

---

# ALLTYPE™ FASTPLEX™ NGS ASSAY on ION TORRENT SEQUENCING SYSTEMS

---

## Application Note

ALLTYPE™ FASTPLEX™ NGS 11 LOCI KIT – 48

Product: AllType™ FASTplex™ NGS 11 Loci Kit – 48

**REF**

Catalog ID: ALL-FAST11LX

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

 **ONE LAMBDA**

A Thermo Fisher Scientific Brand |

## TABLE of CONTENTS

INTRODUCTION	5
PRODUCT DESCRIPTION	5
Official Product Name	5
Catalog Information	5
Product Overview	5
Principles of the Method	6
WARNINGS	7
MATERIALS PROVIDED	8
Package Contents and Storage	8
Components in the AllType FASTplex NGS 11 Loci Kit	8
Instability Indications	9
Electronic Files	9
MATERIALS – REQUIRED but not PROVIDED	10
Supplies and Consumables	10
EQUIPMENT – REQUIRED but not PROVIDED	11
Equipment	11
PRECAUTIONS	12
IMPORTANT GUIDELINES	12
General Laboratory Practice	12
Technical and Equipment	12
Reagents	12
Safe Stopping Point	12
SAMPLE COLLECTION and SAMPLE PREPARATION	13
Sample Type	13
Sample Storage	13
DNA Extraction Methods	13
DNA Storage	14
WORKFLOW for the ASSAYS	15
GENERAL PREPARATION for the ASSAYS	16
AMPLIFICATION	20
Materials and Equipment	20
Part 1: Sample Amplification	20
Part 2: Amplicon Purification	22
Part 3: Amplicon Quantification	24
Part 4: Amplicon Dilution	25

SAMPLE BARCODING	26
Materials and Equipment	26
Part 1: Sample Barcoding (SB) Reaction, Reaction Stop, Sample Pooling, and Purification	26
Part 2: Universal Barcoding (UB) Reaction, Reaction Stop, and Purification	30
LIBRARY AMPLIFICATION	33
Materials and Equipment	33
Part 1: Library Amplification Reaction	33
Part 2: Purification of the Amplified Library	34
Part 3: Quantification of the Final Library	36
PREPARING for the ION GENESTUDIO S5 PLUS or EQUIVALENT SEQUENCER and ION CHEF SYSTEM	38
Materials and Equipment	38
Part 1: Uploading the FASTplex Template	38
Part 2: Creating and Uploading a Sample List	39
Part 3: Creating a Planned Run and Uploading the Sample List	40
RUNNING the ION CHEF SYSTEM	42
Guidelines for Using the Ion Chef System	42
Materials and Equipment	43
Part 1: Setting Up the Ion Chef System	43
Part 2: Starting the Chef ExT Run	44
USING the ION S5 or Ion GENESTUDIO S5	48
Guidelines for Using the Ion S5 or Ion GeneStudio S5	48
Reagents	48
Part 1: Initializing the Ion S5 Sequencer	49
Part 2: Starting the Sequencing Run	51
RESULTS	53
Data Acquisition	53
Data Calculation	53
Data Analysis	53
LIMITATIONS of the PROCEDURE	54
EXPECTED VALUES	56
Sample Amplification	56
Library Preparation	56
DNA Sequencing	56
CONTACT INFORMATION	57
Manufacturer	57
Technical Assistance	57

APPENDICES	58
Appendix 1: PCR Program Reference Guide	58
Appendix 2: FASTplex Sample Plate 48 Worksheet	59
Appendix 3: Quick Guide (Refer to the Application Note for more detail.)	60
BIBLIOGRAPHY	61
TRADEMARKS	62
DISCLAIMERS	62
EXPLANATION of SYMBOLS	63
EXPLANATION of HELPFUL SYMBOLS	64
REVISION HISTORY	64

## INTRODUCTION

This Application Note describes how to prepare compatible libraries from amplicons generated using the AllType™ FASTplex™ NGS 11 Loci Kit for the Ion Torrent Sequencing Systems.

The Application Note contains the steps required to generate the following PCR amplicons:

Locus	Targeted Region
• HLA-A	Full gene
• HLA-B	Full gene
• HLA-C	Full gene
• HLA-DRB1	Exon 1 & Exon 2 thru 3' UTR *
• HLA-DRB3/4/5	Exon 2 thru 3' UTR *
• HLA-DQB1	Exon 1 & Exon 2 thru 3' UTR *
• HLA-DPB1	Exon 1 & Exon 2 thru 3' UTR *
• HLA-DQA1	Full gene
• HLA-DPA1	Full gene

\* Includes intronic sequence

After amplification, the remainder of the Application Note covers the steps for sample barcoding, universal barcoding, library amplification, preparing for the Ion GeneStudio S5 Plus or equivalent sequencer and Ion Chef System, running the Ion Chef System, and using the Ion S5 or Ion GeneStudio S5 series sequencer.

To prevent injury and for optimal use of the AllType FASTplex NGS 11 Loci Kit, read through the entire Application Note.

## PRODUCT DESCRIPTION

### Official Product Name

AllType™ FASTplex™ NGS 11 Loci Kit – 48

### Catalog Information

<b>REF</b>	<b>Catalog ID</b>
	• ALL-FAST11LX

### Product Overview

AllType™ FASTplex™ NGS HLA 11 Loci Kit includes reagents to enrich the 11-loci HLA genes for HLA-A, B, C, DRB1, DRB345, DQB1, DPB1, DPA1, and DQA1 by a long-range multiplex polymerase chain reaction (PCR) method, purify the amplification products, and construct sequencing libraries for Ion Torrent systems for up to 96 samples per kit.

AllType FASTplex NGS HLA 11 Loci products consist of a pre-optimized HLA gene amplification primer solution in aqueous liquid format, PCR enzyme, 5X reaction buffer, and deoxyribonucleotides (dNTP) provided at recommended storage temperature at -20°C. The amplification primer design is based on the known DNA sequences reported in public DNA sequence databases such as Genbank (<http://www.ncbi.nlm.nih.gov/genbank/>) and IMGT (<http://www.ebi.ac.uk/ipd/imgt/hla/>).

AllType FASTplex NGS HLA 11 Loci Products provide a 96-well plate containing 48 unique Sample Barcoding reagents in each well in duplicate and 1 Universal Barcoding reagent (see Product Identification). When combined, a user can prepare a library for 8 to 48 samples with unique barcodes and common sequence for subsequent amplification at the ends of DNA fragments to match Ion GeneStudio S5 or Ion S5 systems. AllType FASTplex NGS HLA 11 Loci products also provide a library amplification primer mix that identifies common sequence at the ends of DNA fragments and amplifies all the barcoded products in the library. The kits also contain reagents to complete the library preparation, including barcoding buffer, stop solution and paramagnetic beads.

### **Throughput for AllType FASTplex NGS HLA 11 Loci Products with Ion Sequencers**

<b>Ion Chip Kit</b>	<b>Number of Samples Maximum Throughput</b>
Ion 530 Chip Kit	48
Ion 520 Chip Kit	24

### **Principles of the Method**

The AllType FASTplex NGS 11 Loci product generates target-specific amplification products for multiple HLA genes in a single multiplex polymerase chain reaction (PCR). DNA sequencing data are obtained by processing the amplified product with a fragmentation library workflow to prepare a library that can be sequenced using Ion Torrent sequencer platforms. The AllType FASTplex NGS 11 Loci Kit has been designed and optimized to produce nearly equimolar quantity of each target to provide adequate sequence coverage. Resulting data are analyzed using TypeStream™ Visual NGS Analysis Software to generate high resolution HLA allele assignments.

## WARNINGS



**FOR RESEARCH USE ONLY.** Not for use in diagnostic procedures.

**WARNING:** Refer to the Safety Data Sheet for detailed information. Individual Safety Data Sheets can be downloaded at [www.onelambda.com](http://www.onelambda.com) and/or [www.thermofisher.com](http://www.thermofisher.com).

## MATERIALS PROVIDED

### Package Contents and Storage



The AllType FASTplex NGS 11 Loci Kit is comprised of two separate boxes. The AllType FASTplex NGS 11 Loci Kit – 48 (Box 1 of 2) can be safely stored at -20°C and the AllType FASTplex NGS Kit (Box 2 of 2) has components to be stored at +2 to 8°C. The recommended storage condition for the FAST-STOP and FAST-BBUF is +20 to 25°C. The -20°C product may be frozen/thawed up to 12 times.



**CAUTION:** Upon receipt, keep each box intact and stored until ready to use. Avoid unnecessary handling.

A full list of the provided materials and their appropriate storage conditions can be found in the [Components Provided in the AllType FASTplex NGS 11 Loci Kit](#) section. The product label also contains individual component storage conditions.

### Components in the AllType FASTplex NGS 11 Loci Kit

#### Box 1 of 2

Component	Catalog ID	Quantity	Amount (µL)	Storage
AllType FASTplex 11 Loci Primer Mix	ALF-P11	1 vial	600	-20°C
AllType FASTplex 11 Loci Exon 1 Primer Mix	ALF-P11E1	1 vial	240	
AllType dNTPs	ALL-NTP	1 vial	200	
AllType Buffer	ALL-BUF	1 vial	500	
AllType LR Polymerase	ALL-LRPOL	1 vial	100	
FASTplex Sample Plate 48	FAST-SP48B	1 vial	16 per well	
FASTplex Univ Barcode P1	FAST-UBP1	1 vial	40	
FASTplex Library Primer Mix	FAST-LPM	1 vial	100	
FASTplex Library Amp Mix	FAST-LAM	1 vial	900	

#### Box 2 of 2

Component	Catalog ID	Quantity	Amount (µL)	Storage
FASTplex Barcoding Buffer	FAST-BBUF	1 vial	1500	+2 to 25°C
FASTplex Stop Solution	FAST-STOP	2 vials	2200	
FASTplex Paramagnetic Beads	FAST-BEAD	1 vial	7,700	+2 to 8°C
FASTplex DNA Suspension Buffer	FAST-SUSP	1 vial	8,000	



## Instability Indications



**WARNING:** Do not use if the boxes are damaged, product is not received at required storage temperature, or the product has leaked. Contact One Lambda Technical Support at [1lambda-TechSupport@thermofisher.com](mailto:1lambda-TechSupport@thermofisher.com).

## Electronic Files

Component	Location
AllType FASTplex Ion ExT template 530	Download from
AllType FASTplex Ion ExT template 520	<a href="http://www.onelambda.com">www.onelambda.com</a>

## MATERIALS – REQUIRED but not PROVIDED

### Supplies and Consumables

Description	Supplier	Catalog No.
96-Well, 0.2 mL, PCR Plates	MLS**	---
96 Well Plastic/Foil Plate Seals	MLS	---
Thermo Scientific Nunc™ 96-Well Polypropylene MicroWell™ Plates	Fisher Scientific	12-565-369
PCR Plate & 0.2 mL Tube Cooler	MLS	---
Eppendorf DNA LoBind Tubes, 5.0 mL	Eppendorf	0030108310
Eppendorf DNA LoBind Tubes, 2.0 mL	Eppendorf	022431048
PCR tube 0.2 mL	MLS	---
50mL Conical Tubes Nuclease free	MLS	---
15mL Conical Tubes Nuclease free	MLS	---
Serological Pipettes, 10 or 25 mL	MLS	---
Pipette Controller	MLS	---
Full array of manual single channel pipettes (20 µL, 200 µL, 1 mL)	MLS	---
20 µL and 200 µL manual multi-channel pipettes	MLS	---
Full array of filtered, pre-sterilized, pipette tips	MLS	---
Sterile Reagent Reservoirs	MLS	---
Strip Tubes / Caps	MLS	---
Thermal Cycler PCR Pressure Pad	MLS	---
Ion 520™ & Ion 530™ ExT Kit-Chef	Thermo Fisher Scientific	A30670
Ion 520 Chip Kit-4 Reactions*	Thermo Fisher Scientific	A27761
Ion 530 Chip Kit-4 Reactions*	Thermo Fisher Scientific	IONS5-530C4
Nuclease Free Water	MLS	---
200 Proof Ethanol, Molecular Biology Grade	MLS	---
PCR Plate Cooler	MLS	---

\* User should determine which size Ion Chip to use based on average sample library size.

\*\*MLS: Major Laboratory Supplier

## EQUIPMENT – REQUIRED but not PROVIDED

### Equipment

Description	Supplier	Catalog No.
TypeStream™ Visual NGS Analysis Software (Version 2.0 or higher)	One Lambda, Inc.	TSVPGR
Ion Chef™ Instrument	Thermo Fisher Scientific	IONCHEF
Ion S5™ System	Thermo Fisher Scientific	IONS5
Ion S5™ XL System	Thermo Fisher Scientific	IONS5XL
Ion GeneStudio™ S5 System	Thermo Fisher Scientific	IONGSS5
Ion GeneStudio™ S5 Plus System	Thermo Fisher Scientific	IONGSS5PL
Magnetic-Ring Stand (96 well)	Thermo Fisher Scientific	AM10027 or AM10050
2.0 mL Tube Magnet Stand	Thermo Fisher Scientific	12321D
Veriti 96-Well Thermal Cycler*	Thermo Fisher Scientific	4375786
Qubit® Fluorometer or equivalent	Thermo Fisher Scientific	Q33216 or Q33226
Qubit dsDNA HS Assay Kit	Thermo Fisher Scientific	Q32854
Qubit Assay Tubes	Thermo Fisher Scientific	Q32856
Plate Centrifuge capable of 1,500 x g	MLS**	---
Mini/Micro Centrifuge	MLS	---
Vortex	MLS	---

\*The Veriti 96-Well Thermal Cycler may be substituted with a thermal cycler capable of temperature settings optimized for the PCR reaction – 0.8 °C per second heating rate and 1.6°C per second cooling rate – and reaction volumes of 100 µL.

\*\*MLS: Major Laboratory Supplier

## PRECAUTIONS



**SAFETY PRECAUTION:** Wear gloves and appropriate PPE.

**CAUTION:** Follow clean bench work techniques to reduce the risk of contamination.

Clean the workbench thoroughly with a DNA-removing agent (e.g., DNA Away™, Termi-DNA-Tor, 10% bleach followed by 70% alcohol, or equivalent) to reduce the risk of sample contamination.

While working at the bench, wipe gloves frequently with a DNA-removing agent to reduce the risk of sample and reagent cross-contamination. Alternatively, change gloves frequently.

## IMPORTANT GUIDELINES

### General Laboratory Practice

- Use of manual multi-channel pipettes is recommended. Single channel pipettes are only recommended for workflows containing a small quantity of samples.
- All instruments and pipettes should be calibrated and maintained per the manufacturer's guidelines.


### Technical and Equipment

- Use of dedicated equipment in both the pre- and post-PCR environment is advised.
- For convenience, set up all the thermal cycler programs before starting.
- Use the Veriti 96-Well Thermal Cycler or a model capable of 9600 emulation speed or +0.8°C/sec heating and -1.6°C/sec cooling and a heated cover at 105°C or equivalent for all programs.
  - If a thermal cycler other than the Veriti-96 will be used, validate the thermal cycler.
- If a quantification method other than a Qubit Fluorometer will be used, validate the quantification method.

### Reagents

- The items in Box 1 of 2 of the kit should be kept on ice during use and returned to -20°C immediately after use.
- Store pre-amplification (Box 1 of the AllType FASTplex NGS 11 Loci Kit) and post-amplification reagents separately.
- Master mix preparation and dispensation should be done on ice and at a reasonably fast pace to avoid unintended results.
- Use FASTplex DNA Suspension Buffer for all gDNA and amplicon dilution and library elution.
- Do not use EDTA-containing solutions (e.g., TE buffer or Low TE buffer) as it may inhibit the reactions in this assay.

### Safe Stopping Point

- For optimal results, perform all the library preparation steps in one day.
  - But if more time is needed, look for the  **SAFE STOPPING POINT** to store an intermediate material at -20°C.
- Due to an unknown amount of left-over enzyme activities, do not to keep the materials for more than 72 hours at -20°C; longer storage time would require validation.

## SAMPLE COLLECTION and SAMPLE PREPARATION

### Sample Type

Specimens, including anticoagulated peripheral blood, cultured lymphocytes, and buccal epithelial cells, have been tested and verified for use using the AllType FASTplex NGS 11 Loci Kit.

DNA can be isolated from samples as long as 2 weeks after the initial blood draw or buccal epithelial cell collection, although it is recommended that samples be processed within 2 to 3 days of collection (4, 5). DNA may be kept the storage condition and length as validated by the individual laboratory.

### Sample Storage

Whole blood must be collected in ACD or EDTA anticoagulants and stored at 4°C. Samples containing heparin are not suitable for use.

Buccal swab must be collected using swabs that are validated for epithelial cell collection and should be stored at ambient temperature in a sealed container to avoid excessive drying.

### DNA Extraction Methods

The following extraction methods have been tested with the AllType NGS 11 Loci Amplification Kit:

- Maxwell 16 Blood DNA Purification Kit (Promega Corporation, Cat No. AS1010)
- QIAamp DNA Blood Mini Kit (QIAGEN, Cat No./ID: 51104)
- Automated MagMAX™ DNA Multi-Sample Ultra Kit (Thermo Fisher Scientific, Cat. No. A25597) using KingFisher Flex Purification System (Thermo Fisher Scientific, Cat. No. 5400630, 5400610)

Other DNA extraction methods can be used, but the methods and equipment must be validated by the end-user.

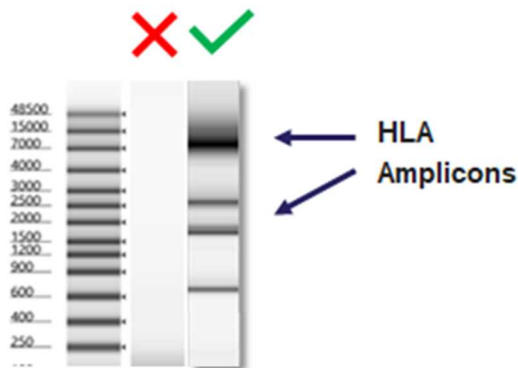
Make sure the samples are of acceptable integrity, purity and free from any inhibitors of DNA polymerase.

DNA integrity may be determined by 0.8% agarose gel electrophoresis to verify that the DNA for each sample is of high molecular weight and double stranded. A single band above the location of the 20 KB band Ladder indicates good integrity. The DNA should also have a single band of high molecular weight. If a high molecular weight band is not present and a significant amount of DNA is smeared below the 5 KB band (from DNA ladder, size marker), the sample is degraded and will not generate good results when used for creating NGS libraries.

Following DNA extraction, samples should be measured for purity using the A 260/280 ratio for which a ratio of ~1.8 is generally accepted as “pure” DNA. Abnormal 260/280 ratios usually indicate that a sample is contaminated by residual phenol, guanidine, or other reagent used in the extraction protocol, in which case the ratio is normally low. Inaccurate ratios may also be encountered at very low concentrations (< 10 ng/μl) of nucleic acids (6). High 260/280 purity ratios are not indicative of an issue. An A260/A230 ratio higher than 2.2 may indicate the presence of unwanted organic compounds such as Trizol, phenol, Guanidine HCL and guanidine thiocyanate that may result in PCR inhibition.

It is not required but recommended as a best practice to investigate the quality of the amplification products by gel electrophoresis. Such methods are useful in determining the presence of PCR products based on molecular weight and may provide valuable information regarding the integrity of a DNA amplicon, including the potential for a fragmented template. The use of an automated gel electrophoresis is preferred over traditional methods to assess for amplicon quality (e.g. Agilent BioAnalyzer or

TapeStation). Below is an example of a poor amplification product (left) and high-quality amplification product (right). The product on the left would not provide sequencing results.



**Figure 1.** Gel images of a poor amplification product, which is the gel image on the left under the **X**, and a high-quality amplification product, which is the gel image on the right under the **✓**.

Do not resuspend the samples in solutions containing chelating agents, such as EDTA, above 0.1 mM in concentration.

Resuspend the DNA samples to be used for PCR in in Low (below 0.1mM) EDTA, DNA Suspension buffer, sterile water or in 10 mM Tris-HCl, pH 8.0 to 9.0 at an optimal concentration of 25 ng/μl with a fluorometric method such as Qubit™ Fluorometer and Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific). Other specifications can be used but must be validated by the laboratory.

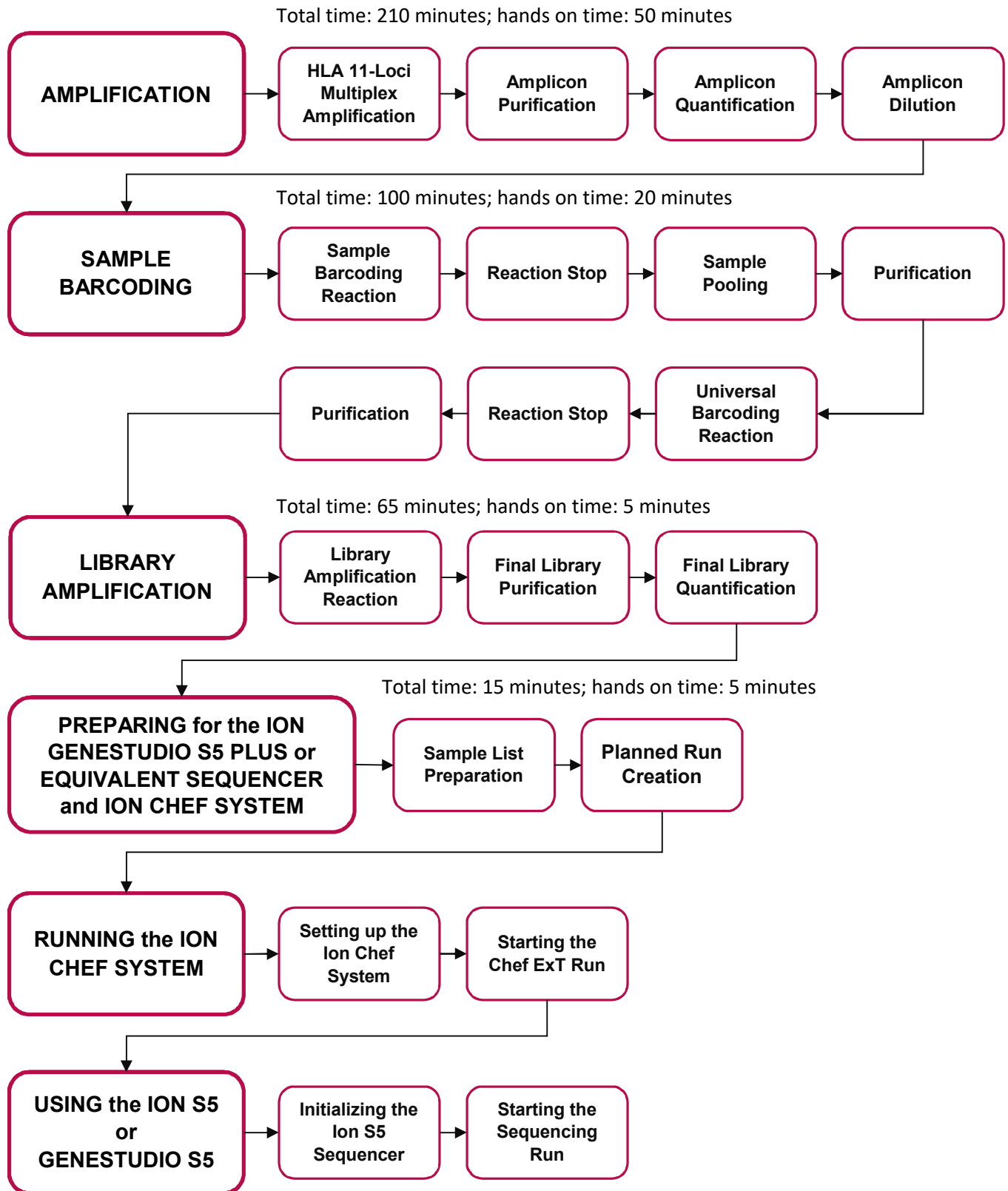
**IMPORTANT:** Only in the dilution of the genomic DNA can the above reagents be used. In all subsequent steps, when DNA Suspension Buffer is listed, only the FASTplex DNA Suspension Buffer can be used.

## DNA Storage

- Use DNA samples immediately after isolation or store DNA at -20°C or below.
- Ship DNA samples at 4°C or below to preserve their integrity during transport.

Extracted DNA has been tested that has been stored up to 14 days using the AllType FASTplex NGS 11 Loci Kit. Additional DNA storage may be validated by the individual laboratory.

## WORKFLOW for the ASSAYS



## GENERAL PREPARATION for the ASSAYS

1. **Separate** pre- and post-PCR areas.
2. **Gather** the following items and reagents so that they are accessible throughout the workflow:
  - A full range of filtered pipette tips
  - Single and multi-channel pipettes
  - 96-Well 0.2 mL PCR Plates
  - Eppendorf LoBind® tubes
  - Magnetic racks for a 96-well plate and a 2.0 mL tube
  - Nuclease-free water
  - Ice
  - PCR plate cooler
  - Tube-labeling supplies
  - FASTplex DNA Suspension Buffer
  - 200-proof Ethanol
  - Sterile reagent reservoirs

3. **Resuspend** DNA samples in FASTplex DNA Suspension Buffer, Low (below 0.1mM) EDTA DNA Suspension buffer, sterile water, or in 10 mM Tris-HCl, pH 8.0 to 9.0 at an optimal concentration of 25 ng/μL using a Qubit dsDNA HS Assay Kit and Qubit Fluorometer for measurement.

An acceptable range of genomic DNA concentrations is 3.00 ng/μL to 50 ng/μL.

Optical density (OD) methods should not be used to measure DNA concentration; the readings could be overestimated. If optical density (OD) method is used to measure DNA quality, the expected A260/A280 ratio is 1.80.

4. **Record** each sample location, the volume of the FASTplex Sample Plate 48 (Refer to [Appendix 2](#) for a diagram of the plate.), and the Univ Barcode P1 (UB) reagent to be used for library prep.
5. **Before starting the procedure, make** a map with the PCR 8-tube strip(s) or 96-well plate clearly labeled.
6. **Keep track** of which wells from the FASTplex Sample Plate 48 and how much UB reagent has been consumed in the library preparation. Users may refer to the plate grid at the end of this document.
7. **Do not discard** reagents in the kit until empty since they are intended for multiple uses.
8. **Avoid** contamination of all kit reagents/components.
9. **Return** the unused portions to storage at the temperature indicated on the label.
10. **Take** precautions to avoid well-to-well cross-contaminations.

The FASTplex Sample Plate 48 supplied in the kit contains enough sample-barcoding (SB) reagent to prepare a 48-sample library twice or up to twelve 8-sample libraries, for a total of 96 samples. The FASTplex kit is also compatible with any library size between 8 and 48 samples. The plate is designed to allow for columns of barcodes (wells) to be separated; the remaining contents of the FASTplex Sample Plate should be stored at -20°C until the next use. Care must be taken to avoid well-to-well cross-contaminations.



The FASTplex Sample Plate contains 48 unique barcodes which is dispensed in duplicate in a 96 well plate. See the plate layout in [step 21](#) of this section.

11. **Program** the thermal cyclers(s).
12. **Set up** all the thermal cyclers programs before starting.
13. **Use** the following programs:

- **HLA 11-loci Amplification Ion Program:**

Temperature	Time	Cycle
94°C	2 min	1
98°C	10 sec	22
69°C	3 min	
98°C	10 sec	8
60°C	3 min	
4°C	HOLD	1

**9600 emulation speed and heated cover on**

- **TAG Program:** 55°C for 15 min., 25°C hold, heated cover on
- **STOP Program:** 68°C for 10 min., 25°C hold, heated cover on
- **FASTplex Ion Library Amplification Program:**

Temperature	Time	Cycle
72°C	10 min	1
98°C	3 min	1
98°C	15 sec	9
66°C	30 sec	
72°C	1 min	
72°C	5 min	1
4°C	HOLD	1

**9600 emulation speed and heated cover on**

**IMPORTANT:** At the completion of each PCR step, select the next thermal cyclers program to be used, so the thermal lid is prewarmed. The thermal cyclers lid should be at the appropriate target temperature before placing the reaction. Avoid placing reactions onto a cold thermal cyclers.

14. **Pulse-spin** the kit components in a suitable centrifuge to gather the reagents at the bottom of the well or tube before every use of the FASTplex Sample Plate and before dispensing from the reagent tubes, since liquids can condense or shift locations inside containers during shipment or storage.
15. **If the Room Temperature kit components freeze during shipment or storage, perform** the following steps before use:
  - a) Thaw the kit components.
  - b) Mix the kit components.
  - c) Pulse-spin the kit components.

**16. Equilibrate** FASTplex Paramagnetic Beads to room temperature. FASTplex Paramagnetic Beads can be stored for up to 2 weeks at room temperature or for longer periods at 2 - 8°C.

- **If the FASTplex Paramagnetic Beads are stored cold, perform** the following steps before use:
  - a) Set the beads at room temperature for 30 minutes.
  - b) Vortex the beads thoroughly to resuspend.

**IMPORTANT:** To transfer volumes accurately, do not pre-wet pipette tips and pipette slowly.

**17. Check** the Stop Solution for precipitate before use.

- **If a precipitate is visible, perform** the following steps:
  - a) Incubate the tube at 37°C for 5 minutes.
  - b) Mix gently by inversion until the precipitate dissolves.

**IMPORTANT:** Do not vortex. Stop Solution contains SDS and will precipitate if stored below room temperature. Overly vigorous mixing will cause foaming.

**18. Store** the FASTplex Barcoding Buffer at room temperature when in use because it is viscous.

**IMPORTANT:** To transfer Barcoding Buffer accurately, pipette slowly and do not pre-wet pipette tips. While adding Barcoding Buffer to reactions, mix in the Barcoding Buffer completely by pipetting up and down several times with the same pipette tip(s) that was used for addition.

**19. Prepare** 80% ethanol daily.

**20. Plan** sample barcoding before starting the assay.

**21. When planning for sample barcoding, keep** the following in mind:

- The kit contains a total of 48 unique IonXpress Barcodes as Sample Barcodes (SB) and a unique FASTplex Univ Barcode P1 (UB), to prepare up to two 48-sample libraries.
- A library can contain between 8 to 48 samples and is barcoded with one unique UB.
- When pooling multiple samples, be sure to select non-overlapping SB. Do not mix wells 1-48 with its duplicated barcode in wells 49-96. Each well contains enough barcoding reagents for one reaction.
- The AllType FASTplex NGS 11 Loci Kit (Cat. No. ALL-FAST11LX) has been tested using Ion GeneStudio S5 Plus System or equivalent and Ion Chef Instrument using the Ion 520 Chip (8 to 24 samples) and Ion 530 Chip (8 to 48 samples) with the Ion 520 & Ion 530 ExT Kit-Chef sequencing reagents.

**IMPORTANT:** Alternative configurations, kits, and sequencing systems are not supported by this application and must be determined and validated by the user.

- Figure 2 provides the layout for a 48-sample library. Wells A7 through H12 are a duplicate of the wells shown here. IonXpress barcode IDs and its positions are shown. The TypeStream Visual (TSV) Calculation Tool has a tab for the sample sheet that reflects this barcode order.

	1	2	3	4	5	6
A	lonXpress_001	lonXpress_009	lonXpress_017	lonXpress_050	lonXpress_033	lonXpress_041
B	lonXpress_002	lonXpress_010	lonXpress_018	lonXpress_026	lonXpress_034	lonXpress_042
C	lonXpress_003	lonXpress_011	lonXpress_019	lonXpress_027	lonXpress_035	lonXpress_043
D	lonXpress_004	lonXpress_012	lonXpress_020	lonXpress_028	lonXpress_036	lonXpress_049
E	lonXpress_005	lonXpress_013	lonXpress_021	lonXpress_029	lonXpress_037	lonXpress_045
F	lonXpress_006	lonXpress_014	lonXpress_022	lonXpress_030	lonXpress_038	lonXpress_046
G	lonXpress_007	lonXpress_015	lonXpress_023	lonXpress_031	lonXpress_039	lonXpress_047
H	lonXpress_008	lonXpress_016	lonXpress_024	lonXpress_032	lonXpress_040	lonXpress_048

**Figure 2.** Barcode sample plate layout for a 48-sample library. This layout is duplicated across columns 7-12. Do not mix duplicated barcodes.

- The TypeStream Visual (TSV) NGS Calculator has a tab for the sample sheet that reflects this barcode order.

**IMPORTANT:** Alternative configurations, kits, and sequencing systems are not supported by this application and must be determined and validated by the user.

**22. If working with various sample sizes, keep** the following in mind for dealing with reagent volumes:

- The AllType FASTplex NGS 11 Loci Kit allows for preparation of a library using the minimum of 8 and up to 48 samples. When processing different sample sizes, different volumes of the pooled SB reaction and of the FASTplex Paramagnetic Beads will be used after Sample Barcoding step.
- The TSV NGS Calculator displays the required reagent volumes. In the “SB UB Lib Amp” tab, the total number of samples will be automatically updated based off of the number of samples added in the “Pool Configuration” tab. In the “SB UB Lib Amp” tab, the required reagent volumes are displayed.
- The kit contains enough barcoding reagents, Barcoding Buffer, and Stop Solution to process 12 runs of 8 samples per run.
- To ensure sample IDs are properly populated throughout, please refer to the TypeStream Visual NGS Analysis Software User Manual.

## AMPLIFICATION

### Materials and Equipment

- Diluted genomic DNA (25ng/μL)
- Eppendorf DNA LoBind Tubes, 2.0 mL
- 96-Well, 0.2 mL, PCR Plates
- AllType FASTplex 11 Loci Primer Mix (Cat. ID ALF-P11)
- AllType™ FASTplex™ 11 Loci Exon 1 Primer Mix (Cat. ID ALF-P11E1)
- AllType Buffer (Cat. ID ALL-BUF)
- AllType dNTPs (Cat. ID ALL-NTP)
- AllType LR Polymerase (Cat. ID ALL-LRPOL)
- Nuclease Free Water
- FASTplex Paramagnetic Beads (Cat. ID FAST-BEAD)
- FASTplex DNA Suspension Buffer (Cat. ID FAST-SUSP)
- 200 Proof Ethanol, Molecular Biology Grade
- Nunc 96-Well Polypropylene MicroWell Plates
- Magnetic Plate Stand
- 50 mL Polystyrene Reagent Reservoirs
- Qubit dsDNA High Sensitivity Assay Kit
- Qubit Fluorometer
- Full array of manual single channel pipettes (20 μL, 200 μL, 1 mL)
- Full array of filtered, pre-sterilized, pipette tips
- Serological Pipettes, 10 or 25 mL
- PCR Plate Centrifuge
- Mini Centrifuge
- Vortex
- Ice or Cold Block
- Thermal cycler PCR Pressure Pad
- [Veriti 96-Well Thermal Cycler or equivalent](#)
- TSV NGS Calculator

### Part 1: Sample Amplification

1. **Turn on** a thermal cycler.
2. **Program** the thermal cycler to run the following HLA 11-loci Amplification Ion Program:

### HLA 11-loci Amplification Ion Program

Temperature	Time	Cycle
94°C	2 min	1
98°C	10 sec	22
69°C	3 min	
98°C	10 sec	8
60°C	3 min	
4°C	HOLD	1

- Use 9600 emulation speed or +0.8°C/sec heating and -1.6°C/sec cooling with the heated cover set to 105°C.

**IMPORTANT:** Check the thermal cycler lid temperature before adding the reaction. Make sure the thermal cycler lid is at the appropriate target temperature. Do not place the reaction onto a cold thermal cycler.

- Use the “gDNA Dilution” tab in the TSV NGS Calculator to calculate the original sample volumes and DNA Suspension Buffer volumes to prepare 25 ng/μL dilutions.



**Note:** To ensure sample IDs are properly populated throughout, please refer to the TypeStream Visual NGS Analysis Software User Manual.

- Thaw AllType FASTplex 11 Loci Primer Mix, AllType FASTplex 11 Loci Exon 1 Primer Mix, AllType dNTP, and AllType Buffer at room temperature.
- Keep the AllType LR Polymerase tube on ice.
- Briefly vortex and spin-down all reagents EXCEPT polymerase.
- Keep all reagents on ice until ready for use.
- In a 96-Well 0.2 mL PCR Plate, aliquot 4.0 μL of genomic DNA with the concentration adjusted to 25 ng/μL.
- Record the sample IDs and well positions in the PCR plate.
- Go to the “Amp-Clean Up” tab of the TSV NGS Calculator, which will automatically calculate the volumes of each amplification reagent based on the number of samples input into the “gDNA Dilution” tab.
- Check the “Exon 1” box if using the Exon 1 primer mix.
- In a 2.0 mL LoBind Tube, prepare an Amplification Master Mix based on the reagent volumes shown in the “Amp Clean Up” tab of the TSV NGS Calculator.
- Prepare reagents in the order shown in the “Amplification” table of the “Amp-Clean Up” tab in the TSV NGS Calculator.
 

**IMPORTANT:** At this stage, only prepare the Amplification Mix inclusive of the first five components. Do not add the Polymerase at this stage.
- Vortex and pulse-spin the mix for 10 seconds.
- Keep the mix on ice.

17. **Quick spin** the Polymerase.
18. **Add** the Polymerase to the Master Mix according to the “Amplification” table in the TSV NGS Calculator.
19. **Mix well** by pipetting 15-20 times with the pipet volume set to half the total volume of the Master Mix.
20. **Aliquot** 16 µL of the Master Mix to each well with DNA.
21. **Seal** the plate with a tray seal.
22. **Pulse-spin** the plate.
23. **Load** the plate into a pre-warmed thermal cycler.
24. **Cover** the plate with a pressure pad.
25. **Run** the HLA 11-loci Amplification program.

**IMPORTANT:** Check the thermal cycler lid temperature before adding the reaction. Make sure the thermal cycler lid is at the appropriate target temperature. Do not place the reaction onto a cold thermal cycler.



**SAFE-STOPPING POINT:** Proceed immediately to the next step or store the amplicon at -20°C.

## Part 2: Amplicon Purification

1. **Equilibrate** the FASTplex Paramagnetic Beads to room temperature for 30 minutes.
2. **Prepare** fresh 80% ethanol solution by measuring ethanol and nuclease-free water separately and mixing. Refer to the TSV NGS Calculator and see the “Amp Clean Up” tab in the Amplification Purification section.
3. **Vortex** the FASTplex Paramagnetic Beads at medium speed for 30 seconds to fully resuspend the beads.
4. **In a Nunc LoBind 96-well plate, aliquot** 12 µL of the beads to each well. Use a multi-channel pipette and reagent reservoir for convenience.
5. **Transfer** all amplified product (~20 µL) to the corresponding wells.
6. **Mix** by pipetting up and down 10 times.

**IMPORTANT:** Change tips for each sample. Avoid producing bubbles.
7. **Incubate** at room temperature for 5 minutes.
8. **Place** the plate on the magnetic ring stand. Adjust the plate as needed so the beads form a pellet on one side of each well. Let stand for ~3 minutes or until clear.
9. **Using a pipette set to 28 µL, carefully remove and discard** the supernatant from each well without disturbing the bead pellet.
10. **With the plate on the magnet, add** 100 µL of 80% ethanol to each well.
11. **Incubate** at room temperature for 30 seconds or longer until the solution clears.
12. **With a pipette set to 110 µL, remove and discard** the supernatant.

**IMPORTANT:** Be careful not to disturb the bead pellet.

13. **Repeat** steps 10 through 12 above for a second ethanol wash.
14. **With the plate on the magnet, remove** visible ethanol residue with a P-20 pipette.
15. **Air-dry** the beads at room temperature for ~ 2 minutes on the magnet.

**IMPORTANT:** Avoid over-drying.

16. **With the plate on the magnet, add** 27  $\mu$ L of FASTplex DNA Suspension Buffer to each well.
17. **Remove** the plate from the magnet.
18. **Mix** each sample by pipetting up and down 10 times avoiding making any bubbles.
19. **Incubate** at room temperature for 3 minutes.
20. **Place** the plate back on magnet for ~ 3 minutes or until clear.
21. **With a pipette volume set to 25 $\mu$ L, transfer** the supernatant to a new 0.2 mL 96-well PCR plate without disturbing the beads.



**SAFE-STOPPING POINT:** Proceed immediately to the next step or store the purified products at -20°C.

### Part 3: Amplicon Quantification

1. **Label** Qubit Assay Tubes for each sample and two additional tubes for Qubit Standard 1 and 2.
2. **Prepare** a Working Qubit Solution using the volumes per sample shown below plus 15% overage by mixing the equivalent of:
  - a) 199  $\mu$ L of Qubit dsDNA HS Buffer (Component B) per sample.
  - b) 1  $\mu$ L of Qubit dsDNA HS Reagent (Component A) per sample. Vortex prior to use.
3. **Vortex** the Working Qubit Solution.

**IMPORTANT:** Protect the Working Qubit Solution from light. Use it within 2 hours.
4. **Add** 198  $\mu$ L of Working Solution to each labeled Qubit Assay Tube.
5. **Add** 190  $\mu$ L of Working Solution to the two tubes for the Standards.
6. **Add** 2  $\mu$ L of the corresponding purified amplicon to each labeled tube.
7. **Add** 10  $\mu$ L of the corresponding Standard to appropriate, labeled tube.
8. **Briefly vortex** and pulse-spin all tubes.
9. **Protect** all tubes from light.
10. **Incubate** at room temperature for 2 minutes.
11. **Turn on** the Qubit Fluorometer.
12. **Select** DNA from the home screen.
13. **Select** dsDNA High Sensitivity.
14. **Press** the appropriate button to begin reading the standards.
15. **Measure** Standards 1 and 2 to complete the calibration.
16. **Begin reading** the amplicon tubes.
17. **When prompted, set** the volume of sample used to 2  $\mu$ L and the units to ng/ $\mu$ L.
18. **Record** the concentration of sample.
19. **Repeat** steps 16 through 18 for all samples.
20. **If any sample exceeds the upper detection, perform** the following steps:
  - a) Dilute the samples.
  - b) Remeasure the diluted samples.
  - c) Multiply the reading by the dilution factor. See the Qubit protocol for additional clarification.



## Part 4: Amplicon Dilution

Lower concentration samples may not contain an adequate quantity of the amplicons. The steps in this section will help calculate a volume of FASTplex DNA Suspension Buffer to dilute amplicons.

1. Use the “Amplicon Dilution” and “Plate Layout” tabs of the TSV NGS Calculator for amplicon dilution calculation.

### Functions of the “Amplicon Dilution” and “Plate Layout” Tabs

Calculation Tool Tab ID	Functions
Amplicon Dilution	Calculation of 48 (max) amplicon dilution factors and diluent volumes to prepare 3-30ng input DNA for Sample Barcoding reaction
Plate Layout	View the result of input DNA and FASTplex DNA Suspension Buffer volumes in an 8 x 6 plate format

2. Open the TSV NGS Calculator.

Sample IDs will be automatically populated from the sample IDs input into the “gDNA Dilutions” tab.

3. In the “Amplicon Dilution” tab, enter the amplicon concentrations in the column labeled “Amplicon Conc. (ng/μL).”

- The table will display a volume of FASTplex DNA Suspension Buffer to add to 2 μL of each amplicon [“Sample (μL)” column].
- Samples whose amplicon concentrations, after dilution, fall outside of the desired 3-30ng input into Library Preparation will be highlighted in orange (“High”) or red (“Low”).
- The “Range” column will show either “High” or “Low” for the respective sample, and volume of sample and buffer to use.
- The stock sample volume cell in the calculator is adjustable if more volume is needed.

4. In a clean 96-well plate, add the indicated volume of FASTplex DNA Suspension Buffer and 2 μL of the stock amplicon.

- For any outlier samples, add volumes of the FASTplex DNA Suspension Buffer and stock amplicon that are indicated in the Sample (μL) and FASTplex DNA Suspension Buffer (μL) columns for each sample. The optimal input amount is 10 ng in an 8 μL volume at 1.25 ng/μL.
- Use the “Plate Layout” tab to locate positions of outlier samples in the plate

**IMPORTANT:** For any samples that fall outside the normal range of concentrations, it is important to add the indicated volume.

5. Carefully mix the diluted amplicon well by pipetting.

**IMPORTANT:** Avoid cross-contamination.

6. Seal the plate

7. Pulse-spin the plate.

8. Store on ice for immediate use or store at -20°C.



**SAFE-STOPPING POINT:** Proceed immediately to the next step or store the purified products at -20°C.

## SAMPLE BARCODING

### Materials and Equipment

- Diluted amplicons
- Eppendorf DNA LoBind Tubes, 2.0 mL
- PCR tube 0.2 mL
- 96-Well, 0.2 mL, PCR Plates
- 2.0 mL Tube Magnet Stand
- Serological Pipettes, 10 or 25 mL
- FASTplex Sample Plate 48 (Cat. ID FAST-SP48B)
- FASTplex Univ Barcode P1 (Cat. ID FAST-UBP1)
- FASTplex Barcoding Buffer (Cat. ID FAST-BB)
- FASTplex Stop Solution (Cat. ID FAST-STOP)
- FASTplex Paramagnetic Beads (Equilibrated to room temperature) (Cat. ID FAST-BEAD)
- FASTplex DNA Suspension Buffer (Cat. ID FAST-SUSP)
- 200 Proof Ethanol, Molecular Biology Grade
- Full array of manual single channel pipettes (20 µL, 200 µL, 1 mL)
- Full array of filtered, pre-sterilized, pipette tips
- 8 tube strips
- Thermal cycler PCR Pressure Pad
- [Veriti 96-Well Thermal Cycler or equivalent](#)
- TSV NGS Calculator

### Part 1: Sample Barcoding (SB) Reaction, Reaction Stop, Sample Pooling, and Purification

**IMPORTANT:** Before starting this step, make sure the thermal cycler lid is at the appropriate target temperature. Do not place the reaction onto a cold thermal cycler.

**IMPORTANT:** Before starting this step, ensure the “SB UB Lib Amp” tab of the TSV NGS Calculator has been populated.



**Useful Tip:** In the following Sample Barcoding section below through step 20, the individual sample must be treated separately. An 8-channel pipette and use of an 8-tube strip can make this step faster and less error-prone. Common reagents, Barcoding Buffer and Stop Solution, can be pre-aliquoted into an 8-tube strip or equivalent for use with the 8-channel pipette. The volume of reagents to aliquot using an 8-tube strip are summarized below.

For two runs of a 48-sample assay, use two 8-tube strips. Add the volume of reagents to each well of each strip.

#### Barcoding and Stop Buffer Volume (µL) per Well in 8-Tube Strip (W/Overage)

Reagent	8 Samples	16 Samples	24 Samples	48 Samples
Barcoding Buffer	14.0	27.0	41.0	83.0
Stop Solution	20.0	41.0	61.0	124.0

1. **Pulse-spin** the FASTplex Sample Plate 48 in a centrifuge.
2. **After centrifugation, carefully remove** the plate seal from the Sample Plate.
3. **Discard** the plate seal  
**IMPORTANT:** Do not reuse the plate seal.
4. **Place** the FASTplex Sample Plate 48 on ice.
5. **Equilibrate** the FASTplex Paramagnetic Beads at room temperature.
6. **Set up** the SB reaction at ambient temperature.
7. **Transfer** 16 µL of the SB reagent from the FASTplex Sample Plate 48 to a pre-labeled PCR plate or an 8-tube PCR strip(s) using an accurate pipette.  
**IMPORTANT:** Use clean tips for each transfer.
8. **Visually confirm** that the volume of SB reagent appears equal.
9. **After dispensing the sample barcode reagent, break-away** used rows of the plate.
10. **If all barcodes are not used, return** the plate to the freezer (-20°C storage).
11. **Transfer** 8 µL of diluted input DNA (1.25 ng/µL) to each well (one sample per well) or tube using an accurate pipette.
12. **Mix** the DNA thoroughly with the sample barcode reagent in each tube by pipetting up and down ten times at 8µL.  
**IMPORTANT:** Be careful not to introduce excessive bubbles.
13. **Use** clean tips for each sample.
14. **Carefully pipette** 12 µL of Barcoding Buffer to each well or tube using new pipette tips for each transfer
15. **Mix thoroughly but slowly by pipetting up and down** ten times at 12 µL.  
**IMPORTANT:** Be careful not to introduce excessive foam. Barcoding Buffer is very viscous and is subject to foaming.
16. **Carefully and completely seal or cap** the SB reactions to prevent evaporation.
17. **Pulse-spin** the plate.
18. **Transfer** the plate to a thermal cycler.
19. **Place** a PCR pressure pad over the plate.
20. **Run** the following program (TAG) with heated cover on:  
**TAG program:** 55°C for 15 min., 25°C hold, Reaction volume: **36 µL**  
**IMPORTANT:** Check the thermal cycler lid temperature before adding the reaction. Make sure the thermal cycler lid is at the appropriate target temperature. Do not place the reaction onto a cold thermal cycler.
21. **Take** the plate out of the thermal cycler.

22. **Pulse-spin** the plate.
23. **Add** 18 µL of FASTplex Stop Solution to each SB reaction.
24. **Slowly pipette** up and down 5 times to mix.
 

**IMPORTANT:** Be careful not to introduce excessive bubbles.
25. **Use** clean tips for each transfer.
26. **After mixing with the Stop Solution, securely re-seal** the PCR plate.
27. **Pulse-spin** the PCR plate.
28. **Transfer** the PCR plate to a thermal cycler
29. **Run** the following STOP program with the heated cover on:
 

**STOP program:** 68°C for 10 min., 25°C hold, Reaction volume: **54 µL**


**IMPORTANT:** Check the thermal cycler lid temperature before adding the reaction. Make sure the thermal cycler lid is at the appropriate target temperature. Do not place the reaction onto a cold thermal cycler.
30. **Vortex** (or vigorously pipette) room temperature FASTplex Paramagnetic Beads to ensure that the beads are fully resuspended.
31. **After thermal cycling, pulse-spin** the PCR plate containing the stopped SB reactions.
32. **Using an appropriately sized pipette, transfer** 25 µL of each SB reaction into a single 2 mL LoBind tube, label this tube as “Tube A” and gently mix by pipetting up and down slowly.
 

The final volume of the combined SB reaction pool is determined by the batch size.
33. **Once pooling is complete, refer** to the “Sample and Universal Barcoding” section of the “SB UB Lib Amp” tab of the TSV NGS Calculator for the volume of FASTplex Paramagnetic Beads to be added to the pooled SB reaction.
34. **In a new 2.0mL LoBind tube, transfer** the volume specified in the calculator cell “Volume of stopped, pooled SB reaction” from “Tube A” over to “Tube B”. Not all of Tube A volume will be used.
35. **Label** this tube as “Tube B.”
36. **Proceed** to the next step with “Tube B” and its contents.

Volumes of the pool and paramagnetic bead for common sample sizes are listed in the following SB Reaction Pool and FASTplex Paramagnetic Bead Volumes table. The volumes for different sample sizes can be displayed using the TSV NGS Calculator.

**SB Reaction Pool and Paramagnetic Bead Volumes**

Batch Size (Samples per Pool)	8 Samples	16 Samples	24 Samples	48 Samples
Volume of stopped SB reaction pool	144 µL	288 µL	432 µL	864 µL
Add FASTplex Paramagnetic Beads (1 vol. equivalent)	144 µL	288 µL	432 µL	864 µL

37. **Go to** the “SB UB Lib Amp” tab to find the volumes of the pool, paramagnetic bead, and the reagents for the rest of workflow.
  38. **Add** the appropriate volume of FASTplex Paramagnetic Beads to the stopped SB reaction pool in “Tube B.”
  39. **Mix** thoroughly by pipetting up and down 10x.
  40. **Incubate** in a tube rack (non-magnetic) on the bench for 5 minutes to allow the DNA to bind.
  41. **Transfer** the tube to a magnetic stand for ~ 5 minutes or until clear. A bead pellet will form.
  42. **Slowly remove** the supernatant with a pipette and discard.  
**IMPORTANT:** Be careful not to disturb the bead pellet.
  43. **With the tube in the magnetic stand, add** 2.0 mL of 80% ethanol to completely immerse the bead pellet without disturbing the beads.  
**IMPORTANT:** Be careful not to disturb the beads.
  44. **After 30 seconds, slowly remove and discard** the supernatant.  
**IMPORTANT:** Be careful not to disturb the bead pellet.
  45. **Repeat** steps 43 and 44 for a total of two washes with 80% ethanol. Use a small pipette (e.g., P20) to remove the residual ethanol after the second wash.
  46. **Air-dry** beads by leaving the tube uncapped on the magnetic stand for 2 minutes.
  47. **Check** to ensure that there are no visible ethanol droplets in the tubes at end of 2 minutes.
    - **If ethanol droplets are still visible, air-dry** the beads longer.
-  **CAUTION:** Do not dry the pellet longer than 3 minutes in total or the DNA recovery will be compromised.
48. **Remove** the tube from the magnetic stand.
  49. **Pipette** 50 µL of FASTplex DNA Suspension Buffer on top of the bead pellet.
  50. **Pipette** the buffer-bead solution along the inner wall of the tube multiple times to thoroughly mix and resuspend the bead pellet.
  51. **Incubate** the tube in a tube rack (non-magnetic) on the bench for at least 5 minutes to elute the purified SB reaction pool from the beads.
  52. **Return** the tube to a magnetic stand and allow a bead pellet to reform on the inner wall of the tube ~2 minutes or until clear.
  53. **When the supernatant has cleared completely, carefully transfer** the full library eluate to a PCR tube. The transferred eluate contains the purified SB reaction pool.



**SAFE-STOPPING POINT:** Proceed immediately to the next step or store the purified products at -20°C.

## Part 2: Universal Barcoding (UB) Reaction, Reaction Stop, and Purification

**IMPORTANT:** Before starting this step, make sure the thermal cycler lid is at the appropriate target temperature. Do not place the reaction onto a cold thermal cycler.

1. **Set up** a UB reaction in a 0.2 ml PCR tube using the purified SB reaction pool from the last step.
2. **Add** the amounts, depending on the pool size, of UB P1 and Barcoding Buffer, by using the TSV NGS Calculator or according to the Universal Barcoding (UB) Reaction Set-up table.

### Universal Barcoding (UB) Reaction Set-up

Batch Size (Samples per Pool)	8 Samples	16 Samples	24 Samples	48 Samples
Purified SB reaction	48.0 µL	48.0 µL	48.0 µL	48.0 µL
UB P1	1.7 µL	3.3 µL	5.0 µL	10.0 µL
Barcoding Buffer	25.0 µL	26.0 µL	26.0 µL	29.0 µL
<b>Total Reaction Volume</b>	74.7 µL	77.3 µL	79.0 µL	87.0 µL

3. **If the pool size is outside of 8, 16, 24, and 48, use** the “UB reaction set-up” of the “SB UB Lib Amp” tab of the TSV NGS Calculator Tool
4. **Using a pipette set at 50 µL, mix** the UB reaction thoroughly by pipetting up and down 10 times.
5. **Cap** the PCR tube containing the UB reaction.
6. **Pulse-spin** the PCR tube.
7. **Transfer** the tube to a thermal cycler.
8. **Use** a PCR pressure pad.
9. **Run** the following program (TAG) with appropriate reaction volume and lid-heating on:

**TAG program:** 55°C for 15 min., 25°C hold, **Reaction Volume:** Set the reaction volume to the appropriate sample size listed in the Universal Barcoding (UB) Reaction Setup table above or from the TSV NGS Calculator.

**IMPORTANT:** Check the thermal cycler lid temperature before adding the reaction. Make sure the thermal cycler lid is at the appropriate target temperature. Do not place the reaction onto a cold thermal cycler.

10. **After the TAG program, remove** the tube from thermal cycler.
11. **Add** Stop Solution according to the Stop Solution Volumes table or from the TSV NGS Calculator.

### Stop Solution Volumes

Batch Size (Samples per Pool)	8 Samples	16 Samples	24 Samples	48 Samples
Stop Solution	37.0 µL	38.0 µL	40.0 µL	44.0 µL
<b>Total Reaction Volume</b>	111.7 µL	115.3 µL	119.0 µL	131.0 µL

12. **With the pipette set at 50 µL, mix** thoroughly by pipetting up and down 10 times.

**IMPORTANT:** Be careful not to produce excessive foaming.

13. **Re-cap** the PCR tube containing the UB reaction and pulse-spin the PCR tube.
14. **Transfer** the tube to a thermal cycler.
15. **Run** the following program (STOP) with the appropriate reaction volume and lid-heating on:  
**STOP program:** 68°C for 10 min., 25°C hold. Use 100 µL volume in the PCR program.  
**IMPORTANT:** Check the thermal cycler lid temperature before adding the reaction. Make sure the thermal cycler lid is at the appropriate target temperature. Do not place the reaction onto a cold thermal cycler.
16. **Transfer** the entire volume of the stopped UB reaction to a single 2.0 ml LoBind tube.
17. **Vortex** room temperature FASTplex Paramagnetic Beads to ensure they are completely resuspended.
18. **Measure** the volume of the UB reaction using a pipette.
19. **Add** 1 volume equivalent of FASTplex Paramagnetic Beads according to the volumes in the table below or from the TSV NGS Calculator to the stopped UB reaction in the 2.0 mL LoBind tube from the previous step.

**IMPORTANT:** Evaporation during reaction may reduce its volume.

- **If evaporation occurs, adjust** the Paramagnetic bead volume from the table below accordingly.

**FASTplex Paramagnetic Beads Purification of Stopped UB Reaction**

Pool Size (Samples per Pool)	8 Samples	16 Samples	24 Samples	48 Samples
Volume of UB reaction	112.0 µL	115.0 µL	119.0 µL	131.0 µL
Volume of FASTplex Paramagnetic Beads	112.0 µL	115.0 µL	119.0 µL	131.0 µL

20. **For a sample size other than those shown below, refer** to the “SB UB Lib Amp” tab of the TSV NGS Calculator.
21. **If evaporation during reaction reduces the volume, perform** the following steps:
  - a) Measure the volume of UB reaction.
  - b) Add an equal volume of FASTplex Paramagnetic Beads.
22. **Mix** thoroughly by pipetting.
23. **Incubate** at room temperature in a tube rack (non-magnetic) on the bench for 5 minutes to allow the DNA to bind.
24. **Transfer** the 2.0 ml LoBind tube to a magnetic stand.
25. **Let** the beads separate completely for 3 minutes. A bead pellet will form along one side of the tube and the supernatant should appear completely clear after ~ 3 minutes.
26. **Slowly remove** the supernatant with a pipette and discard.  
**IMPORTANT:** Be careful not to disturb the bead pellet.
27. **With the tube in the magnetic stand, add** 500 µL of 80% ethanol, ensuring the bead pellet is submerged.

**IMPORTANT:** Be careful not to disturb the bead pellet.

**28. Slowly remove and discard** the supernatant after 30 seconds or until clear.

**IMPORTANT:** Be careful not to disturb the bead pellet.

**29. Repeat** steps 27 and 28 for a total of 2 washes with 80% ethanol.

**30. Use** a large pipette to remove most of the ethanol waste.

**31. Use** a smaller pipette (e.g., P20) to remove the residual ethanol that collects at the bottom of the tube.

**32. Air-dry** beads by leaving the tube uncapped on the magnetic stand for 2 minutes.

**33. Check** to ensure that there are no visible ethanol droplets in the tube at end of 2 minutes.

- **If ethanol droplets are still visible, air-dry** the beads longer.



**CAUTION:** Do not dry the pellet longer than 3 minutes in total or the DNA recovery will be compromised.

**34. Remove** the tube from the magnetic stand.

**35. Add** 13 µL of FASTplex DNA Suspension Buffer.

- **Pipette** the liquid along the inside of the tube several times to thoroughly resuspend the bead pellet.

**36. Incubate** in a tube rack (non-magnetic) on the bench for at least 5 minutes to elute the purified DNA from the beads.

**37. Return** the tube to the magnetic stand.

**38. Allow** the bead pellet to form on the inner wall of the tube for ~2 minutes or until clear.

**39. When the supernatant has cleared completely, carefully transfer** 10 µL of DNA eluate to a clean 0.2 mL PCR tube. The transferred eluate contains the DNA purified from the UB reaction and is now ready for library amplification.



**SAFE-STOPPING POINT:** Proceed immediately to the next step or store the purified products at -20°C.



## LIBRARY AMPLIFICATION

### Materials and Equipment

- UB Barcoded library
- 0.2 mL PCR tube
- FASTplex Library Amp Mix (Cat. ID FAST-LAM)
- FASTplex Library Primer Mix (Cat. ID FAST-LPM)
- FASTplex Paramagnetic Beads (Cat. ID FAST-BEAD)
- FASTplex DNA Suspension Buffer (Cat. ID FAST-SUSP)
- Manual single channel pipettes (20 µL, 200 µL, 1 mL)
- Filtered, pre-sterilized, pipette tips
- 200 Proof Ethanol, Molecular Biology Grade
- [Veriti 96-Well Thermal Cycler \(or equivalent\)](#)
- 2.0 mL Tube Magnet Stand
- Nuclease Free Water
- Eppendorf DNA LoBind Tubes, 2.0 mL
- Qubit® Fluorometer or equivalent
- Qubit dsDNA HS Assay Kit
- Qubit Assay Tubes
- Calculation Tool

### Part 1: Library Amplification Reaction

**IMPORTANT:** Before starting this step, make sure the thermal cycler lid is at the appropriate target temperature. Do not place the reaction onto a cold thermal cycler.

#### Reagent Volumes for the Steps Below

Reagent	Volume
FASTplex Library Primer Mix	15 µL
Barcoded Library from <a href="#">step 39</a> in Part 2 of the Sample Barcoding section	10 µL
FASTplex Library Amp Mix	75 µL

1. **As described in the table above, add 15 µL** of Library Primer Mix to the 10 µL Barcoded Library eluate in the 0.2 mL PCR tube from [step 39](#) in Part 2 of the Sample Barcoding process.
2. **Add 75 µL** of Library Amp Mix.
3. **Mix** well by pipetting.
4. **Cap** the PCR tub and pulse spin.
5. **Use** an appropriate PCR pressure pad.

6. Run the following FASTplex Ion Library Amplification PCR program with a heated cover on and 9600 emulsion mode or +0.8°C/sec heating and -1.6°C/sec cooling with the heated cover set to 105°C. The total reaction volume is 100 µL.

• **FASTplex Ion Library Amplification Program:**

Temperature	Time	Cycle
72°C	10 min	1
98°C	3 min	1
98°C	15 sec	9
66°C	30 sec	
72°C	1 min	
72°C	5 min	1
4°C	HOLD	1

**9600 emulsion speed and heated cover on**

**IMPORTANT:** Check the thermal cycler lid temperature before adding the reaction. Make sure the thermal cycler lid is at the appropriate target temperature. Do not place the reaction onto a cold thermal cycler.

7. After PCR, pulse-spin the library amplification reaction.
8. Transfer 95 µL of the library amplification reaction to a 2.0 mL LoBind tube.
9. If it's less than 95 µL, note the volume for PART 2: Purification of the Amplified Library, [step 1](#).

**IMPORTANT:** The volume will normally decrease due to evaporation during thermal cycling, so it is important to measure the volume prior to the purification steps.



**SAFE-STOPPING POINT:** Proceed immediately to the next step or store the purified products at -20°C.

## Part 2: Purification of the Amplified Library

- Transfer 95 µL of the amplified product to a fresh 2.0 ml LoBind tube.
- Add 305 µL of FASTplex DNA Suspension Buffer to a final volume of 400 µL.
  - If less than 95 µL is available, increase the FASTplex DNA Suspension Buffer volume accordingly to make the final volume to 400 µL.
- Vortex room temperature FASTplex Paramagnetic Beads to ensure they are completely resuspended.
- Add 240 µL of FASTplex Paramagnetic Beads (0.6x volume equivalents) to the diluted multiplexed library.
- Mix thoroughly by pipetting up and down.
- Incubate in a tube rack (non-magnetic) on the bench for 5 minutes to allow the DNA to bind.
- Transfer the 2.0 ml LoBind tube to a magnetic stand and let the beads settle completely for approximately 3 minutes. A bead pellet will form along one side of the tube and the supernatant should appear completely clear blue after ~ 3 minutes.

8. **Slowly transfer** 580 µL the supernatant to a fresh 2.0 ml LoBind tube.

**IMPORTANT:** Do not discard the supernatant. Be careful not to disturb the bead pellet.

9. **Add** 65.5 µL of FASTplex Paramagnetic Bead to the supernatant in the new 2.0 ml LoBind tube.

10. **Mix** well by pipetting up and down.

11. **Incubate** at room temperature on the bench for 5 minutes in a tube rack (non-magnetic) to allow the DNA to bind.

12. **Transfer** the 2.0 ml LoBind tube to a magnetic stand.

13. **Let** the beads separate for ~3 minutes or until clear.

14. **With a pipette set to 600 µL, remove** and discard the supernatant.

**IMPORTANT:** Do not disturb the bead pellet.

15. **With the tube in the magnetic rack, add** 500 µL of 80% Ethanol, ensuring the bead pellet is submerged.

**IMPORTANT:** Be careful not to disturb the beads.

16. **Incubate** for 30 seconds.

17. **Keep** the tube on the magnetic rack.

18. **Carefully remove and discard** the supernatant.

19. **Repeat** steps 15 through 18 for a total of two washes with 80% ethanol.

20. **Use** a small pipette (e.g., P20) to remove the residual ethanol after the second wash.

21. **Air-dry** the beads by leaving the tube uncapped on the magnetic stand for 2 minutes.

22. **Check** to ensure that there are no visible ethanol droplets in the tubes after 1 minute.

- **If ethanol droplets are still visible, air-dry** the beads longer.



**CAUTION:** Do not dry the pellet longer than 3 minutes in total or the DNA recovery will be compromised.

23. **Remove** the tube from the magnetic stand.

24. **Add** 35 µL of FASTplex DNA Suspension Buffer.

25. **Pipette** the liquid along the inside of the tube several times to thoroughly disperse the beads.

26. **Incubate** for 5 minutes in a non-magnetic tube rack on the bench to elute the multiplexed library from the magnetic beads.

27. **Return** the tubes to magnetic stand.

28. **Allow** the bead pellet to form on the inner wall of the tube for ~ 2 minutes or until clear.

**29. When the supernatant has cleared completely, carefully transfer** 33 µL of DNA eluate to a new 2.0 ml LoBind tube. The transferred eluate contains the final library.



**SAFE-STOPPING POINT:** Proceed immediately to the next step or store the purified products at -20°C.

### Part 3: Quantification of the Final Library



**Useful Tip:** To combine multiple libraries of non-overlapping SB, follow the protocol below for each library. For the libraries containing the same number of samples, mix the final library 1:1. For the libraries of different sample number, follow the protocol below, but mix the libraries using volumes proportional to the sample number. For example, if two libraries have the sample sizes of 8 and 20, mix 8 µL and 20 µL or 4 µL and 10 µL of the libraries at the final concentration of 0.045 ng/µL.



**Note:** The recommended library concentration is the same whether using the Ion 520 or 530 Chip

1. **Label** five Qubit Assay Tubes – three for triplicate quantification of the library and two additional tubes for Qubit Standards 1 and 2.
2. **Prepare** a Working Qubit Solution in a 5 ml tube for 10 samples (to account for the measurement in this step and [step 25](#) for post-dilution samples) by mixing the equivalent of:
  - a) 199 µL of Qubit dsDNA HS Buffer (Component B) per sample
  - b) 1 µL of Qubit dsDNA HS Reagent (Component A) per sample. Vortex the reagent before use.
3. **Vortex** the Working Qubit Solution.
4. **Cover** with foil.

**IMPORTANT:** Use within 2 hours.
5. **Add** 198 µL of Working Solution to the three Qubit Assay Tubes used for triplicate quantification of the library.
6. **Add** 190 µL of Working Solution to the two tubes used for the Qubit Standards.
7. **Add** 2 µL of the purified library to the three Qubit Assay Tubes used for triplicate quantification of the library.
8. **Add** 10µL of the corresponding Qubit Standard to the two tubes used for the Qubit Standards.
9. **Briefly vortex** and spin all tubes.
10. **Cover** the tubes with foil.
11. **Incubate** the tubes at room temperature for 2 minutes.
12. **Turn on** the Qubit Fluorometer.
13. **Select** DNA from the home screen.
14. **Select** dsDNA High Sensitivity.
15. **Press** the appropriate button to begin reading the standards.
16. **Measure** Standards 1 and 2 to complete the calibration. This calibration can be used for quantification of the final library in [step 25](#) of this section, if the final quantification is performed within 2 hours.

17. **Begin reading** the three library tubes.
18. **When prompted, change** the volume of sample used to 2  $\mu\text{L}$  and the units to  $\text{ng}/\mu\text{L}$ .
19. **Record** the concentration of the library in triplicate.
20. **In the TSV NGS Calculator, open** the tab named “Final Quant.” The Calculator will automatically display a desired target concentration of 0.045  $\text{ng}/\mu\text{L}$ ; the user may adjust this if an intermediate dilution is needed.
21. **Input** the average concentration of the triplicate readings from the Qubit in  $\text{ng}/\mu\text{L}$  in the cell “Pool Quant Values ( $\text{ng}/\mu\text{L}$ ).”

The calculator will calculate the amount of FASTplex DNA Suspension Buffer and sample pool volume that should be combined in a new 2.0 mL LoBind tube to achieve the proper library concentration.

22. **If not immediately using the library for loading on the Ion Chef™ System, freeze** the undiluted or intermediate diluted library at  $-20^{\circ}\text{C}$  for 72 hours or less.



**Useful Tip:** The “Diluted Pool Volume ( $\mu\text{L}$ )” can be adjusted as needed, but the final desired target concentration cannot be edited in the calculator. It may be helpful to make an intermediate dilution to make it easier to reach the final concentration of 0.045  $\text{ng}/\mu\text{L}$ .

23. **Quantify** the final diluted library in triplicate using the Qubit Working Solution in order to verify that it is at the appropriate concentration.
24. **Adjust** the concentration as needed by adding FASTplex DNA Suspension Buffer or undiluted library to achieve the proper concentration.
25. **Repeat Quantitation** until the correct concentration is achieved. Record the final concentration in the TSV NGS Calculator text box.

**IMPORTANT:** Use 195  $\mu\text{L}$  of the Qubit Working Solution with 5  $\mu\text{L}$  of the library for triplicate Qubit reading because of the low concentration.

26. **Keep** the library on ice for immediate loading to the Ion Chef Instrument.

## PREPARING for the ION GENESTUDIO S5 PLUS or EQUIVALENT SEQUENCER and ION CHEF SYSTEM

### Materials and Equipment

- Final library with the concentration adjusted for Ion 520 & Ion 530 ExT Kit-Chef kit
- Ion 520 & Ion 530 ExT Kit-Chef
- Ion 520 or 530 Chip Kit

### Part 1: Uploading the FASTplex Template



**Note:** Templates are available for download at [www.onelambda.com](http://www.onelambda.com). If experiencing trouble locating or downloading the appropriate template, please contact One Lambda Technical Support at [1lambda-techsupport@thermofisher.com](mailto:1lambda-techsupport@thermofisher.com).

1. To upload the FASTplex Ion Sequencing Templates (FASTplex Ion ExT Template 530 or FASTplex Ion ExT Template 520), log into the Torrent Browser for the Torrent Server connected to the Ion S5 or Ion GeneStudio S5 System and Ion Chef System.
2. Select the “Plan” tab, which will take you to the “Templates” selection screen.
3. Click the blue “Upload” button, as shown in figure 3.

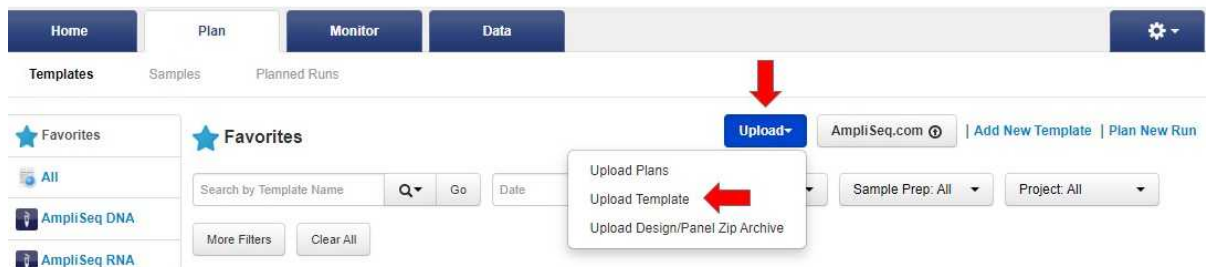


Figure 3. Screenshot of the “Upload” drop down menu

4. Select “Upload Template” from the drop-down menu, as shown in figure 3. An “Import Plan Template” window will pop up, as shown in figure 4.

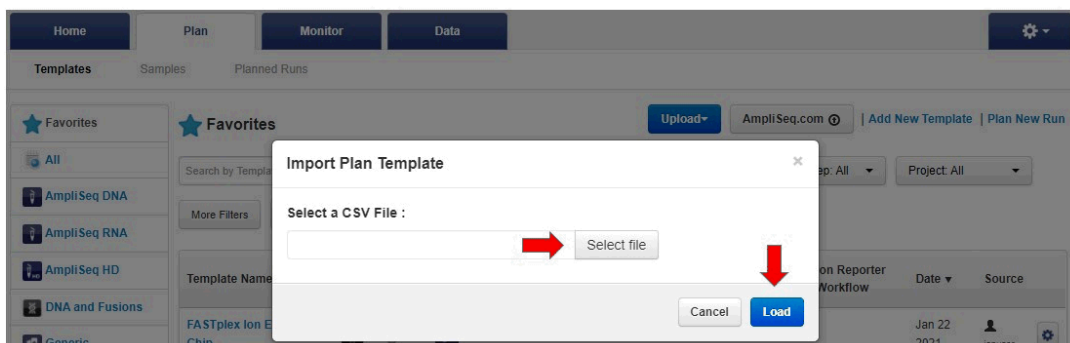
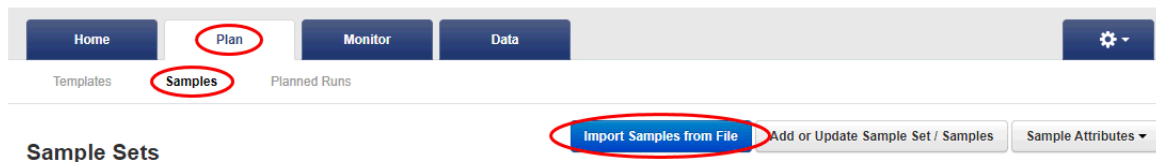


Figure 4. Screenshot of the “Import Plan Template” window

5. **Click** “Select file” and choose the downloaded FASTplex Ion ExT template 530 or FASTplex Ion ExT template 520 that matches your Torrent Suite Software version. The file should be saved to a location somewhere on the computer or in a USB drive.
6. **Click** the blue “Load” button after choosing the desired template(s). The template will show as imported successfully.

## Part 2: Creating and Uploading a Sample List

1. **Open** the “Sample Sheet” tab in the TSV NGS Calculator to find the sample sheet. The Sample Sheet “Sample\_Name” and “Sample\_ID” columns will automatically populate from the information that was input earlier.
2. **Export** the completed sample sheet using the “Export” button and name the sample sheet.
3. **Copy** the file on a USB memory drive if desired.
4. **To upload this Sample List for a Planned Run set up, log into** the Torrent Browser for the Torrent Server connected to the Ion S5 or Ion GeneStudio S5 System.
5. **Select** the “Plan” Tab, and then select “Samples” from submenu.
6. **Click** the “Import Samples from File” button on the right, as shown in figure 5.



**Figure 5.** Screenshot of the “Plan” tab, “Samples” submenu, and “Import Samples from File” button

7. **Follow** the Import Samples prompt.
8. **Select** the new “Sample List for Upload” to import.
9. **Using the “Select File” button, browse** for the sample list, as shown in figure 6.
10. **Select** the “Add Sample Set.,” as shown in figure 6.
11. **Enter** the “Sample Set Name,” as shown in figure 6.

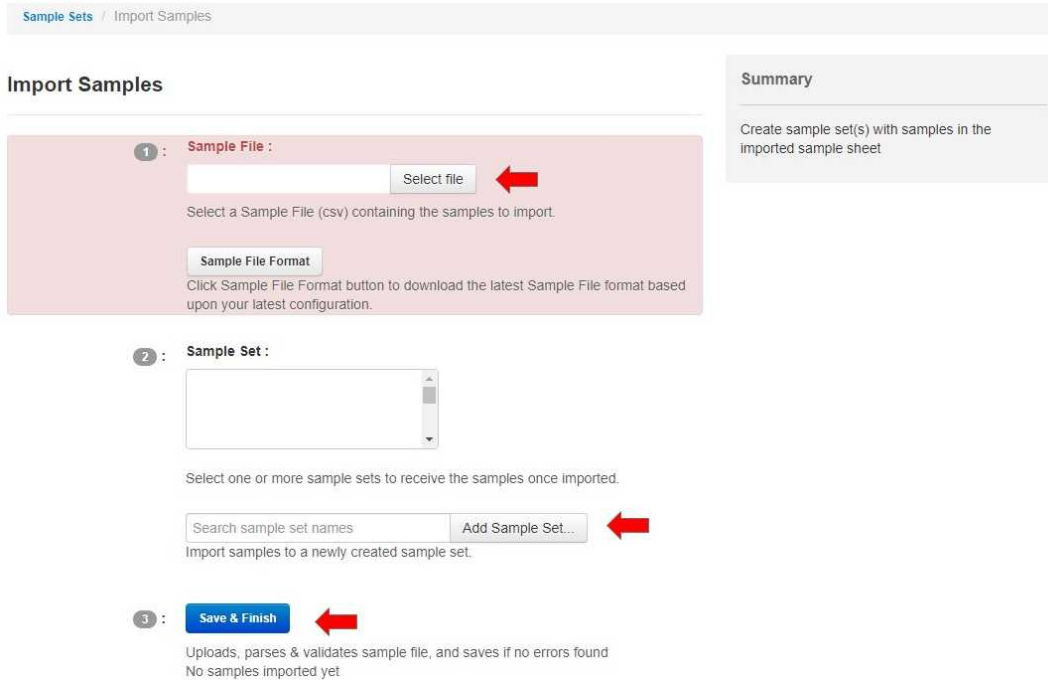


Figure 6. Screenshot for adding a new sample set name

12. Click “Save & Finish.”

### Part 3: Creating a Planned Run and Uploading the Sample List

1. To create a Planned Run, log into the Torrent Browser for the Torrent Server connected to the Ion S5 or Ion GeneStudio S5 System and Ion Chef System.
2. Select the “Plan” Tab.
3. Select “Samples” in the sub-menu.
4. Locate the sample set that was uploaded for the run.
5. Click the corresponding gear icon on the right.
6. Choose “Plan Run,” as shown in figure 7.

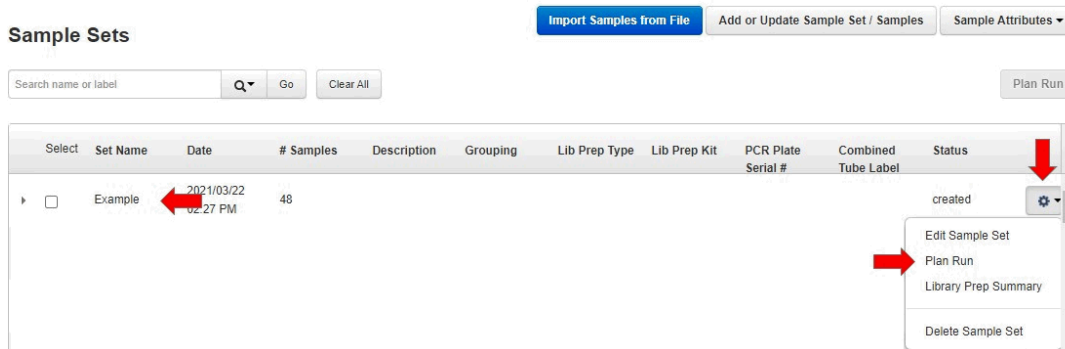


Figure 7. Screenshot of the “Plan run” drop down menu



7. **In the pop-up window, mark “Show All Plan Templates.”**
8. **Select** the “FASTplex Ion ExT” from the drop-down menu.
9. **Click** “Plan Run” button.
10. **Click** “Next” on the “Barcoding” tab and the “Projects” tab.
11. **On the “Save & Finish” tab, name** the run in the field “enter a plan name.”
12. **Click** “Save & Finish.”



**Note:** If the Torrent Suite version is prior to 5.10, an error message rejecting the template provided may occur. If an error message occurs, please contact One Lambda Technical Support at [1lambda-techsupport@thermofisher.com](mailto:1lambda-techsupport@thermofisher.com).

## RUNNING the ION CHEF SYSTEM

### Guidelines for Using the Ion Chef System



**Note:** All Ion Chef Instrument components are single use only.

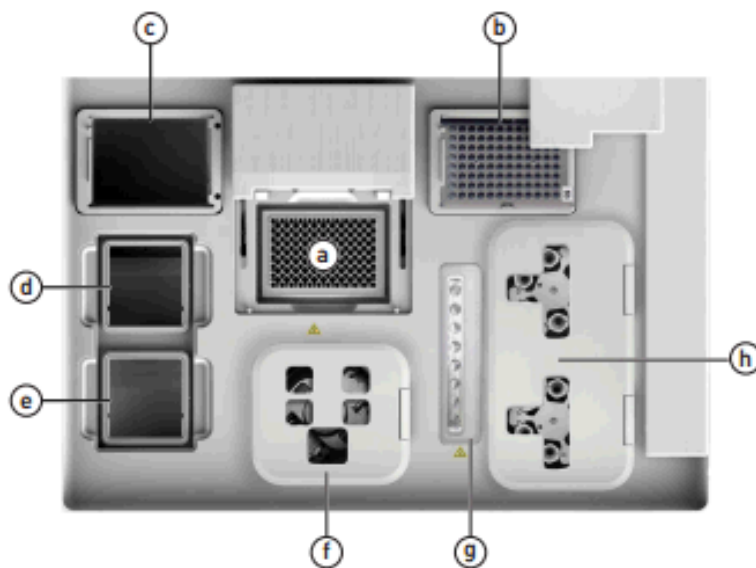
- **Store** all Ion Chef System consumables and cartridges under the recommended conditions and in an upright position.
- **Inspect** all Ion Chef System consumables and cartridges for damage upon arrival and again before use.
- **Hold** sequencing chips by gently gripping them by their edges.
- **When the Ion Chef System is not in use:**
  - a) Remove all consumables and reagents from the deck.
  - b) Close the instrument door.
- **Before use, confirm** that the Ion Chef has been cleaned following the last use.
- **Confirm** that all components are clean and dry before loading them onto the Ion Chef Instrument.
- **Confirm** that the Reagents and Solutions station compartments are dry and free of condensate before loading components.
- **Except for the New Pipette Tip Cartridge v2, do not reuse** any of the Ion Chef System consumables or reagents. After each run, the empty Pipette Tip Cartridge v2 is transferred to the waste tip station.

**IMPORTANT:** Ion Chef chip-loading centrifuge is rated to operate at the listed rotational frequencies with the chip buckets, chips, and adapters. The centrifuge must be load balanced.

- **Remove and sequence** chips within 1 hour after the Ion Chef System finishes loading them.
- **If a loaded chip cannot be sequenced immediately, store** it within a chip storage container at 4°C until ready to run it (up to 6–8 hours maximum).
- **If a loaded chip has been stored, remove** the chip from 4°C storage (but keep it in the chip storage container) at least 20 minutes prior to running it, allowing the chip to warm to room temperature.

## Materials and Equipment

- Ion 520 or 530 Chip Kit
- Ion 520 & 530 ExT Kit-Chef
- Ion S5 Chef Solutions
- Ion S5 Ext Chef Reagents
- Ion S5 Chef Supplies (see figure 8):
  - Chip Adapters
  - Enrichment Cartridge v2
  - Tip Cartridge v2
  - PCR Plate and Frame Seal v2
  - Recovery Station Disposable Lid v2
  - Recovery Tube v2



**Figure 8.** Summary of the supplies that are placed on the Ion Chef, see Part 1 below for instructions corresponding to each labeled item.

### Part 1: Setting Up the Ion Chef System

1. **Press** the Eject button at the top right of the screen to open the front if it is closed.
2. **Remove** all cartridges and consumables from their wrappings and boxes.
3. **Place** them on the bench next to the Ion Chef Instrument.
4. **Place** a new tip rack into Position C. The empty tip rack from the previous run will be placed in Position B.
5. **Place** the semi-skirted 96 well plate onto position A.
6. **Slide** a plate seal underneath the cover that is homed behind the 96 well plate. The notches should be facing out and up and will slide in the slot smoothly.
7. **Uncap** the 4 tubes on the Reagents Cartridge.

8. **Add** 50 µL of Final Diluted Library into the Ion S5 Ext Chef Library Sample Tube (barcoded tube on Reagents Cartridge).
9. **Place** the Ion S5 Ext Chef Reagents Cartridge onto Position D.
10. **Place** the Ion S5 Ext Chef Solutions Cartridge onto Position E after uncapping the four tubes on the cartridge.
11. **Place** Ion torrent Chip in Rotor Bucket and clip it in the chip adapter (see figure 9) and place the assembled chip into the centrifuge in Position F.



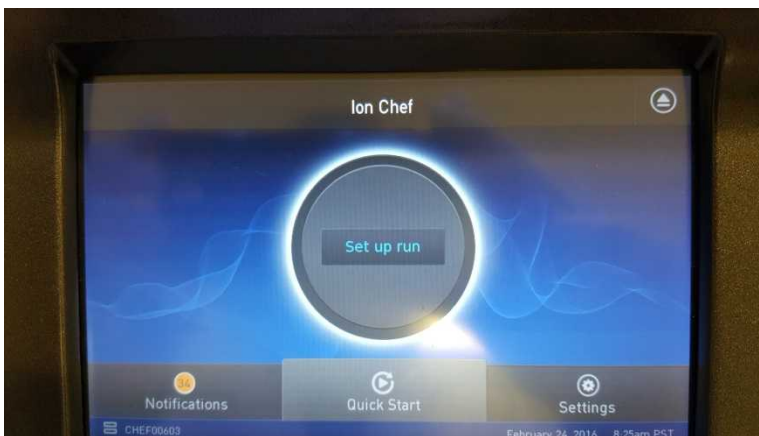
**Figure 9.** Ion torrent Chip in Rotor Bucket

12. **Place** the Enrichment Cartridge onto Position G.
13. **Place** the Recovery Tubes into each empty spot.
14. **Place** the Recovery Station Disposable Lids onto Position H. The barcode should be facing up and the port oriented towards the back of the machine.

## Part 2: Starting the Chef ExT Run

**IMPORTANT:** Create a planned run prior to starting Chef ExT on the Ion Chef. The Ion Chef draws the information from the planned run to template.

1. **Once all the reagents and sample have been set up on the Ion Chef, press** “Set up run,” as shown in figure 10.



**Figure 10.** Screenshot of the “Set up run” button

2. Press “Quick start,” as shown in figure 11.

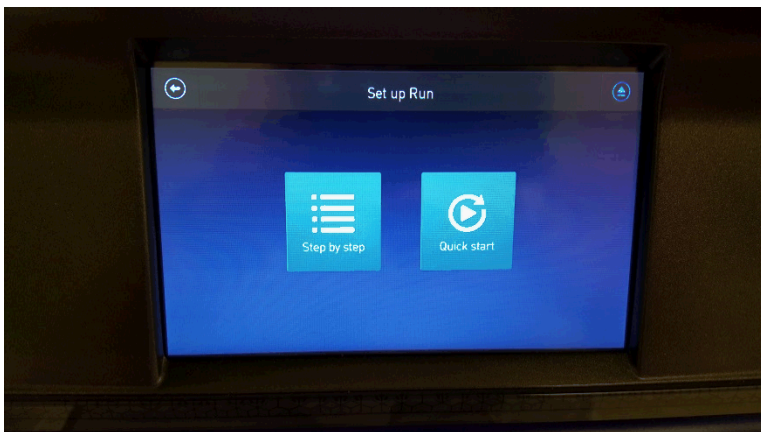


Figure 11. Screenshot of the “Quick Start” button

3. Press “Next” to start the Ion Chef’s tip verification, as shown in figure 12.

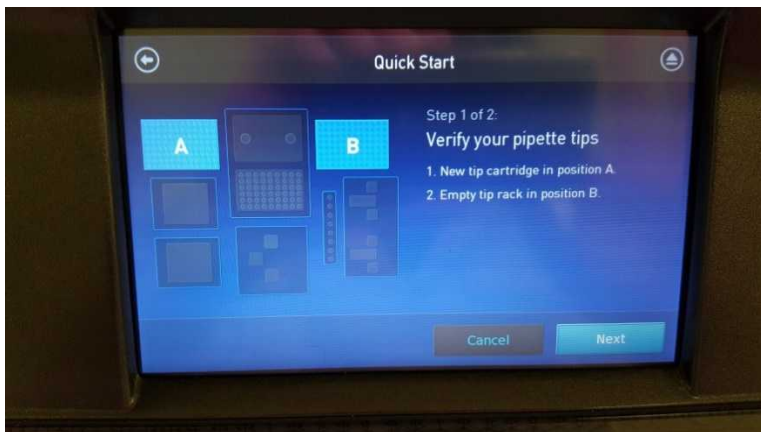


Figure 12. Screenshot of the “Next” button

4. Press “Start check” to begin Deck Scan, as shown in figure 13.



Figure 13. Screenshot of the “Start check” button

5. Choose your run plan selection on the Data Destination screen, as shown in figure 14.



Figure 14. Screenshot of the “Data Destination” screen

6. **If running the Chef through the entire process to chip loading is needed, choose Timer** at the Run Options screen (see figure 15). The Timer option allows for choosing when the run ends. Runs typically take 6 hours and 45 minutes to complete.

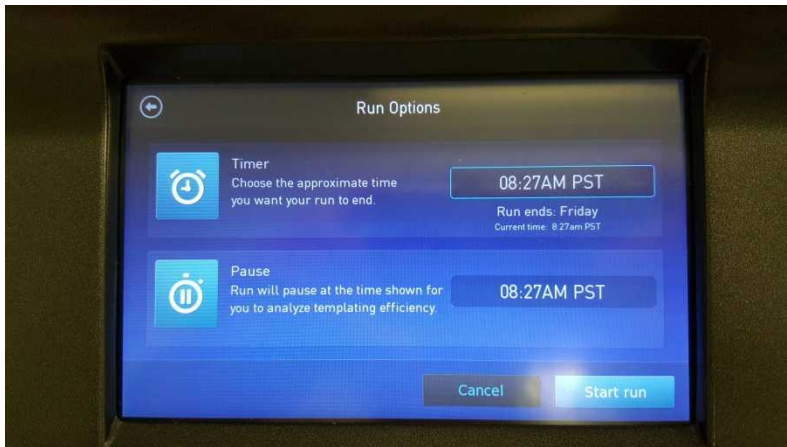


Figure 15. Screenshot of the “Run Options” screen

7. **Press “Start run”** to begin the run.
8. **Once the Chef ExT process completes, remove** the chip adapter and loaded chip from the bucket (see figure 16).

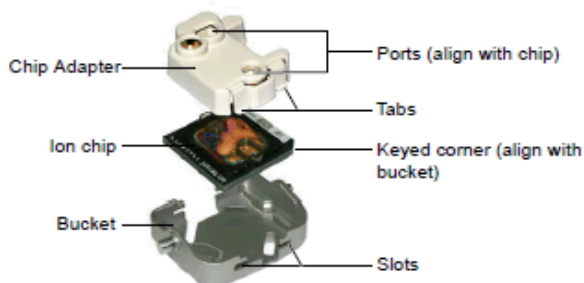


Figure 16. Chip Adaptor, Loaded Chip, and Bucket Configuration

9. **Check** the chip for any excess liquid.
10. **Use** a P-200 pipette to draw off the excess.
11. **Proceed** to “Start the Sequencing Run” for loading the S5 with your chip.
12. After completing the Ion Chef run, the technician who performed and the second technician who verified the documentation shall sign off the Ion Chef tab of ALT-0007FORM-A.

## USING the ION S5 or Ion GENESTUDIO S5

### Guidelines for Using the Ion S5 or Ion GeneStudio S5

**IMPORTANT:** The Ion S5, Ion S5 XL, GeneStudio S5, and GeneStudio S5 Plus Sequencers are equipped to verify the compatibility of each chip and consumable loaded during initialization and sequencing, and to further confirm that these components have not exceeded their expiration date. To avoid exceptions during initialization, inspect the expiration date for each consumable before installing them onto the instrument.



**Note:** Used chips cannot be reused.

- **Unbox** the Ion S5 ExT Sequencing Reagents cartridge (Ion Chef System) 1 hour before use.
- **Allow** the Ion S5 ExT Sequencing Reagents cartridge to equilibrate to room temperature.
- **Do not remove** the Ion S5 [ExT] Sequencing Reagents cartridge from its foil packaging until immediately before loading, so that the unused cartridge may be returned to storage if the sequencing run is delayed.

**IMPORTANT:** The Ion S5, Ion S5 XL, Ion GeneStudio S5, and Ion GeneStudio S5 Plus Sequencers require that a cleaning be performed prior to initialization. This is normally done automatically at the completion of the previous sequencing run. However, if a sequencing run is not completed (due to power failure, a user-aborted run, etc.), the instrument will not allow the subsequent initialization to proceed until a cleaning has been performed. Refer to the User Manual for manual cleaning directions.

- **Recycle or dispose** of all reagents and consumables according to applicable regulations.
- **In the event of a spill or a leak, perform** the following:
  - a) Remove the Ion S5 [ExT] Wash Solution bottle, then remove and empty the waste reservoir.
  - b) Remove the Ion S5 [ExT] Sequencing Reagents cartridge.
  - c) Inspect the waste and nucleotide reagent bays for liquid.
  - d) Using absorbent paper soak up as much liquid as possible. Wash the affected area with 10% bleach solution.
  - e) Wipe the affected surfaces with 70% Isopropanol, and then allow them to air-dry.

### Reagents

- Ion S5 [ExT] Sequencing Reagents
- Ion S5 [ExT] Sequencing Reagents cartridge
- Ion S5 [ExT] Sequencing Solutions
- Ion S5 [ExT] Wash Solution
- Ion S5 [ExT] Cleaning Solution





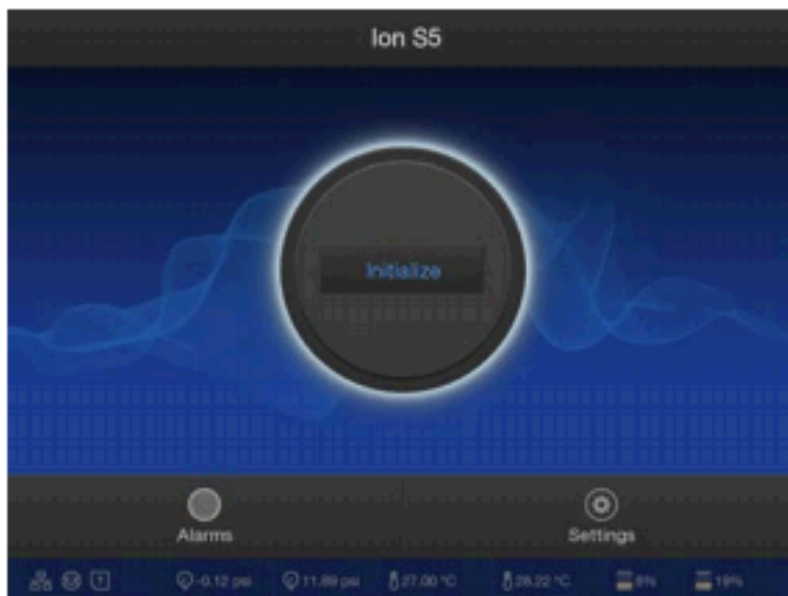
**Figure 17.** The Ion S5 and Ion GeneStudio S5 System Component Positions



**Useful Tip:** It is recommended that sequencing be started as soon as possible after chip loading and instrument initialization are complete; however, successful sequencing runs can be started up to 24 hours after instrument initialization.

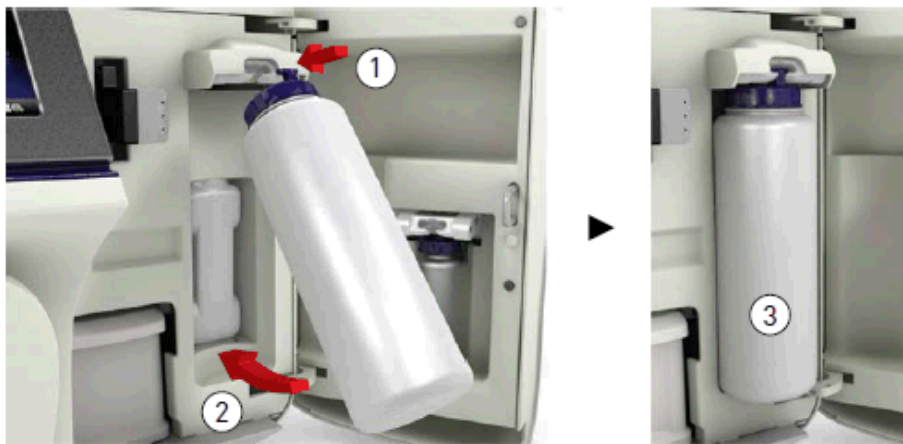
## Part 1: Initializing the Ion S5 Sequencer

1. In the instrument touchscreen main menu, select “Initialize” (see figure 18). The door, chip, and reagent cartridge clamps unlock.



**Figure 18.** Ion S5 and Ion GeneStudio S5 Initialization Home Screen

2. **When prompted, remove** the Ion S5 [EXT] Wash Solution bottle to access the waste reservoir, then remove and empty the waste reservoir.
3. **Reinstall** the empty waste reservoir.
4. **Replace** the expended Ion S5 [EXT] Sequencing Reagents cartridge with a new cartridge equilibrated to room temperature.
5. **Thaw** the cartridge for at least 1 hour prior to initialization to allow the nucleotides to fully thaw.
6. **Remove** the red cap from a new Ion S5 [EXT] Wash Solution bottle.
7. **Install** the new Ion S5 [EXT] Wash Solution bottle, as shown in figure 19.



**Figure 19.** Installing a new Ion S5 EXT Wash Solution Bottle

8. **Ensure** that the used sequencing chip from the previous run is properly seated in the chip clamp and the chip clamp is pushed in all the way.
9. **If necessary, install** a new Ion S5 [EXT] Cleaning Solution bottle.
  - a) Unbox the Ion S5 EXT Wash Solution bottle (Ion Chef System).
  - b) Invert the bottle 5 times.
  - c) Swirl at an angle to mix thoroughly.



**Note:** The Ion S5 [EXT] Cleaning Solution bottle contains enough reagent to complete four cleanings.

10. **Close** the door.
11. **Select** “Next.” The instrument confirms that the consumables and chip are properly installed and that the Ion S5 [EXT] Cleaning Solution bottle contains enough reagent to perform the post-run clean.
12. **Follow** all on-screen recommendations to ensure proper installation of required consumables.



**Note:** If the allowed number of post-run cleans has been met, the instrument will prompt the user to replace the Ion S5 [EXT] Cleaning Solution bottle.

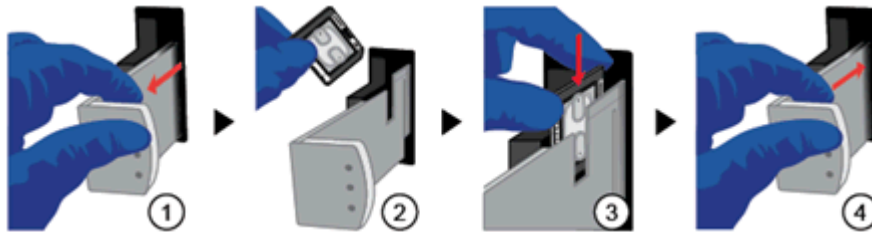
13. **When initialization is complete (~40 minutes), select** “Next” to return to the main menu. The instrument is now ready for a sequencing run.

## Part 2: Starting the Sequencing Run



**Note:** The term S5 Sequencer is used to represent the general S5 Sequencer line, including the Ion S5, Ion S5 XL, Ion GeneStudio, and Ion GeneStudio Plus.

1. **In the instrument touchscreen main menu, press “Run.”** The door and chip clamp will unlock.
2. **Remove** the used sequencing chip then secure the Chef loaded chip with templated ISPs in the chip clamp with the chip notch facing downwards, as shown in figure 20.



**Figure 20.** Placing a Chip in the Chip Clamp.

3. **Slide** the chip clamp out.
4. **Remove** the chip currently in the clamp.
5. **Place** the loaded chip in the chip clamp with the chip notch in the bottom-front corner.

**IMPORTANT:** Do not force the chip into the clamp. If the chip does not fit easily in the clamp, confirm that the notch is oriented as shown on the image.

6. **Slide** the metal tab in fully to engage the clamp.
7. **Close** the instrument door.
8. **Press** “Next.”
9. **Push** the chip clamp all the way in to engage.
10. **Close** the instrument door.
11. **Press** “Next.”
12. **In the drop-down list, select** the Planned Run that was created in the Torrent Suite™ Software.
13. **Press** “Next.”



**Note:** “Planned Run (none)” may also be selected, after which run information should be entered on the following screen.



**Useful Tip:** Selecting a predefined Planned Run is recommended.

14. **Confirm** that the pre-populated settings are correct.
15. **If necessary, make** changes using the buttons and drop-down lists.
16. **Confirm** that the instrument door is closed.
17. **Press** “Next.”

- 18. If the option becomes available, press “Calibrate” to begin the sequencing run. If not, the sequencing run will begin automatically.**



**CAUTION:** During a run, do not open the instrument door. Avoid touching the instrument. Touching the instrument during the sequencing run may reduce the quality of the measurements.

When the sequencing run is complete, the instrument automatically performs the cleaning procedure. After cleaning, the touchscreen returns to the main menu.

- 19. Use the Torrent Browser to review the results.**

## RESULTS

### Data Acquisition

Upon completion of sequencing, the sequencer system software demultiplexes all sequence reads using the barcodes and generates a file per sample. Refer to user manual of the sequencer system software to download sequence data in its default or specified format such as a FASTQ or BAM file for each sample. TypeStream Visual NGS Analysis Software imports a FASTQ or BAM file from the sequencer for analysis.

Sample ID can be embedded in the data file from the sequencer software or can be added at the time of data acquisition through TypeStream Visual NGS Analysis Software.

### Data Calculation

Please see TypeStream Visual NGS Analysis Software User Manual for details. The HLA TypeStream Visual NGS Analysis Software provides all the necessary quality metrics depth of coverage, quality score for the base reads, read alignment and variant call, and the laboratory should determine the acceptable values for each quality metric in order to assure a successful run.

### Data Analysis

HLA TypeStream Visual NGS Analysis Software sorts imported sequence reads using the locus-specific reference sequences from the IPD-IMGT/HLA Database which provides a specific database for sequences of the human major histocompatibility complex, identifies reference alleles that best match the sample reads, quality-trims, maps and assembles contiguous sequences from the mapped reads, and assigns alleles per locus based on the assembled consensus contiguous sequences. The use of other analysis software may lead to incorrect results and is not supported.

## LIMITATIONS of the PROCEDURE

- A. Sub-optimal sample or library quality and/or quantity may cause test failures. The causes of such failures may include low sample quantity and quality, contamination, presence of inhibitors, random enzymatic reaction failures, uncalibrated and malfunctioning instruments, use of expired reagents or third-party reagents, incorrect reagent maintenance, protocol modification, and incorrect quantification or calculation.
- B. Sample DNA should be quantified with a fluorometer and be free from any known PCR inhibitor. PCR inhibitors may be introduced from the original sample source or from various DNA extraction methods. Routine samples should be validated for amplification using the AllType FASTplex NGS 11 Loci Kit reagents.
- C. The PCR and library preparation workflow described in this protocol requires highly controlled conditions. Follow the standard PCR guidelines listed in the section above, [GENERAL PREPARATION for the ASSAYS](#) to minimize contaminations.
- D. The AllType FASTplex NGS 11 Loci Kit – 48 has been tested for use with the Applied Biosystems Veriti 96-Well Thermal Cycler (Cat. No. 4375786), a model capable of 9600 emulation speed or +0.8°C/sec heating and -1.6°C/sec cooling and a heated cover at 105°C or equivalent for all programs. Other thermal cyclers considered for use require end-user evaluation and validation.
- E. The AllType FASTplex NGS 11 Loci Kit – 48 (Cat. No. ALL-FAST11LX) has been tested using Ion GeneStudio S5 Plus System or equivalent and Ion Chef Instrument using Ion 520 Chip (8 to 24 samples) and Ion 530 Chip (8 to 48 samples), and Ion 520 & Ion 530 ExT Kit-Chef sequencing reagents. Alternative configurations, kits, and sequencing systems are not supported by this application and must be determined and validated by the user.
- F. The minimum sample size per an assay is eight .
- G. The AllType FASTplex NGS 11 Loci Kit – 48 (Cat. No. ALL-FAST11LX) has not been tested using any protocol that deviates from the one described above and may lead in erroneous results.
- H. HLA typing at high resolution using the NGS technology is a complex process that requires qualified personnel to review data and final HLA allele assignments.
- I. This test must not be used for making a clinical decision.
- J. Please refer to AllType FASTplex NGS 11 Loci Kit – 48 Ambiguities List for a known list of lot-specific allele assignment ambiguities for polymorphisms located outside of the amplified region. Any allele listed may produce incorrect results and needs to be evaluated before assigning a result.
- K. AllType FASTplex NGS 11 Loci Kit - 48 can detect DRB4\*03:01N and can eliminate DQB1\*03:276N when the supplemental Class II Exon 1 primer mix is used and the test sample is heterozygous in exon 1, using TypeStream Visual 2.1 or later.
- L. Genotype ambiguities are expected from the limitation in the primer design and heterozygous genotype phasing due to limitations in sequencing read length and associated sequence alignment.
- M. The AllType FASTplex 11 Loci primers for HLA-DRB1, -DQB1 and -DPB1 primers do not amplify exon 1. Associated ambiguities are listed in the Ambiguities List. The AllType FASTplex Exon 1 primer mix must be used to resolve Exon 1 ambiguities for these genes. When using the AllType FASTplex 11 Loci Exon 1 Primer Mix with low quality or highly fragmented DNA, users may experience low uniformity across sequence reads.

- N. In rare cases, unknown sequence variants at the amplification primer binding sites in the untranslated regions (UTRs) may affect amplification efficiency of molecular typing reagents listed above. Homozygous results needs be confirmed by a secondary method before assigning a result.
- O. The AllType FASTplex primers were tested using alleles identified in the Nomenclature List in brackets in Index 4 for AllType (e.g., A\*01:01<sup>[1234]</sup>). The reactivity of alleles that were not available has been predicted from its available sequence and may produce false reactions and needs to be evaluated before assigning a result.
- P. The AllType FASTplex primers were tested based on Field-3 (6-digits HLA types) only. There are no performance claims outside this field.
- Q. Software analysis for this kit is only supported with the HLA TypeStream Visual NGS Analysis Software 2.1 or above using AllType FASTplex catalog file. The use of other software is not supported.
- R. Failure to completely read and explicitly follow all the instructions contained herein may result in invalid test results, damage to the product(s), injury to persons, including to users or others, and damage to other property. One Lambda, Inc. does not assume any liability arising out of the improper use of the product(s) described herein (including parts thereof or software).

## EXPECTED VALUES

### Sample Amplification

The AllType NGS 11 Loci Amplification Kit – 48 is expected to amplify and produce HLA locus-specific amplification products consisting of ~5000 base pairs in averaged length (subject to change per lot). Physical separation and monitoring of contamination in a pre-amplification laboratory and equipment is highly recommended. Exon 1 amplicons will range from 1.6-2.2 kb.

### Library Preparation

The instruction provided in this Application Note has been tested to produce a final sequence-compatible barcoded library using amplified DNA from the AllType FASTplex NGS 11 Loci Kit – 48 for the final concentration of 0.045 ng/μL Ion Torrent Sequencing using the Ion 520 or 530 Chip on Ion S5 or Ion GeneStudio S5 series instruments. The expected final library concentration is 1 ng/μL or above, as measured in [LIBRARY AMPLIFICATION, Part 3, step 19](#). A lower concentration library may give incorrect or ambiguous results due to lower reads.

### DNA Sequencing

The AllType FASTplex NGS 11 Loci Kit – 48 has been tested using 48 samples per sequencing run to ensure a minimum average read depth of 50 reads or greater per allele when using Ion 520 or 530 Chip. Alternative sample-per-run throughputs are not supported by this application and must be determined and validated by the user. Due to the barcoding chemistry involved, the reads generated from AllType FASTplex NGS Assay are trimmed by 30 bases from the 3' end in Torrent Suite using the FASTplex Ion ExT Template files (see [PREPARING for the ION GENESTUDIO S5 PLUS or EQUIVALENT SEQUENCER and ION CHEF SYSTEM, Part 1: Uploading the FASTplex Template](#)). This process improves the quality of the reads to ensure reliable results and has no effect on the resolution or quality of results. Elimination of the read processing using an incorrect analysis template may produce erroneous results.

The following sequencing metrics in the Torrent Suite Run Summary report have been tested to produce reliable results for the 48-sample run. Total reads is the number of reads that meets the sequencer QC filters and is a good metric to assess if the run produced enough reads. Mean read length indicates if the sequenced reads meet the minimum length needed to phase the sequence for high-resolution results. % CV barcode read indicates the library contains an even number of reads for each sample. A high %CV with samples with significantly lower reads indicates poor sample input or normalization that may lead to sample-specific issues. A deviation of a single metric does not necessarily indicate a sequencing error. A combination of low total reads and high barcode balance %CV due to multiple low reads samples, or low read length may lead to ambiguous or incorrect results due to insufficient reads.

QC Metrics for Sequencing:

- Total Reads: ≥13 million
- Mean Read Length: >200 bp
- % CV Barcode Reads Balance: ≤30% without excessively low reads sample(s).



## CONTACT INFORMATION

### Manufacturer



One Lambda, Inc.  
22801 Roscoe Blvd, West Hills, CA 91304, USA  
T: 747.494.1000 | F: 747.494.1001

### Technical Assistance

For technical questions or customer support, please contact us at:

One Lambda Technical Support

North America: + 1 747-494-1000 Option #2 (PST)

North America Toll Free: +1 800-822-8824 Option #2 (PST)

International: +49 3302883-426 (CET)

International Toll Free: 00800 6200 0000 (CET)

Web: [www.onelambda.com](http://www.onelambda.com)

Email: [1lambda-TechSupport@thermofisher.com](mailto:1lambda-TechSupport@thermofisher.com)

## APPENDICES

### Appendix 1: PCR Program Reference Guide

The following is a list of PCR programs used for the assays:

A. **HLA 11-loci Amplification Ion Program:**

Temperature	Time	Cycle
94°C	2 min	1
98°C	10 sec	22
69°C	3 min	
98°C	10 sec	8
60°C	3 min	
4°C	HOLD	1

**9600 emulation speed and heated cover on**

B. TAG Program: 55°C for 15 min., 25°C hold, heated cover on

C. STOP Program: 68°C for 10 min., 25°C hold, heated cover on

D. **FASTplex Ion Library Amplification Program:**

Temperature	Time	Cycle
72°C	10 min	1
98°C	3 min	1
98°C	15 sec	9
66°C	30 sec	
72°C	1 min	
72°C	5 min	1
4°C	HOLD	1

**9600 emulation speed and heated cover on**

### Appendix 2: FASTplex Sample Plate 48 Worksheet

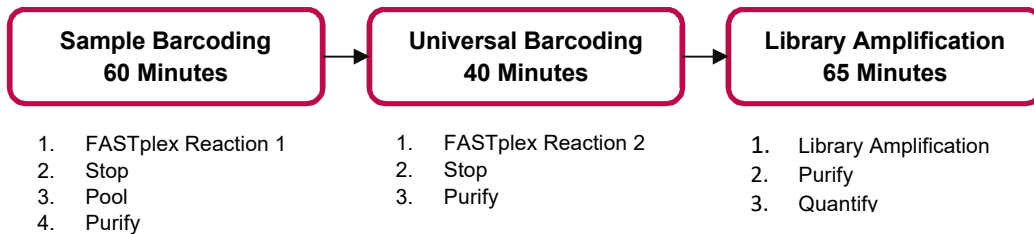
I	G	F	E	D	C	B	A	
								1
								2
								3
								4
								5
								6
								7
								8
								9
								10
								11
								12

**Figure 21.** FASTplex Sample Plate 48, which contains enough sample-barcoding (SB) reagent to prepare a 48-sample library twice or up to twelve 8-sample libraries.

## Appendix 3: Quick Guide (Refer to the Application Note for more detail.)

### Ion – AllType FASTplex NGS Assay Quick Guide

- After amplification of DNA sample, purify with magnetic beads, and measure concentration with standard Qubit protocol.
- Enter the Qubit values into the TSV NGS Calculator. Dilute the amplicons with the FASTplex DNA Suspension Buffer from kit as indicated.
- Diluted amplicons are ready for library preparation.



*Note: Before beginning, thaw reagents and keep on ice. Place FASTplex Paramagnetic Beads at room temp for at least 30 minutes. Ensure that Buffers contain NO crystals.*

**IMPORTANT:** Only use FASTplex DNA Suspension Buffer.

#### Sample Barcoding Steps:

- Centrifuge FASTplex Sample Plate and transfer **16 µL** barcoding reagent into a fresh plate.
- Add **8 µL** diluted amplicon and pipette mix 10x.
- Add **12 µL** FASTplex Barcoding Buffer and pipette mix 10x.
- Seal Plate and pulse centrifuge. Run FASTplex **TAG program**.
- Add **18 µL** FASTplex Stop Solution and pipette mix 5x.
- Seal plate and pulse centrifuge. Run FASTplex **STOP program**.
- Pool **18 µL** from each sample barcoding reaction well.
- Complete Sample Barcode (SB) purification following the TSV NGS Calculator.
- Elute in volume of DNA suspension Buffer **according** to the TSV NGS Calculator.
- Transfer **48 µL** Purified SB pool to 0.2 ml PCR tube.

#### Universal Barcoding (UB) Steps (Refer to the TSV NGS Calculator for appropriate volumes of each reagent):

- Add FASTplex Univ Barcode P1 reagent to **48µL** Purified SB Pool.
- Add FASTplex Barcoding Buffer, pipette mix and pulse centrifuge. Run FASTplex **TAG program**.
- Add FASTplex Stop Solution, pipette mix, and pulse centrifuge. Run FASTplex **STOP program**.
- Pulse centrifuge and complete UB Purification by adding 1 volume equivalent of FASTplex Paramagnetic Beads.
- Elute in **13 µL** of FASTplex DNA Suspension Buffer.
- Transfer **10 µL** Purified UB pool to 0.2 ml PCR tube.

#### Library Amplification Steps:

- Add **15 µL** FASTplex Library Primer Mix to **10µl** purified UB pool.
- Add **75 µL** FASTplex Library Amp Mix and pipette to mix.
- Run **FASTplex Ion Library Amplification Program**.
- Dilute Library – **95 µL** Library in **305 µl** FASTplex DNA Suspension Buffer
- Complete Library Purification with a double size selection, 0.6x volume and 0.113x volume equivalent of FASTplex Paramagnetic Beads.
- Elute in **35 µl** of FASTplex DNA Suspension Buffer and Transfer **33 µL** of final library to a 2ml LoBind Tube.
- Dilute final library for Ion Chef loading to 0.045 ng/µL.

## BIBLIOGRAPHY

1. Jonathan C. Barone, Katsuyuki Saito, Karl Beutner, Maria Campo, Wei Dong, Chirayu P. Goswami, Erica S. Johnson, Zi-Xuan Wang, Susan Hsu, “HLA-genotyping of clinical specimens using Ion Torrent-based NGS,” *Tissue Antigens* (2015) 76:903-909
2. Takashi Shiina, Kazuyoshi Hosomichi, Hidetoshi Inoko and Jerzy K Kulski: “The HLA genomic loci map: expression, interaction, diversity and disease,” *Journal of Human Genetics* (2009) 54:15-39
3. SGE Marsh, ED Albert, WF Bodmer, RE Bontrop, B Dupont, HA Erlich, M Fernández-Vina, DE Geraghty, R Holdsworth, CK Hurley, M Lau, KW Lee, B Mach, WR Mayr, M Maiers, CR Müller, P Parham, EW Petersdorf, T Sasazuki, JL Strominger, A Svejgaard, PI Terasaki, JM Tiercy, J Trowsdale: “Nomenclature for factors of the HLA system,” 2010. *Tissue Antigens* (2010) 75:291-455
4. *American Society for Histocompatibility and Immunogenetics Laboratory Manual*, 4<sup>th</sup> Edition Volume 1, American Society for Histocompatibility and Immunogenetics (2000)
5. *EFI Standards for Histocompatibility & Immunogenetics Testing*, Version 7.0 The European Federation for Immunogenetics, Strasbourg, France (2017)
6. Brian Matlock, “Assessment of Nucleic Acid Purity,” Thermo Fisher Scientific: Technical Note 52646 (2015) Masaharu Yokota, Noriyuki Tatsumi, Oytip Nathalang, Tetsuaki Yamada, Izumi Tsuda, “Effects of heparin on polymerase chain reaction for blood white cells”. *Journal of Clinical Laboratory Analysis* (1999) 13:133-140

## TRADEMARKS

“Eppendorf,” and “LoBind,” are trademarks of Eppendorf AG.













All other trademarks are the property of Thermo Fisher Scientific and its subsidiaries.

## DISCLAIMERS

For Research Use Only. This product is not intended to provide information for the diagnosis, prevention or treatment of disease or to aid in the clinical decision making process. This product is not cleared or approved for clinical use by the FDA or approved in the EU as an in vitro diagnostic assay, nor is it CE marked.




## EXPLANATION of SYMBOLS

Reference EN ISO 15223-1: Medical Devices – symbols to be used with medical device labels, labeling and information to be supplied.

Symbol	Description	Symbol	Description
 <i>ISO 7000 Reg No. 1641</i>	Consult Application Note. An eIFU indicator can be a manufacturer's website URL or some other appropriate indication that the Application Note is available in an electronic format.	 <i>ISO 7000 Reg No. 0518</i>	Contains sufficient for <n> tests
 <i>ISO 7000 Reg No. 2493</i>	Catalog number	 <i>ISO 7000 Reg No. 3082</i>	Manufacturer
 <i>ISO 7000 Reg No. 0434A</i>	Caution	 <i>ISO 7000 Reg No 2497</i>	Date of Manufacture
 <i>ISO 7000 Reg No. 0632</i>	Temperature limitation	 <i>ISO 7000 Reg No 2607</i>	Use by Date
 <i>ISO 7000 Reg No. 0633</i>	Upper limit of temperature	 <i>ISO 7000 Reg No. 2492</i>	Batch code
<b>Other Symbols</b>			
	Irritant (Skin, Eyes)		Carcinogen

The batch field on the label is for traceability of a manufacturing event.

## EXPLANATION of HELPFUL SYMBOLS

Icon	Description
	Safe Stopping Point
	Note
	Useful tip

## REVISION HISTORY

Revision	Date of Issue	Revision Description
01	30 Oct 2020	Initial Release
02	25 May 2021	<ul style="list-style-type: none"> <li>• Update label volume for FASTplex Stop Solution (FAST-STOP)</li> <li>• Clarify language to step in Section F.2.</li> <li>• Updated Eppendorf DNA LoBind Tubes, 2.0 mL CAT number to match supplier provided</li> </ul>
03	19 Aug 2021	<ul style="list-style-type: none"> <li>• Application Note converted to new template layout.</li> <li>• Protocol updated for new product configuration (Lot 003 and greater). See sections for Amplification (Part 1 &amp; 2) and Sample Barcoding (Part 1).</li> </ul>
04	Current	<ul style="list-style-type: none"> <li>• Added best practice recommendations under section: DNA Extraction Methods</li> </ul>