
4. Place the tube on a magnet such as a DynaMag™-2 magnet for 2 minutes, then *carefully* remove and discard the supernatant without disturbing the pellet of Dynabeads™ MyOne™ Streptavidin C1 Beads.
5. Add 130 µL of MyOne™ Beads Wash Solution to the Dynabeads™ MyOne™ Streptavidin C1 Beads.

   Note: You add the resuspended Dynabeads™ MyOne™ Streptavidin C1 Beads in the 130 µL MyOne™ Beads Wash Solution to Well 2 of the 8-well strip.
6. Remove the tube from the magnet, vortex the tube for 30 seconds, and centrifuge for 2 seconds.

Fill the 8-well strip

Note: If the template-positive ISPs were stored at 2°C to 8°C, vortex the tube to resuspend the ISPs and pulse-centrifuge to collect contents. Pipet the solution up and down to resuspend the Ion PGM™ Template IA ISPs and transfer to Well 1 of the 8-well strip.

1. Add the entire volume (~100 µL) of template-positive ISPs from the amplification reaction into Well 1 of the 8-well strip. Well 1 with the ISPs is on the left:



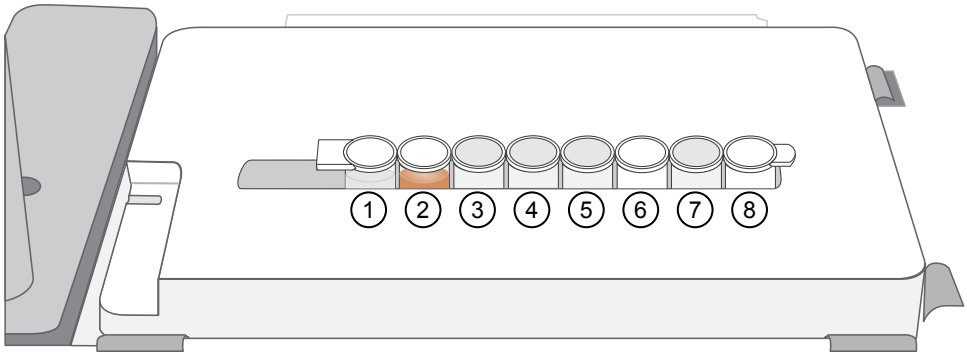
① Well 1
② Square-shaped tab
③ Rounded tab

2. If you have not already assessed the quality of the unenriched, template-positive ISPs, use the following method:

Quality assessment by...	Then...
(Optional) Guava™ easyCyte™ 5 Flow Cytometer	Transfer a 1.0-µL aliquot of the unenriched ISPs to a 1.5-mL Eppendorf LoBind™ Tube. See the *Ion Sphere™ Particles (ISPs) Quality Assessment Using the Guava™ easyCyte™ 5 Flow Cytometer User Bulletin* (Pub. No. 4470082), available on the Ion Community website: **ioncommunity.thermofisher.com**
Demonstrated protocol: Quality assessment by the Applied Biosystems™ Attune™ Acoustic Focusing Cytometer	Transfer a 1.0-µL aliquot of the unenriched ISPs to a 1.5-mL microcentrifuge tube. Put the sample on ice, then see the *Demonstrated Protocol: Ion Sphere™ Particles (ISPs) Quality Assessment using the Applied Biosystems™ Attune™ Acoustic Focusing Cytometer User Bulletin* (Pub. No. 4477181), available on the Ion Community website: **ioncommunity.thermofisher.com**

3. Fill the remaining wells in the 8-well strip as follows (see the following figure):

Well number	Reagent to dispense in well
Well 1 (well closest to the square-shaped tab)	Entire template-positive ISP sample (100 µL; prepared in step 1 of this procedure) (U)
Well 2	130 µL of Dynabeads™ MyOne™ Streptavidin C1 Beads resuspended in MyOne™ Beads Wash Solution [prepared in "Wash and resuspend the Dynabeads™ MyOne™ Streptavidin C1 Beads" on page 20] (B)
Well 3	300 µL of Ion PGM™ Template IA Wash Solution (W)
Well 4	300 µL of Ion PGM™ Template IA Wash Solution (W)
Well 5	300 µL of Ion PGM™ Template IA Wash Solution (W)
Well 6	Empty
Well 7	300 µL of freshly-prepared Melt-Off Solution (prepared in "Prepare Melt-Off Solution" on page 19) (M)
Well 8	Empty



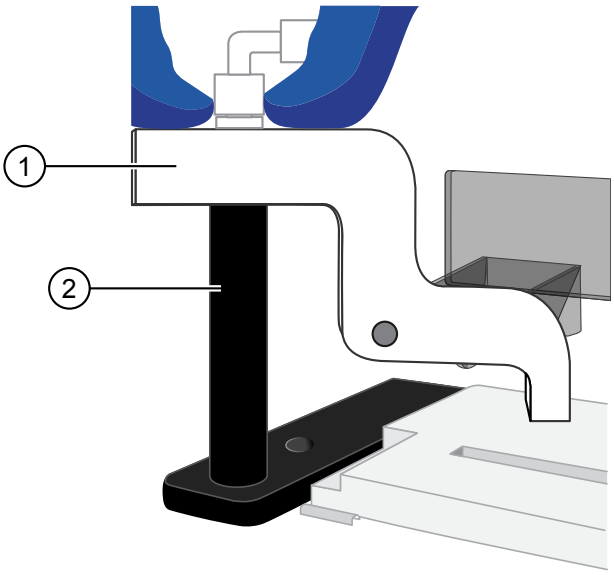
4. Confirm that the square-shaped tab is on the left, then insert the filled 8-well strip with the 8-well strip pushed all the way to the right end of the slot of the Tray.

Prepare the Ion OneTouch™ ES

Before every enrichment performed on the Ion OneTouch™ ES Instrument, install a new Eppendorf™ LoRetention Dualfilter P300 pipette tip.

1. Place a new tip in the Tip Loader. Remove the Tip Arm from the cradle and align the metal fitting of the Tip Arm with the tip.

2. Keeping the fitting on the Tip Arm vertical, firmly press the Tip Arm down onto the new tip until the Tip Arm meets the Tip Loader. Hold the Tip Arm to the Tip Loader for ~1 second to ensure proper installation of the tip.



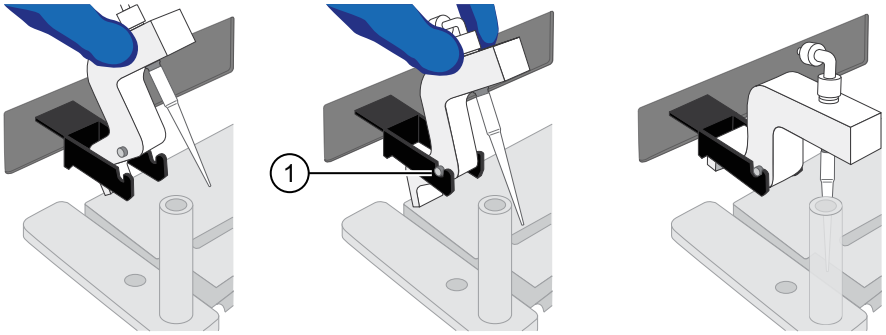
① Tip Arm
② Tip Loader

3. Lift the Tip Arm *straight* up to pull the installed tip from the Tip Loader tube.

4. Return the Tip Arm to the cradle (see the following illustration).
   a. Tilt the Tip Arm back (below left) and align the pins with the round notches in the cradle (below center).

   b. Lower the Tip Arm into position (below center).

c. Move the Tip Arm forward into the working position (below right).

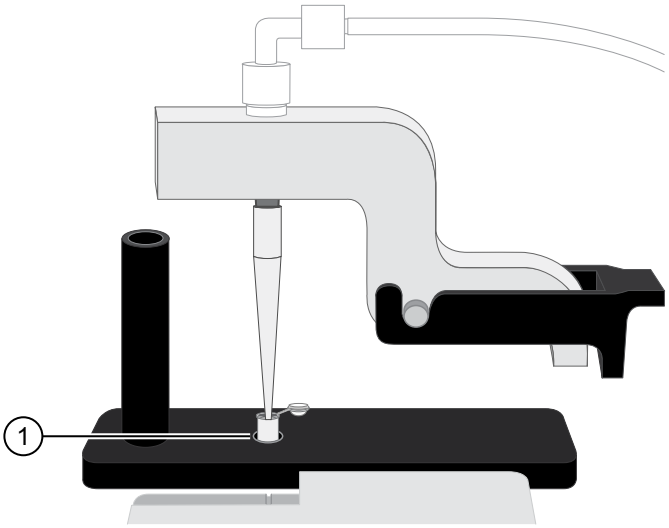


① Tip Arm pins resting in the notches in the cradle

IMPORTANT! Ensure that the back/bottom end of the Tip Arm is not resting on top of the thumb screw, causing the Tip Arm to tilt forward.

5. Add 10 µL of Neutralization Solution to a new 0.2-mL PCR tube.

6. Insert the open 0.2-mL PCR tube containing Neutralization Solution into the hole in the base of the Tip Loader, as shown in the following figure.



① 0.2-mL PCR tube placed in hole at base of Tip Loader

Perform the Ion OneTouch™ ES run

Confirm that a new tip and opened 0.2-mL PCR tube with the Neutralization Solution have been loaded. Ensure that Well 1 (ISP sample) is the left-most well and that the 8-well strip is pushed to the far-right position within the slot.

1. Pipet the contents of Well 2 up and down to resuspend the beads before starting the run. Do not introduce bubbles into the solution.

2. If necessary, turn ON the Ion OneTouch™ ES and wait for the instrument to initialize. The screen displays "rdy". The Tip Arm performs a series of initialization movements and returns to the home position (~5 seconds).

3. Press **Start/Stop**. The screen displays "run" during the run. The run takes ~35 minutes.

   Note: If necessary to stop a run, press **Start/Stop**. The instrument completes the current step, then stops the run and displays "End". Press **Start/Stop** again to return the Tip Arm to the home position. It is not possible to restart (where you left off) after stopping a run.

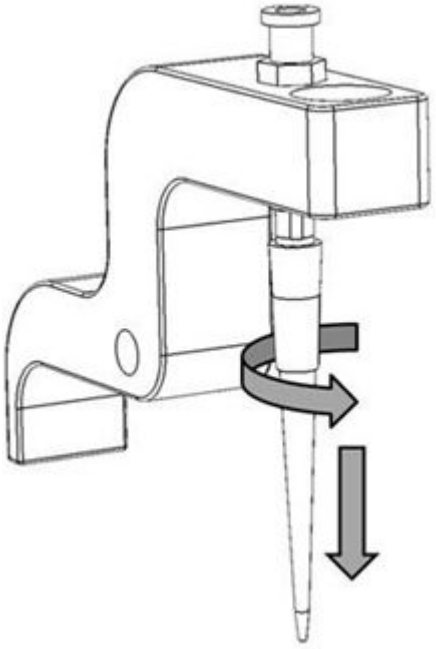
4. At the end of the run, the instrument displays "End" and beeps every 60 seconds. Press the **Start/Stop** button to silence this alarm and reset the Ion OneTouch™ ES for the next run. The instrument can be left on between runs.

5. ***Immediately after the run***, securely close and remove the PCR tube containing the enriched ISPs.

6. Mix the contents of the PCR tube by gently inverting the tube five times.

   Ensure that the 0.2-mL PCR tube has >200 µL of solution containing the enriched ISPs. After a successful run on the instrument, the sample is in ~230 µL of Melt-Off Solution, Ion PGM™ Template IA Wash Solution, and Neutralization Solution. If the tube has <<200 µL of solution containing the enriched ISPs, contact Technical Support.

7. Remove the used tip: While you are standing above the Tip Arm, and with the Tip Arm in its cradle, twist the tip counterclockwise and pull it downward to remove and discard the tip:



IMPORTANT! Improper removal of tips can loosen the metal tip adapter fitting on the Tip Arm and affect instrument operation.

8. Remove and discard the used 8-well strip.

Sequence or store the template-positive ISPs

- Sequence using the Ion PGM™ Hi-Q™ View Sequencing Kit (Cat. No. A30044). Proceed to Chapter 4, "Create a Planned Run" for Ion PGM™ Template IA 500 Kit-specific information for setting up a Planned Run. For more information on performing a sequencing run on the Ion PGM™ System, see the *Ion PGM™ Hi-Q™ View Sequencing Kit User Guide* (Pub. No. MAN0014583).

  or

- Store the material at 2°C to 8°C for up to 3 days.

Perform Ion Sphere™ Particles quality control

Determine the enrichment efficiency using one of the following methods:

Quality assessment by...	Then...
Guava™ easyCyte™ 5 Flow Cytometer	Transfer a 1.0-µL aliquot of the enriched ISPs to a 1.5-mL Eppendorf LoBind™ Tube. Refer to the *Ion Sphere™ Particles (ISPs) Quality Assessment Using the Guava™ easyCyte™ 5 Flow Cytometer User Bulletin* (Pub. No. MAN0015799).
Demonstrated protocol: Quality assessment by the Applied Biosystems™ Attune™ Acoustic Focusing Cytometer	Transfer a 1.0-µL aliquot of the enriched ISPs to a 1.5-mL Eppendorf LoBind™ Tube. Put the sample on ice, then refer to *Demonstrated Protocol: Ion Sphere™ Particles (ISPs) Quality Assessment using the Applied Biosystems™ Attune™ Acoustic Focusing Cytometer User Bulletin* (Pub. No. 4477181).

Create a Planned Run

About Planned Runs

Planned Runs contain all the settings used in a sequencing run, including number of flows, kit types, barcodes used (if any), run type (e.g., DNA, RNA, amplicons), and reference file (if any). They provide a fast and convenient way to set up and organize your runs.

You create a Planned Run using Torrent Browser on the Torrent Server connected to your sequencer, and then select the appropriate plan in the **Select Planned Run** screen of the sequencer touchscreen when you start the run.

You can also create a Planned Run on one Torrent Server and then transfer it to another server for sequencing. See the *Ion PGM™ Hi-Q™ View Sequencing Kit User Guide* (Pub. No. MAN0014583; Appendix B), for more information.

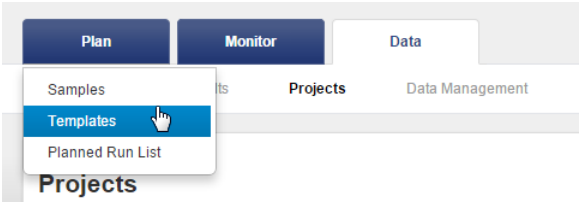
Note: For additional information, see the *Torrent Suite™ Software User Interface Guide*, available on the Ion Community at **ioncommunity.thermofisher.com**.

Create a Planned Run in Torrent Suite™ Software

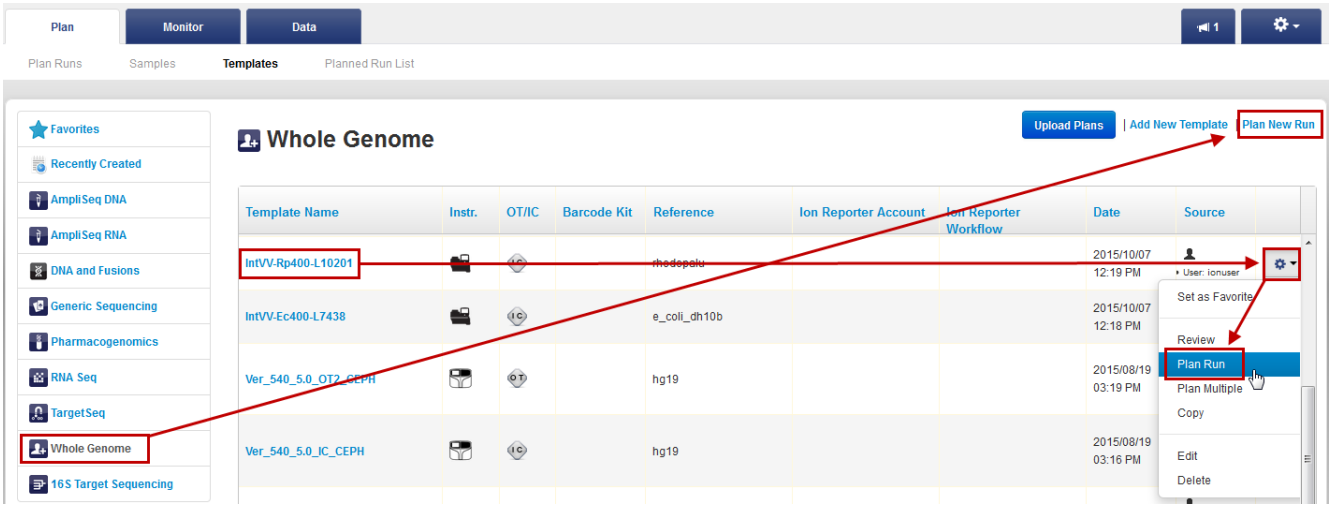
The following provides a summary of steps for creating a Planned Run in Torrent Suite™ Software, for use on the Ion PGM™ System.

For more detailed instructions, see the *Torrent Suite™ Software User Interface Guide*, available on the **Ion Community**.

1. Open the Torrent Browser on the Torrent Server connected to your sequencer.
2. Select the **Plan** tab, then select **Templates**.

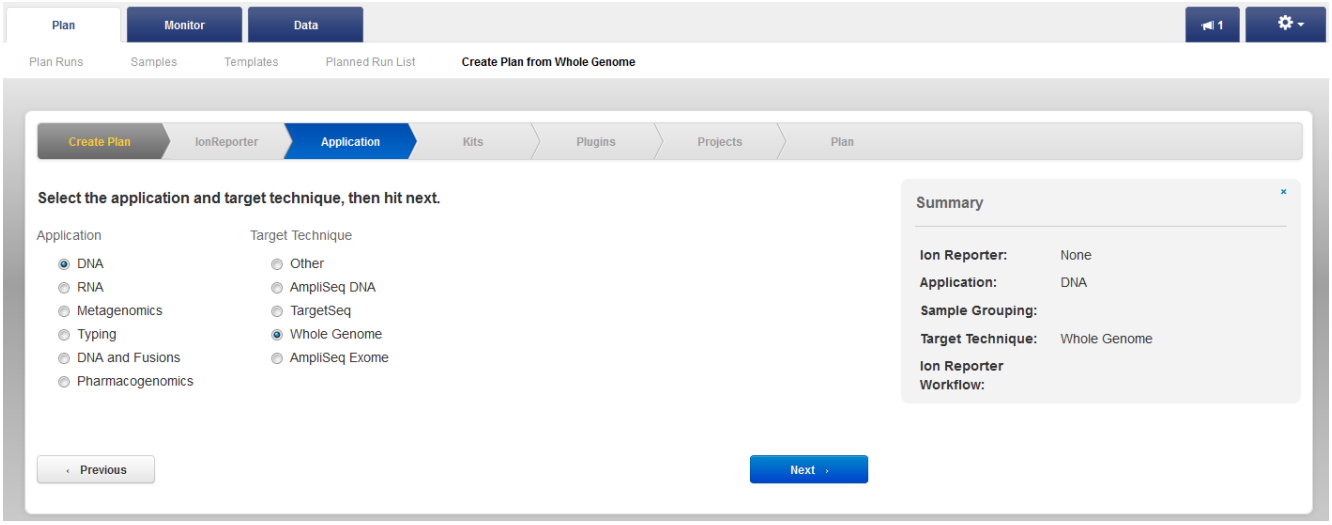


3. Select an application in the left navigation bar (for example, **Whole Genome**). A list of existing Planned Run templates for that application will appear. To create a new Planned Run, select one of the following options:
   - To create a new Planned Run without using an existing template, click **Plan New Run**.
   - To create a new Planned Run from an existing template, click the ⚙ button for the template, then select **Plan Run** from the dropdown menu.
   - Other options are available depending on the selected application, such as downloading templates from AmpliSeq.com.



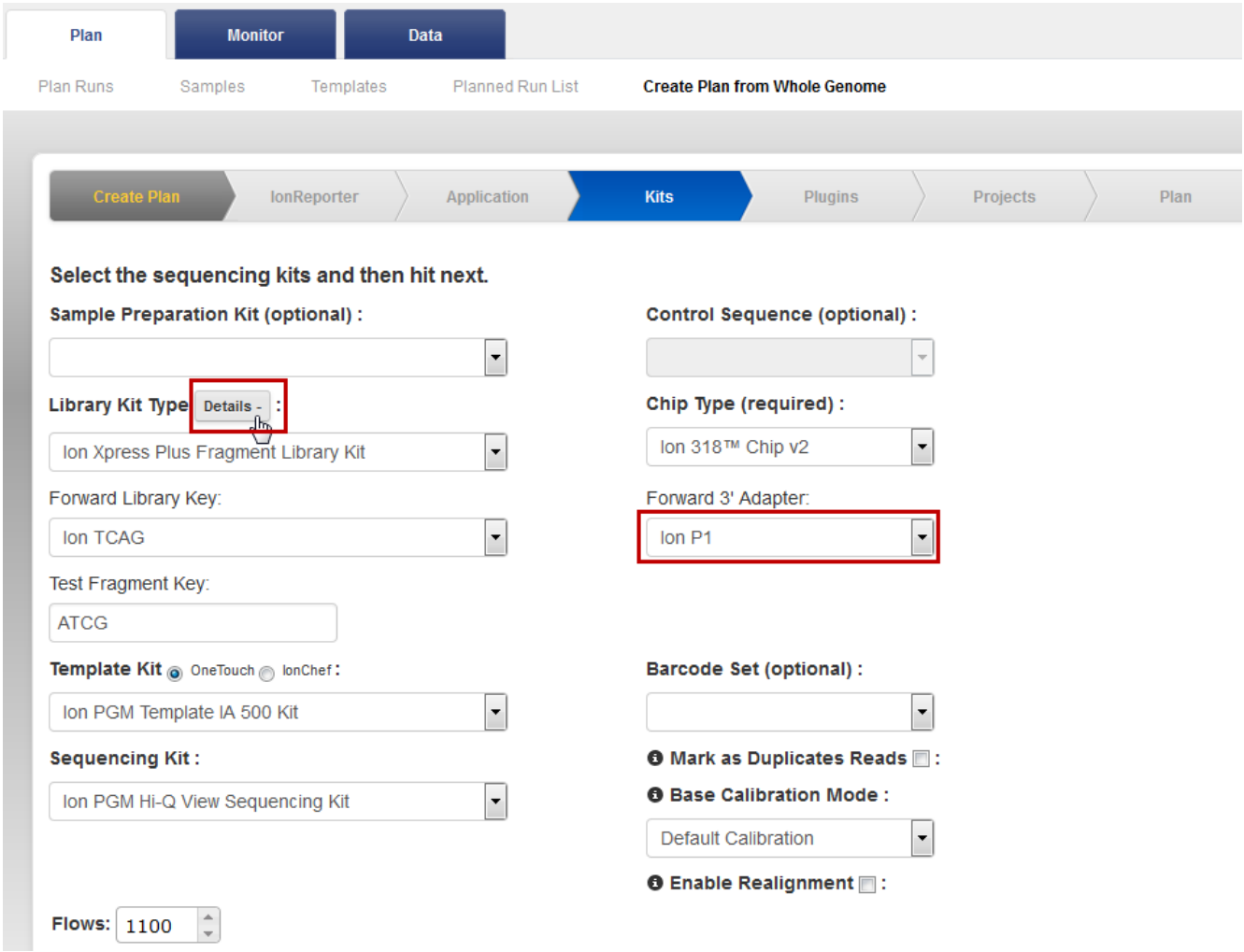
4. In the wizard, make your selections on each screen, then click **Next** to go to the next screen.

   Note: For a complete description of each option, see the *Torrent Suite™ Software User Interface Guide*.



5. On the **Kits** screen, select library, template and sequencing kits, and other settings appropriate to the run.

   IMPORTANT! Click the **Details +** button to the right of Library Kit Type to view more fields, then select **Ion P1** from the Forward 3' Adapter dropdown menu.



6. After you have completed your selections on the **Plugins**, **Projects**, and **Plan** screens, click **Plan Run** at the lower right corner of the **Plan** screen.
   The run is listed on the Planned Runs screen under the name you specified, and is available on the sequencer when you are setting up the run.

Planned Run wizard: key fields

Field name	Description
IonReporter	If used, select the account, then select workflow from the Existing Workflow menu. To create a new workflow, click **Create New Workflow**.
Application	Select the sequencing application you are performing: for example, **DNA Whole Genome**.
Library Kit Type	Select the library kit used.
Template Kit	Select **Ion PGM Template IA 500 Kit**.
Sequencing Kit	Select **Ion PGM Hi-Q View Sequencing Kit**.
Flows	Enter the number of flows appropriate for the read length. For example, enter • 500 flows for 200-base read sequencing • 850 flows fo 400-base read sequencing • 1100 flows for 500-base read sequencing
Chip Type	Select the chip type you are using from the dropdown menu: Ion 314™ Chip v2, Ion 316™ Chip v2, or Ion 318™ Chip v2.
Forward 3' Adapter	Select **Ion P1**.
Barcode Set	Select the barcode set, if used.
Project	Select or add a project in which to group your run data.
Run Plan Name	Enter a name for the Planned Run.
Reference Library	Select a reference library uploaded to the Torrent Server.
Target Regions and Hotspot Regions	Select appropriately.
Enter a sample name	Enter a Sample Name, and assign unique Analysis IDs for each sample in the run (number of samples will change based on the number of barcodes selected, if used). Avoid using the default names "Sample 1", "Sample 2", etc.
Monitoring Thresholds	Set thresholds for Bead Loading, Key Signal, and Usable Sequence. In the Torrent Browser **Monitor ▸ Runs in Progress** tab, an alert is displayed if the values for a run fall below the selected thresholds.

A

Troubleshooting

Ion PGM™ Template IA reaction

Observation	Possible cause	Recommended action
>2% Adapter dimer identified in the ISP Summary of a sequencing run Summary Report	Adapter dimer leftover in the library preparation even though it may not be visible/detectable by Bioanalyzer™ analysis	Further purify your library using the Agencourt™ AMPure™ XP Reagent. 1. Dilute the library to 50–100 µL with TE or Nuclease-free Water. 2. Add AMPure™ XP Reagent to clean up the library. For 400–500-base-read libraries, add 1X library sample volume. 3. Complete the AMPure™ XP bead washing and elution steps as described in the *Ion Xpress™ Plus gDNA Fragment Library Preparation User Guide* (Pub. no. MAN0009847) or *Ion AmpliSeq™ Library Preparation User Guide* (Pub. no. MAN0006735).

Ion OneTouch™ ES

For Ion OneTouch™ ES vertical and horizontal axis calibration and residual volume test procedures, see Chapter 3 of the *Ion OneTouch™ 2 System User Guide* (Pub. No. MAN0014388).

Observation	Possible cause	Recommended action
Excessive foaming occurs	• Instrument is improperly calibrated resulting in inadequate volume in one or more wells. • Fitting is loose. • Pipette tip is cracked.	1. Use the recommended volumes for all wells. 2. Ensure that fittings are tight, especially at the elbow fitting, and the pipette tip is not cracked. 3. If necessary, perform the residual volume test. If the residual volume test fails, then calibrate the instrument.

Observation	Possible cause	Recommended action
Brown pellet is present in centrifuged tube of enriched ISPs	Residual Dynabeads™ MyOne™ Streptavidin C1 Beads are present.	1. Pipet the suspension with the brown pellet up and down 10 times to resuspend the pellet. 2. Place the 0.2-mL PCR tube against a magnet such as a DynaMag™-2 magnet for 4 minutes. 3. Transfer the supernatant with the enriched ISPs to a new 0.2-mL PCR tube without disturbing the pellet of Dynabeads™ MyOne™ Streptavidin C1 Beads. 4. Sequence or store the enriched ISPs.

Observation	Possible cause	Recommended action
E12, E22, or E23 errors display during the run or during calibration	Calibration values are out of range.	1. Power OFF the instrument and wait 3 seconds. 2. While holding down **Vert. Adjust**, power ON the instrument. This step restores the factory default settings. 3. Recalibrate the vertical axis: **Note:** The default setting for the vertical axis is 310. If the setting is <310, the instrument will likely display an error, because the Tip Arm position is too high. a. Press the ▼ (minus) button to lower the Tip Arm until the tip touches the shelf. b. Press the ▼ (minus) button *8* more times. Typical vertical axis settings are ~340–370. 4. Recalibrate the horizontal axis: Press the ▲ (plus) button to move the Tip Arm to the right until the tip touches the left tab of the strip. **Note:** The default setting for the horizontal axis is 625. Typical horizontal axis settings are ~640–670.
	AC line voltage module is installed incorrectly.	1. Determine the voltage of the electrical outlet to plug in the Ion OneTouch™ ES. 2. Align the arrow by the correct voltage on the AC line voltage module with the adjacent white arrow in the lower-right corner of the fuse socket. If the AC line voltage module is installed incorrectly: 1. Gently remove the module with your fingernail or a small flathead screwdriver. 2. Rotate the module so that the correct voltage on the module is aligned and adjacent to the white arrow in the lower right-hand corner of the fuse socket. 3. Insert the AC line voltage module into the fuse socket.

Observation	Possible cause	Recommended action
E12 or E22 error is displayed when the unit is initializing	• Fuse is installed incorrectly. • Unit is below operating temperature. • Program or calibration setting is bad, *or* • Tip Arm is not moving.	1. Ensure that the fuse module is installed correctly and that the unit is at its recommended operating temperature. 2. Reboot the instrument: Power OFF the instrument, wait 3 seconds, then power ON the instrument. 3. If the error persists, restore the factory defaults, then re-calibrate the instrument: a. Power OFF the instrument and wait 3 seconds. b. While holding down **Vert. Adjust**, power ON the instrument. This step restores the factory default settings. c. Repeat 3a–3b as needed to restore the factory defaults. d. Calibrate the vertical and horizontal axes.
Either of the following: • E12 or E22 errors are displayed. • Tip Arm does not move or moves slightly.	AC line voltage module is installed incorrectly.	1. Determine the voltage of the electrical outlet serving the Ion OneTouch™ ES. 2. Align the arrow by the correct voltage on the AC line voltage fuse module with the adjacent white arrow in the lower-right corner of the fuse socket. If the AC line voltage fuse module is installed incorrectly: 1. Gently remove the module with your fingernail or a small flathead screwdriver. 2. Rotate the module so that the correct voltage on the module is aligned and adjacent to the white arrow in the lower right-hand corner of the fuse socket. 3. Insert the AC line voltage fuse module into the fuse socket.
	Instrument is not at the recommended operating temperature	Ensure that the Ion OneTouch™ ES is at an operating temperature of 60°F to 77°F (15°C to 25°C).
Solution overflows during run	Reagent volumes are overloaded.	Repeat with reagent volumes described in enrichment procedure.
Tip is causing 8-well strip to lift out of tray slot during run	Tip is not aligned vertically.	Perform the vertical calibration procedure.
Percent template-positive ISPs after enrichment is <50% as measured by flow cytometry	Multiple causes are possible.	Contact Technical Support.

Observation	Possible cause	Recommended action
Problems with the strip position • Strip lifts up during strip push. • Strip lifts up when tip is raised from well. • Immediately after strip push, the strip is not in contact with the magnet.	Instrument is not calibrated properly.	• Perform horizontal calibration. • Perform vertical calibration.
Tip grinds into base of instrument and Code "1999" displays	• Unit is not calibrated properly. • Vertical calibration setting is too low or out-of-range.	1. Restore the factory default settings on the instrument: Hold down the vertical adjust button while powering ON the instrument. The instrument beeps several times. 2. Re-calibrate the instrument. 3. Perform a residual volume test.
Tip hits the top of the tray at start of run	Tray is not properly seated in the instrument.	Check for debris between the tray and the instrument, then reinstall the tray. Press down firmly to ensure that tray is fully seated in the instrument.
Error messages display	Various causes are possible.	1. Power the instrument OFF, then ON. 2. If the error continues to display, restore the factory default settings on the instrument. Hold down the vertical adjust button while powering ON the instrument. The instrument beeps several times. 3. Re-calibrate the instrument. 4. Perform a residual volume test.
Instrument does not aspirate or dispense liquids	Fitting(s) are loose.	• Ensure that the Luer-Lok™ connections at the elbow on the Tip Arm and at the tubing on the rear syringe pump are finger-tight. • Ensure that the metal tip adapter fitting on the Tip Arm is finger-tight. **IMPORTANT!** After any adjustments to the metal tip adapter, recalibrate the Ion OneTouch™ ES.

B Supplementary procedure

Purify Ion AmpliSeq™ libraries before templating with the Ion PGM™ Template IA 500 Kit

Use the following procedure as an additional round of purification for Ion AmpliSeq™ libraries quantified by either qPCR or with the Qubit™ Fluorometer or Agilent™ 2100 Bioanalyzer™ instrument. At the start of this procedure you have

- purified libraries with one round of AMPure™ XP reagent purification following the Ion AmpliSeq™ ligation reaction. You intend to use qPCR to quantify the unamplified libraries, *or*
- purified amplified libraries with two rounds of AMPure™ XP reagent purification following library amplification. You intend to use the Qubit™ Fluorometer or Agilent™ 2100 Bioanalyzer™ instrument to quantify your amplified libraries.

1. Add 30 µL of Low TE to the AMPure™ XP reagent beads to disperse the beads and elute the library.
2. Seal the plate with MicroAmp™ Adhesive Film, vortex thoroughly, then centrifuge to collect droplets. Alternatively, mix by pipetting at least half the total volume up and down at least five times prior to sealing the plate.
3. Place the plate on the magnet for at least 2 minutes.
4. Carefully transfer the supernatant from each well to a single well of a new 96-well PCR plate, without disturbing the pellet.
5. Add 45 µL (1.5X sample volume) of Agencourt™ AMPure™ XP Reagent to each library and pipet up and down five times to thoroughly mix the bead suspension with the DNA.
6. Incubate the mixture for 5 minutes at room temperature.
7. Place the plate in a magnetic rack such as the DynaMag™-96 Side Magnet (Cat. No. 12331D) and incubate for 2 minutes or until solution clears. Carefully remove and discard the supernatant without disturbing the pellet.
8. Add 150 µL of freshly prepared 70% ethanol and move the plate side-to-side in the two positions of the magnet to wash the beads, then remove and discard the supernatant without disturbing the pellet.

   Note: If your magnet does not have two positions for shifting the beads, remove the plate from the magnet and gently pipet up and down five times (with the pipettor set at 100 µL), then return the plate to the magnet and incubate for 2 minutes or until the solution clears.
9. Repeat step 8 for a second wash.

10. Ensure that all ethanol droplets are removed from the wells. Keeping the plate in the magnet, air-dry the beads at room temperature for 5 minutes. Do not overdry.

11. Remove the plate from the magnet, and add 50 µL of Low TE to the pellet to disperse the beads and elute the library.

12. Seal the plate with MicroAmp™ Adhesive Film, vortex thoroughly, then centrifuge to collect droplets. Alternatively, mix by setting a pipettor to 40 µL and pipet the mixture up and down at least five times prior to sealing the plate.

13. Place the plate in the magnet for at least 2 minutes.

14. Proceed either to quantification of the unamplified library by qPCR, or to quantification of the amplified library with the Qubit™ Fluorometer or Agilent™ 2100 Bioanalyzer™ instrument.

IMPORTANT! The supernatant contains the eluted library. Do not discard!

C

Safety

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 hazard symbol is used along with one of the following user attention words:

- **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.

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.

Symbol	English	Français
	Protective conductor terminal (main ground)	Borne de conducteur de protection (mise à la terre principale)
	Do not dispose of this product in unsorted municipal waste **CAUTION!** 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. **CAUTION!** 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.

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.

Physical injury

CAUTION! Moving Parts. Moving parts can crush, pinch and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing.

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. Use only the cleaning and decontamination methods specified in the manufacturer's user documentation. It is the responsibility of the operator (or other responsible person) to ensure the following requirements are met:

- No decontamination or cleaning agents are used that could cause a HAZARD as a result of a reaction with parts of the equipment or with material contained in the equipment.
- The instrument is properly decontaminated a) if hazardous material is spilled onto or into the equipment, and/or b) prior to having the instrument serviced at your facility or sending the instrument for repair, maintenance, trade-in, disposal, or termination of a loan (decontamination forms may be requested from customer service).
- Before using any cleaning or decontamination methods (except those recommended by the manufacturer), users should confirm with the manufacturer that the proposed method will not damage the equipment.

Safety and electromagnetic compatibility (EMC) standards

The instrument design and manufacture complies with the standards and requirements for safety and electromagnetic compatibility as noted in the following table:

Safety

Reference	Description
EU Directive 2006/95/EC	European Union "Low Voltage Directive"
IEC 61010-1 EN 61010-1 UL 61010-1 CSA C22.2 No. 61010-1	*Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements*
IEC 61010-2-010 EN 61010-2-010	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-010: Particular requirements for laboratory equipment for the heating of materials*
IEC/EN 61010-2-020	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-020: Particular requirements for laboratory centrifuges*
IEC 61010-2-081 EN 61010-2-081	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes*

EMC

Reference	Description
Directive 2004/108/EC	European Union "EMC Directive"
EN 61326-1	*Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 1: General Requirements*
FCC Part 18 (47 CFR)	U.S. Standard "Industrial, Scientific, and Medical Equipment"
AS/NZS 2064	*Limits and Methods of Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radiofrequency Equipment*
ICES-001, Issue 3	*Industrial, Scientific and Medical (ISM) Radio Frequency Generators*

Environmental design

Reference	Description
Directive 2012/19/EU	European Union "WEEE Directive" – Waste electrical and electronic equipment

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.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Safety equipment also may include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.