# Illumina TruSight® HLA Sequencing Panel Automated on the Biomek FX$^P$ HLA SP Liquid Handler

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## Introduction

The Human Leukocyte Antigen (HLA) system consists of a collection of 21 genes located on chromosome 6 that display high levels of polymorphism, resulting in over 12,500 HLA alleles characterized in the IPD-IMGT/HLA database as of January 2015.[1] The variability observed in the HLA system allows the immune system to discriminate between host cells, non-host cells, infected cells, and malfunctioning cells. In addition, certain HLA alleles have been shown to be associated with a number of auto-immune disorders including Type 1 Diabetes, rheumatoid arthritis, and inflammatory bowel diseases[2] as well as certain cancers.[3] Therefore, HLA typing has a number of valuable applications, including reducing the risk of transplant rejection by matching transplant recipients with donors that are close HLA matches in addition to providing a potential diagnostic tool for assessing the risk of certain HLA-associated disorders.

TruSight® HLA Sequencing Panel

In this technical note, we describe the automation of the Illumina TruSight® HLA Sequencing Panel on the Beckman Coulter Biomek FX$^P$ HLA SP. An application specific configuration of the Biomek FX$^P$ Dual Mutli-96 Span-8 automated liquid handler (Figure 8). DNA libraries prepared for next-generation sequencing (NGS) using the TruSight® HLA Sequencing Panel enable high-resolution, unambiguous HLA typing with a single assay. Briefly, three Class I HLA loci (HLA-A, -B, and -C) as well as eight Class II HLA loci (HLA-DPA1, -DPB1, -DQA1, -DQB1, and -DRB1/3/4/5) are amplified using locus specific primers. These amplicons, which range from 2.6kb to 10.3kb in size, are normalized, tagmented, and amplified into uniquely indexed libraries for each locus in each genomic DNA sample. Finished libraries are then pooled and loaded onto the Illumina MiSeq® system for sequencing, with the option of using either a single MiSeq® v2 standard flow cell or MiSeq® v2 Nano flow cell, depending on the number of samples per run.

The Biomek FX$^P$ TruSight® HLA automation method standardizes library preparation and significantly reduces manual labor. Up to 192 Illumina TruSight® HLA libraries can be generated from 24 genomic DNA samples in approximately 11 hours. By limiting the need for user interactions, the automated method reduces the incidence of costly pipetting errors and provides for more uniform sample processing. The resulting library pool is ready to load directly onto the MiSeq® Reagent Cartridge while advanced data handling features speed the preparation of the required Sample Sheet by reporting out the indexing i5 and i7 primers associated with each library.

Biomek FXP Dual- Arm Multi-96 and Span-8 Liquid Handler

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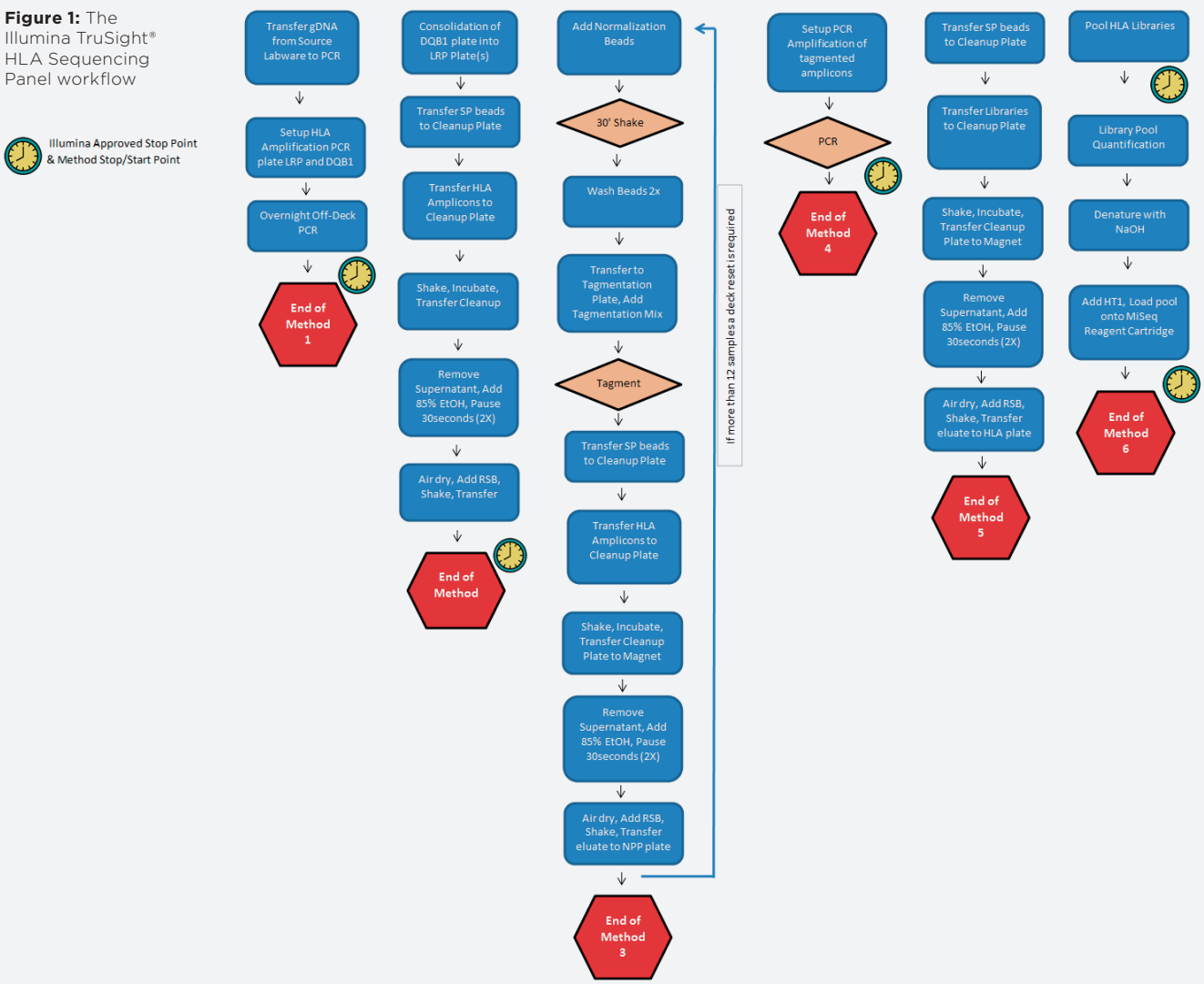
For added convenience, the Biomek FX$^P$ TruSight® HLA automation method is preconfigured to employ fully skirted PCR plates or non-skirted PCR plates to accommodate a range of off-deck thermocyclers. For greater hands-off time, the method also includes the option of utilizing an integrated Biometra TRobot thermocycler for on-deck incubations and thermocycling. The method employs individual Span-8 probes to deliver enzyme and reagent transfers while steps involved in sample cleanups using Sample Preparation Beads, are performed using the multi-channel 96 pipetting head. This provides significant time and consumables savings. On-deck peltier units or chill-blocks ensure automation plates are kept cool during master mix transfers. The method also incorporates all recommended stop points described in the Illumina TruSight® HLA Sequencing Panel Library Preparation Guide to allow users the maximum amount of flexibility in planning their experiments.

## Protocol

The Biomek FX$^P$ TruSight® HLA automation method utilizes the Beckman Coulter Biomek FX$^P$ HLA SP (hereafter Biomek FX$^P$) in conjunction with the Illumina TruSight® HLA Sequencing Panel Library Reference Guide (15056536 Rev. A). Automation consumables, instrument configuration, and other details can be found at the end of this document. The automation method consists of six sub-methods operated by a single HTML-driven User Interface (UI). The Illumina TruSight® HLA Sequencing Panel workflow can be seen in Figure 1. The automation workflow can be seen in Figure 2.

**Figure 1:** The Illumina TruSight® HLA Sequencing Panel workflow



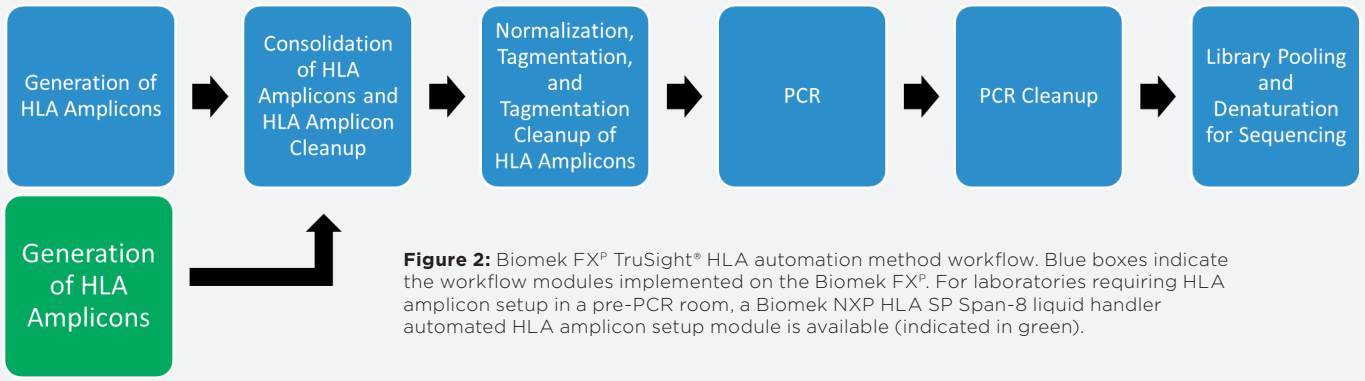



**Figure 2:** Biomek FX$^P$ TruSight® HLA automation method workflow. Blue boxes indicate the workflow modules implemented on the Biomek FX$^P$. For laboratories requiring HLA amplicon setup in a pre-PCR room, a Biomek NXP HLA SP Span-8 liquid handler automated HLA amplicon setup module is available (indicated in green).

The automation method utilizes an HTML-driven UI that offers users a number of different options for customizing their workflow (Figure 3). For scheduling flexibility, the UI also allows the user to select between any one of the six sub-methods that make up the automation method. In each sub-method, the user can select to process any number of genomic DNA samples between 1 and 24. Depending on the sub-method selected and the number of genomic DNA samples being processed, the UI will update to include features specific to the sub-method automatically. For example, when the PCR sub-method is selected, the user is prompted to select which Nextera® XT v2 primer sets (A, B, C, or D) will be used for each Nextera® PCR Plate (NPP). This information is then used not only to populate the HTML-driven reagent calculator, but also to generate two .csv files that record which i5/i7 primer combinations were delivered to each well of the NPP plates. These files can then be used to generate the Sample Sheet for the sequencing run in Illumina Experiment Manager. The PCR sub-method also gives the user the option to perform the library amplification using an on-deck TRobot thermocycler or to use an off-deck thermocycler. Another sub-method with multiple options in the UI can be found in the Normalization, Tagmentation, and Tagmentation Cleanup sub-method, where the tagmentation incubation can be performed either on an on-deck TRobot thermocycler or an off-deck thermocycler. Finally, the Library Pooling sub-method offers the user the choice of whether to pool the libraries or to pool, dilute, and denature the pooled libraries. The number of library pools is defined by the type of MiSeq® flow cell being used (either Version 2 Standard or Version 2 Nano) as outlined in the Illumina TruSight® HLA Sequencing Panel Library Reference Guide. If the user chooses to dilute and denature the resulting library pool(s), the resulting denatured library pool(s) can be loaded directly onto the MiSeq® Reagent Cartridge.

In addition to the UI, the automation method provides an HTML-driven Reagent Calculator (Figure 4) that provides the user with the final volumes of all reagents and master mixes required on the deck as well as instructions on how to generate the various master mixes based upon the number of samples to be processed and which sub-method is selected.

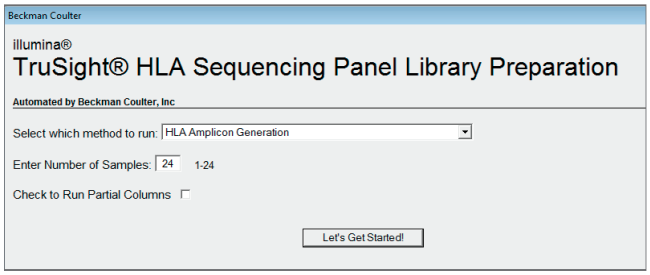



**Figure 3:** Biomek FX$^P$ TruSight® HLA automation method UI.

## Experimental Design and Results

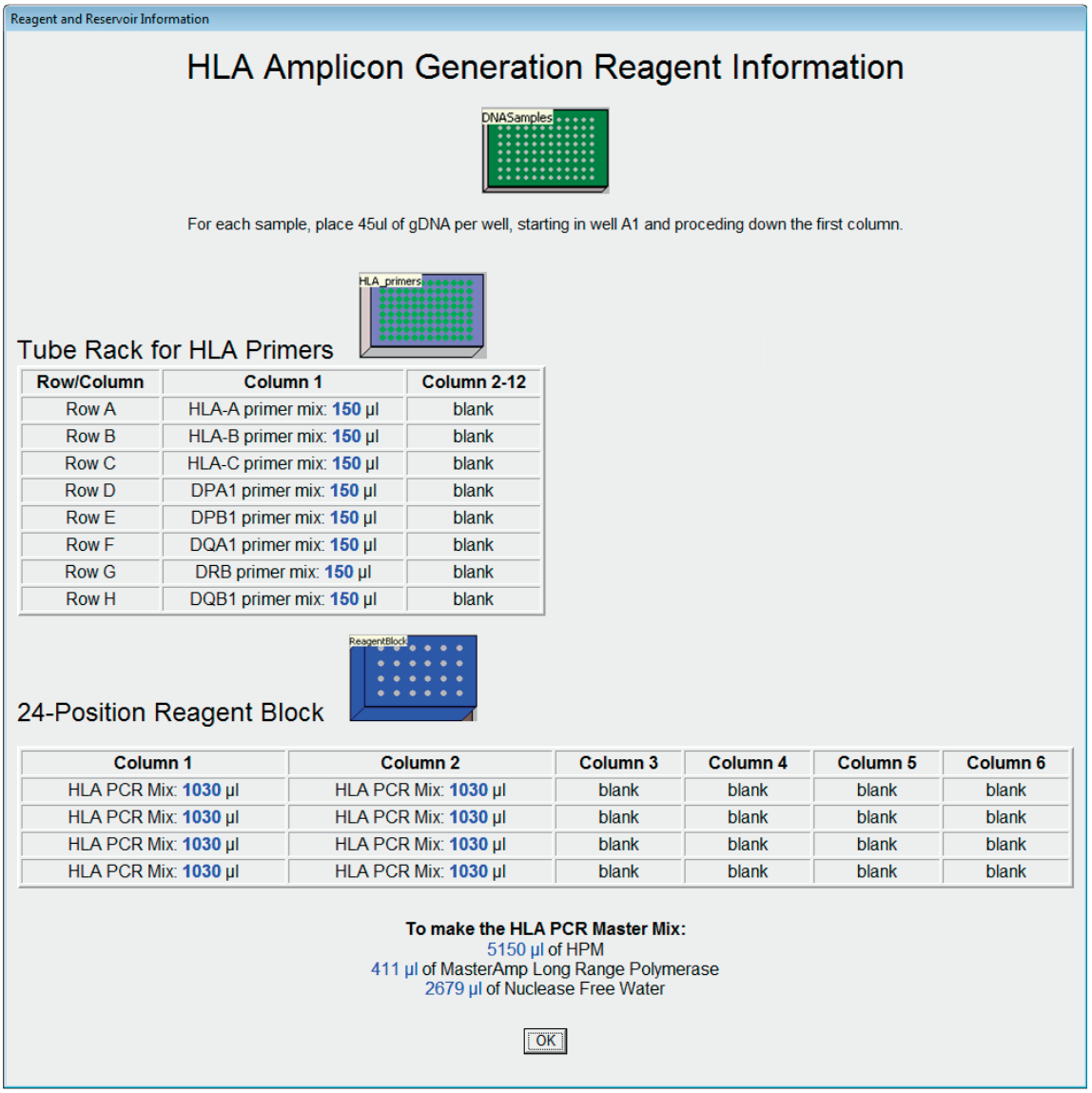
Twenty-three genomic DNA samples obtained from the International Histocompatibility Working Group (IHWG)[4] were quantified using Quant-iT™ Picogreen (Life Technologies), normalized to a final concentration of 10ng/µl, and arrayed in a 96 well BioRad Hard-Shell PCR plate (BioRad). The HLA amplification reactions were set up with the Biomek FX$^P$ using the HLA Amplification sub-method and the HLA amplification reactions were performed in BioRad S1000 thermocyclers. A negative

control sample, ultrapure water, was also included. Following amplification cleanup using the using the HLA Cleanup sub-method, selected samples were assayed using the Bioanalyzer 2100 with a DNA 12000 chip (Agilent), the results of which are presented in Figure 5. The purified HLA amplicons were stored overnight at 4˚C.

Using the Normalization, Tagmentation, and Tagmentation Cleanup sub-method in conjunction with the PCR sub-method, the HLA amplicons were normalized, tagmented, cleaned, and used as template for the library amplification reactions. Thermocycling was performed off-deck in BioRad S1000 thermocyclers and reactions were allowed to hold at 10˚C overnight. The following day, the PCR Cleanup sub-method was used to purify the HLA amplicon libraries. Selected samples were diluted 1:10 in ultrapure water and assayed on the Bioanalyzer 2100 using a DNA High Sensitivity chip (Agilent), the results of which are presented in Figure 6. 192 libraries were pooled, diluted, and denatured using the Library Pooling sub-method and a 2 x 251 paired end sequencing run was performed using a 500-cycle MiSeq® v2 Standard Reagent Kit.

Three 23-sample runs with a negative control sample were performed using the automation method and the workflow described above. FASTQ reads for each run were transmitted to Illumina for analysis using Conexio Assign v1.0.0.719 (Beta 2) (Conexio Genomics) software with default parameters. The sample data

**Figure 4:** Biomek FX$^{P}$ TruSight® HLA automation method reagent calculator for the HLA Amplification sub-method.



generated across the three automated method runs was highly reproducible when compared with the manual sample data generated internally at Illumina as well as with the known HLA types of the IHWG genomic DNA samples. As shown in Figure 7, 99% of the HLA allele typing results generated with the automated method matched HLA typing results generated with the manual preparation of TruSight® HLA libraries as well as with known reference alleles in the 410 alleles that were analyzed. Examination of negative controls showed that all three runs were devoid of contaminating sequences from neighboring wells, indicating that the automation method does not produce sample cross-contamination.

**Figure 5:** Agilent DNA1200 gel image following HLA Amplicon purification.


**Figure 6:** Agilent High Sensitivity gel image following HLA library purification.

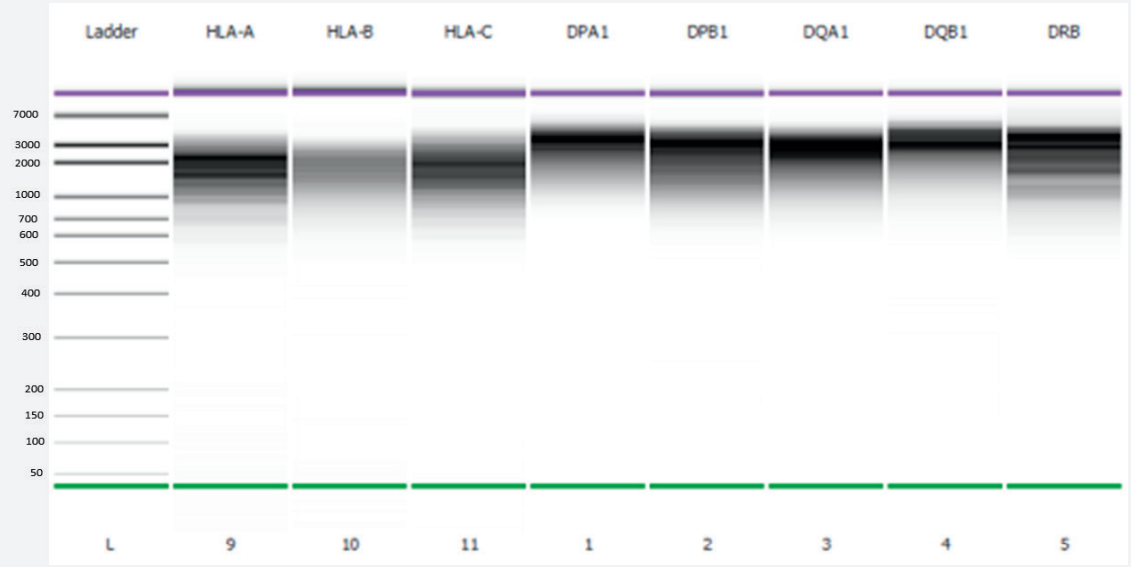



**Figure 7:** Comparison between automated TruSight® HLA for Biomek FX$^P$ automation method and manual TruSight® HLA sequencing data.

| | Total | HLA-A | HLA-B | HLA-C | HLA-DPA1 | HLA-DPB1 | HLA-DQA1 | HLA-DQB1 | HLA-DRB1 | HLA-DRB3 | HLA-DRB4 | HLA-DRB5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Allele Reproducibility | 99% | 99% | 98% | 100% | 100% | 100% | 100% | 100% | 99% | 97% | 96% | 92% |
| Alleles Analyzed | 410 | 46 | 46 | 46 | 46 | 46 | 46 | 42 | 46 | 22 | 16 | 8 |
| Bases Analyzed | 6,822,330 | 486,312 | 369,012 | 463,128 | 1,349,088 | 961,584 | 904,452 | 955,962 | 736,368 | 275,352 | 222,096 | 98,976 |
| Bases Mismatched | 18 | 4 | 8 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 2 |
| Samples Analyzed | 23 | | | | | | | | | | | |

## Conclusion

The Biomek FX$^{P}$ TruSight® HLA automation method streamlines the intensive workflow of the Illumina TruSight® HLA Sequencing Panel, while providing highly reproducible and accurate HLA typing results with a complete sample to sequence workflow.

### References


1. Robinson J, Halliwell JA, Hayhurst JH, Flicek P, Parham P, Marsh SGE. The IPD and IMGT/HLA database: allele variant databases  Nucleic Acids Research (2015) 43:D423-431

2. Fernando MMA, Stevens CR, Walsh EC, De Jager PL, Goyette P, et al. (2008) Defining the Role of the MHC in Autoimmunity: A Review and Pooled Analysis.PLoS Genet 4(4): e1000024. doi: 10.1371/journal.pgen.1000024

3. Madeleine MM, Johnson LG, Smith AG, Hansen JA, Nisperos BB, Li S, et al. Comprehensive analysis of HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 loci and squamous cell cervical cancer risk. Cancer Res (2008) 68(9):3532–910.1158/0008-5472.CAN-07-6471

4. www.ihwg.org


### Software

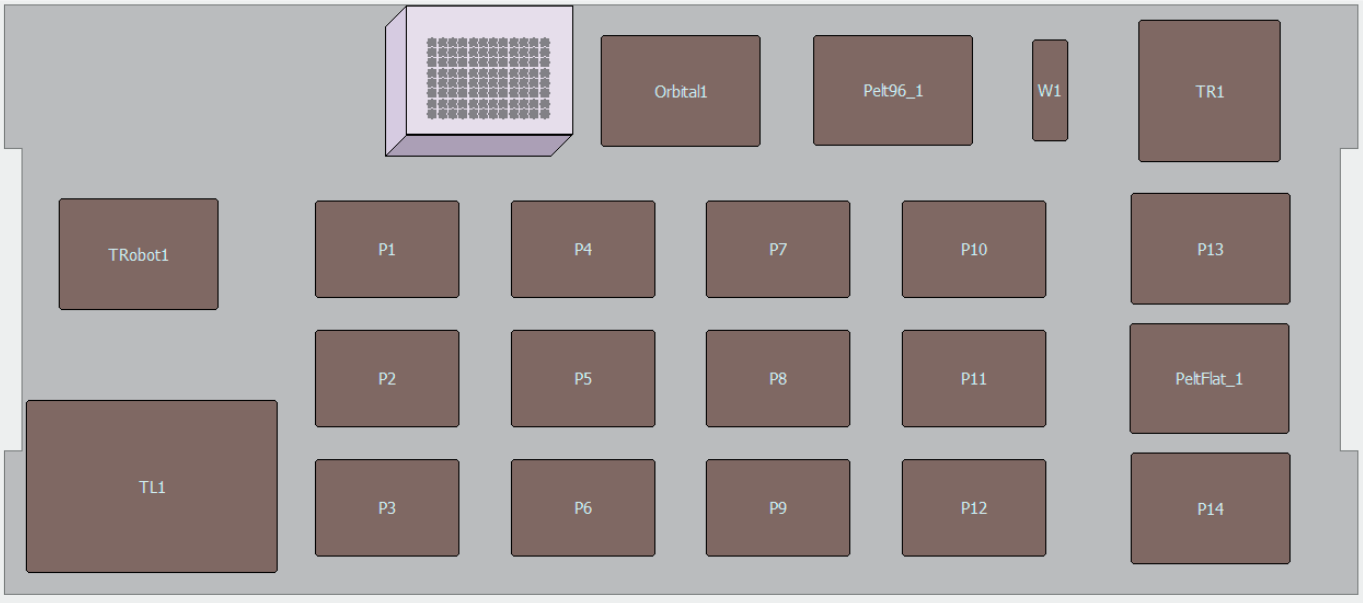
Conexio Assign v1.0.0.719 (Beta 2) www.conexio-genomics.com

## Reagent Details (if applicable)

## ALPs/ Devices Required

| PART NUMBER | QTY | MANUFACTURER | DESCRIPTION |
|---|---|---|---|
| 719948 | 1 | Beckman Coulter, Inc. | ALP, High-Density, 12-Position, 4 x 3 |
| 379448 | 1 | Beckman Coulter, Inc. | ALP, Shaking, Orbital, Single-Position |
| 719357 | 2 | Beckman Coulter, Inc. | ALP, Standard Single-Position |
| A93938* | 2 | Beckman Coulter, Inc. | Static Peltier ALP |
| 719590 | 1 | Beckman Coulter, Inc. | Waste, Span-8, ALP |
| 719356 | 1 | Beckman Coulter, Inc. | Disposable Tip Loader ALP |
| 719654 | 1 | Beckman Coulter, Inc. | ALP, Tip Wash, 8-Channel |
| 719363 | 1 | Beckman Coulter, Inc. | Wash Station including pump and tubes |
| 719366 | 1 | Beckman Coulter, Inc. | Biomek FX Device Controller |
| Contact Beckman Coulter, Inc. | 1 | Biometra | (Optional) Biometra T-Robot for On-Deck incubations |

*Method can be configured to use a chill-block on a 1X1 in place of one Static Peltier ALP.




Deck configuration of the Biomek FX$^{P}$ Dual Arm Multi -96 and Span 8 Liquid Handler.

## Labware Required

| MANUFACTURER PART # | PER 24 SAMPLE RUN | MANUFACTURER | MANUFACTURERS NAME |
|---|---|---|---|
| B01124 | 5 | Beckman | Biomek Span-8 P1000 Tips, Pre-sterile with Barrier |
| 379503 | 15 | Beckman | Biomek Span-8 P250 Tips, Pre-sterile with Barrier |
| A21586 | 15 | Beckman | Biomek P50 Tips, Pre-sterile with Barrier |
| 717256 | 6 | Beckman | Biomek AP96 P20 Tips, Pre-sterile with Barrier |
| 717253 | 8 | Beckman | Biomek AP96 P250 Tips, Pre-sterile with Barrier |
| 372790 | 4 | Beckman | Quarter Reservoir |
| 534681 | 3 | Beckman | Reservoir, Half |
| 372788 | 2 | Beckman | Quarter Reservoir, Divided by Length |
| 372795‡ | 1 | Beckman | Frame for Reservoirs |
| 4900‡ | 1 | ThermoScientific | Empty Latch Racks for 500 µl ScrewTop Tubes |
| A32782‡ | 1 | Beckman | Agencourt® SPRIPlate® 96R - Ring Super Magnet Plate |
| A83054‡ | 1 | Beckman | BCI Tube Block |
| AB-1127 | 8 | Fisher Scientific | Abgene 96-Well Storage Plate, Square Well, 1.2 mL |
| 16466-042 | 14 | VWR | 2mL SuperClear™ Screw Cap Microcentrifuge Tubes - Conical Bottom |
| HSP-9641 | 12 | Bio-Rad | Hard-Shell® Thin-Wall 96-Well Skirted PCR Plates |
| MSL-2022* | 4 | Bio-Rad | Arched Auto-Sealing Lids |
| 4312063**‡ | 4 | Bio-Rad | MicroAmp® Splash-Free 96-Well Base |
| HSS-9641** | 12 | Bio-Rad | Hard-Shell® Thin-Wall 96-Well Non Skirted PCR Plates |
| C5064‡ | 1 | Acme Automation | ReactorAdaptor96Flat |

* Optional: for On-Deck Thermo cycling only **Non-skirted plate option only, not compatible with TRobot ‡ One time purchase

## User-Supplied Consumables

| PART NUMBER | MANUFACTURER | DESCRIPTION |
|---|---|---|
| AB00138-01000 | American Bioanalytical | Ethanol |
| FC-142-1001 | Illumina | Illumina TruSight® HLA Sequencing Panel |
| FC-131-200(1,2,3,4) | Illumina | Nextera® XT Index Kit v2 (Set A, B, C, or D) |
| MS-102-2003 or MS-103-1003 | Illumina | MiSeq® Reagent Kit v2 (500 cycles) or MiSeq® Reagent Nano Kit v2 (500 cycles) |

## Auxiliary Equipment for QC Testing

| PART NUMBER | MANUFACTURER | DESCRIPTION |
|---|---|---|
| G2940CA | Agilent Technologies | Agilent 2100 Bioanalyzer |
| 5067-1508 | Agilent Technologies | Agilent DNA 12000 Kit |
| 5067-4626 | Agilent Technologies | Agilent High Sensitivity DNA Kit |
| SY-410-1003 | Illumina | MiSeq® System |



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