Biomek i7 Hybrid Liquid Handler Automated Method for QIAseq® miRNA UDI Library Kit

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Life Sciences

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QIAseq® miRNA UDI Library Kit

- Qiagen's QIAseq® miRNA UDI Library kit enables Sample to Insight®, precision next-generation sequencing (NGS) of mature miRNAs on Illumina® NGS instruments.
- QIAseq® miRNA UDI Library Kit does not require gel purification, excision, and elution, which substantially reduces the required hands-on time and noticeably shortens the length of the whole workflow.
- The QIAseq® miRNA UDI Library Kit uses mature miRNA and contains the following steps:
    1. 3' ligation
    2. 5' ligation
    3. cDNA synthesis
    4. cDNA cleanup
    5. library amplification
    6. library cleanup

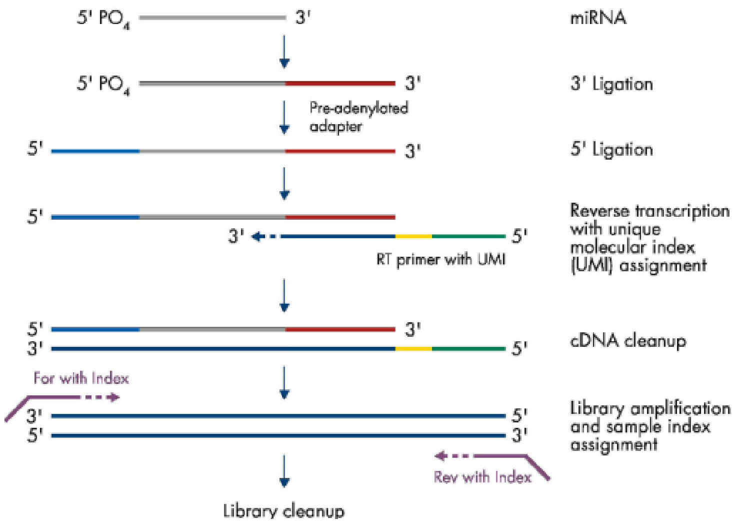


Figure 1: QIAseq® miRNA UDI Library Kit Workflow

Biomek i7 Hybrid Liquid Handler automated method for QIAseq® miRNA UDI Library Kit

Automated Method Description:

- Sample number ranging from 1-96
- On-Deck or Off- Deck Thermocycler
- RNA sample inputs of 500ng, 100ng, 10ng, 1ng, or serum/plasma
- User defined starting well position for UDI index plate
- Optimization of PCR cycles

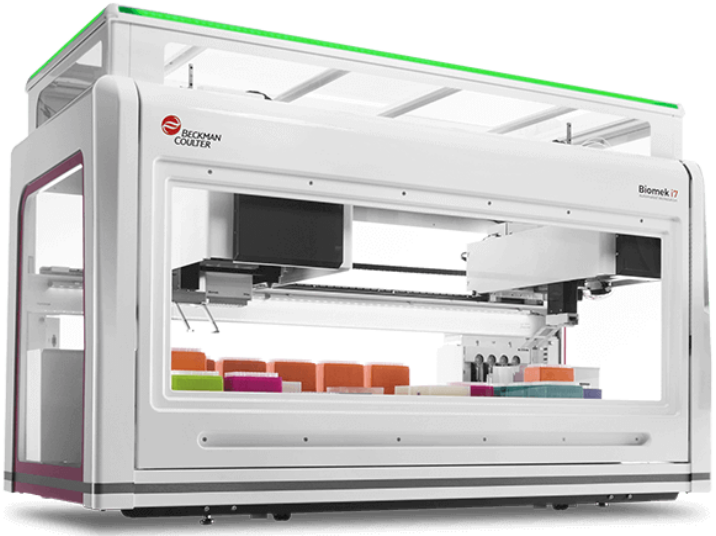


Figure 2: Biomek i7 Hybrid Liquid Handler

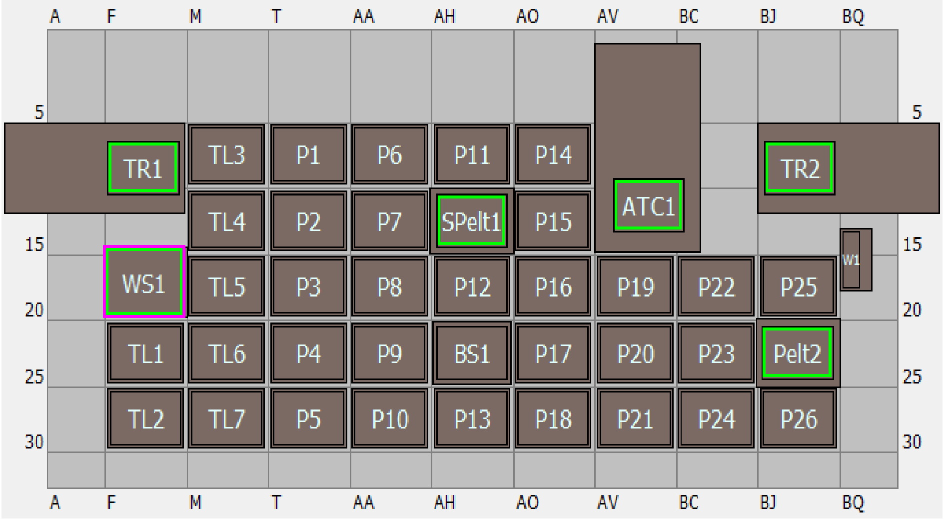


Figure 3: Biomek i7 Hybrid Liquid Handler Deck Layout

Biomek i7 Hybrid Liquid Handler automated method for QIAseq® miRNA UDI Library Kit

Part Number	Qty	Manufacturer	Description
B87585	1	Beckman Coulter	Biomek i7 Hybrid (MC + Span-8) with Enclosure
	1	Beckman Coulter	Pipetting head, MC-96, 5 - 1200 µL
	1	Beckman Coulter	Pipetting head, Span-8, 1mL
C02613	1	Beckman Coulter	Mobile Workstation, i7
719366	2	Beckman Coulter	Device Controller I/O Box
B87483	2	Beckman Coulter	ALP, Trash
C02867	7	Beckman Coulter	ALP, Tip Load 1x1
B87477	23	Beckman Coulter	ALP, Static 1x1
B87478	1	Beckman Coulter	ALP, 1x3 static
A93942	1	Beckman Coulter	ALP, Shaking Peltier Kit
C05041	1	Beckman Coulter	Kit, Integration - Bioshake on i-Series
C02750	2	Beckman Coulter	Vibration isolation mounting plate
A93938	1	Beckman Coulter	ALP, Static Peltier Kit
B87485	1	Beckman Coulter	ALP, Mounting Plate
B87689	1	Beckman Coulter	Wash station, MC-96 pipette tips
719654	1	Beckman Coulter	Wash station, Span-8 pipette tips, Passive
C05021	1	Beckman Coulter	Kit, Integration, Deck - ATC on i-Series
C05753	1	Beckman Coulter	Kit, Installation, i-series Workstation (including Biomek Config Tool Software)
Contact Manufacturer	1	Thermo Fisher	Automated Thermocycler (ATC), 96 well block

Table 1: Biomek i7 Hybrid Liquid Handler Part List

Biomek i7 Hybrid Liquid Handler automated method for QIAseq® miRNA UDI Library Kit

The QIAseq® miRNA UDI Library Kit automation method for the Biomek i7 Liquid Handler can be completed as 2 sections run independently or combined for the complete workflow.

The method utilizes a single user interaction for the guided deck setup and can process up to 96 samples. The completion of the method will result in sequence-ready libraries.

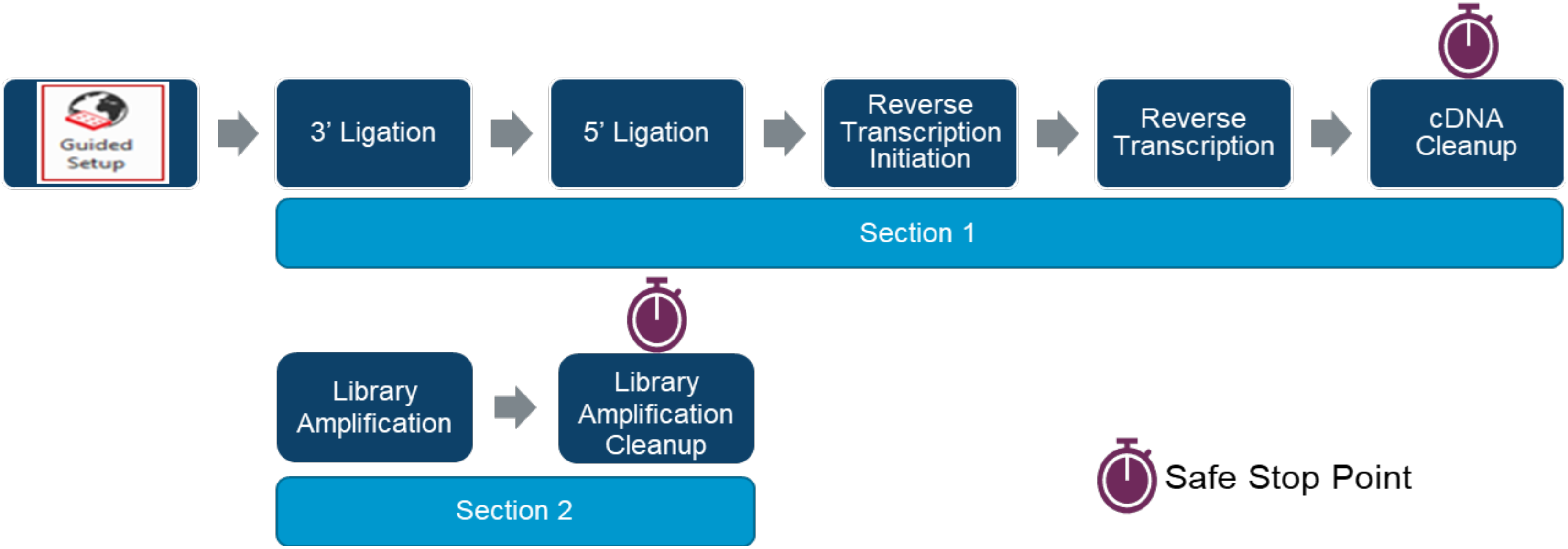


Figure 4: QIAseq® miRNA UDI Library Kit automated workflow for the Biomek i7 Hybrid Liquid Handler

Biomek i7 Hybrid Liquid Handler automated method for QIAseq® miRNA UDI Library Kit

Section Information	Timing for 24 Samples	Timing for 96 Samples
Instrument Setup	10 minutes	15 minutes
Section 1: 3’ Ligation, 5’ Ligation, Reverse Transcriptase, and Cleanup	6 hours	7 hours
Section 2 : Library Amplification and Cleanup	2 hours, 30 minutes	3 hours
Total Run Time	8 hours, 40 minutes	10 hours, 15 minutes
*Total time estimates do not include reagent thawing and preparation. One user interaction is needed for the instrument setup.		

Table 2: QIAseq® miRNA UDI Library Kit automation timing estimates

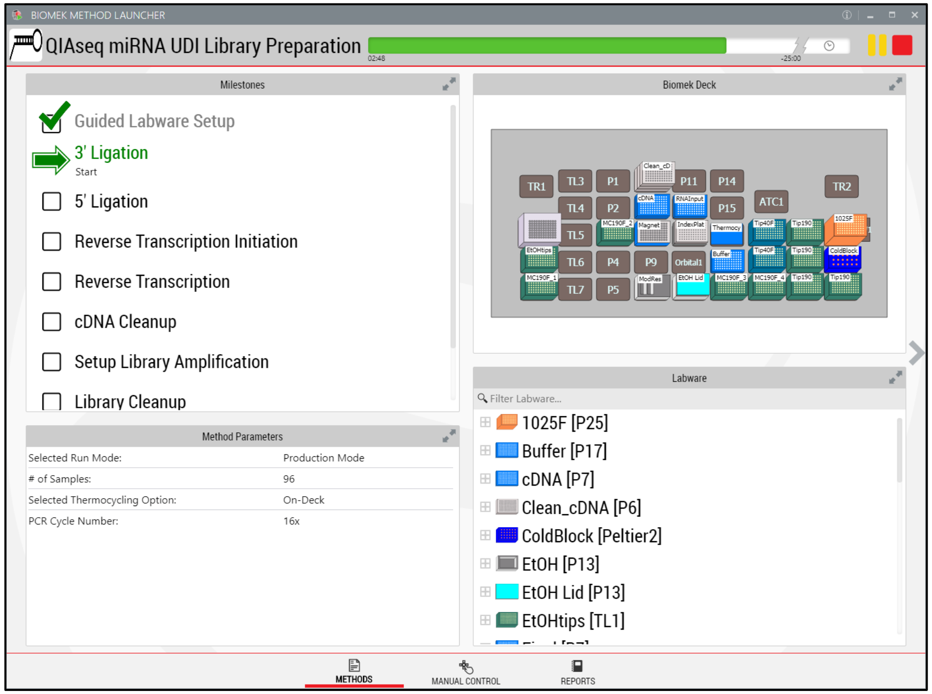
Biomek i7 Hybrid Liquid Handler automated method for QIAseq® miRNA UDI Library Kit

Demonstrated Method Interface

Biomek Method Launcher

1. Makes method available outside of the editor

2. Protects against accidental changes from multiple users



QIAseq miRNA UDI Library Preparation

☑ Guided Labware Setup
3' Ligation
Start
☐ 5' Ligation
☐ Reverse Transcription Initiation
☐ Reverse Transcription
☐ cDNA Cleanup
☐ Setup Library Amplification
☐ Library Cleanup

Selected Run Mode:	Production Mode
# of Samples:	96
Selected Thermocycling Option:	On-Deck
PCR Cycle Number:	16x

1025F [P25]
Buffer [P17]
cDNA [P7]
Clean_cDNA [P6]
ColdBlock [Peltier2]
EtOH [P13]
EtOH Lid [P13]
EtOHtips [TL1]

Figure 5: QIAseq® miRNA UDI Library Kit automated workflow for the Biomek i7 Hybrid Liquid Handler as shown on the Biomek Method Launcher interface

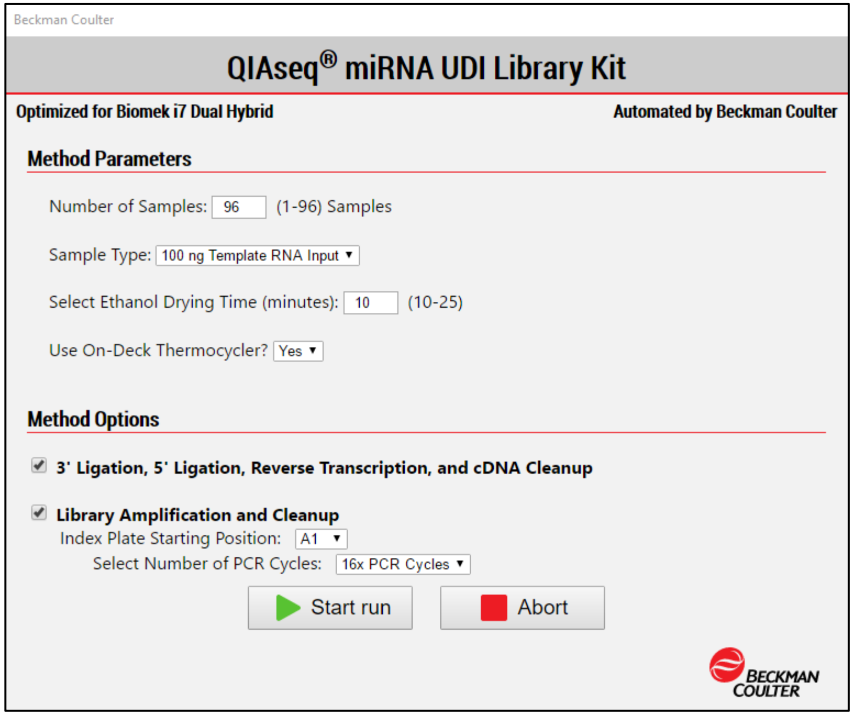
Biomek i7 Hybrid Liquid Handler automated method for QIAseq® miRNA UDI Library Kit

Demonstrated Method Interface

Method Option Selector (MOS)

Dynamic, HTML-driven interface allows the user to select a number of options to configure the workflow as desired.

Full walk away capability from beginning to end of the protocol with multiple restart locations.



Beckman Coulter

QIAseq® miRNA UDI Library Kit

Optimized for Biomek i7 Dual Hybrid

Automated by Beckman Coulter

Method Parameters

Number of Samples: 96 (1-96) Samples

Sample Type: 100 ng Template RNA Input

Select Ethanol Drying Time (minutes): 10 (10-25)

Use On-Deck Thermocycler? Yes

Method Options

☑ **3' Ligation, 5' Ligation, Reverse Transcription, and cDNA Cleanup**

☑ **Library Amplification and Cleanup**

Index Plate Starting Position: A1

Select Number of PCR Cycles: 16x PCR Cycles

Figure 6: QIAseq® miRNA UDI Library Kit automated workflow for the Biomek i7 Hybrid Liquid Handler Method Option Selector

Biomek i7 Hybrid Liquid Handler automated method for QIAseq® miRNA UDI Library Kit

Demonstrated Method Interface

MOS options that enable flexibility in configuring a run:

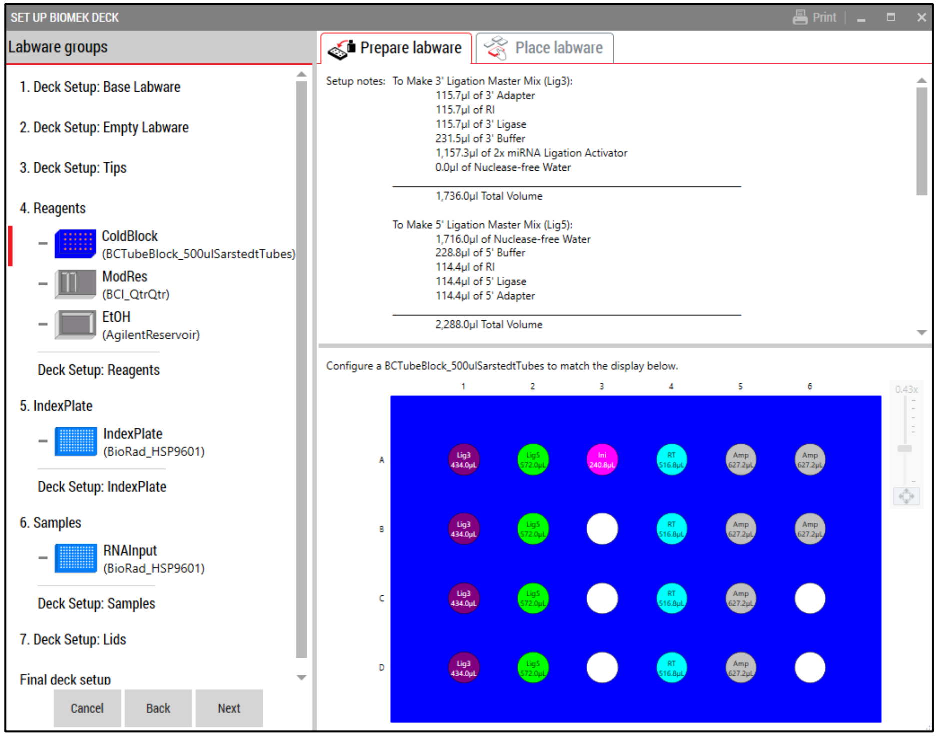
1. **Number of Samples:** Allows the user to manually enter the number of samples (1-96) to be processed.
2. **Sample Type:** Options include 500ng, 100ng, 10ng, 1ng, or serum/plasma.
3. **Ethanol Drying Time**: User can define the drying time for Ethanol depending on lab conditions. Options range from 10-25 minutes.
4. **Use On-Deck Thermocycler**: Results in a drop-down menu of either "yes" or "no" to match the deck layout of the user's Biomek i7 hybrid Automated Workstation.
5. **Index Plate Starting Position**: Drop-down menu that allows the user to specify the starting location within the 96-well Index plate.
6. **Select Number of PCR Cycles:** Allows the user to set the number of PCR cycles run for Library Amplification. The number of cycles are supported from 12-26.

Biomek i7 Hybrid Liquid Handler automated method for QIAseq® miRNA UDI Library Kit

Demonstrated Method Interface

Guided Labware Setup (GLS) and Reagent Calculation

- Provides clear instructions for setup of labware
- Provides calculated volumes of reagents based on the number of samples processed



Labware groups

1. Deck Setup: Base Labware
2. Deck Setup: Empty Labware
3. Deck Setup: Tips
4. Reagents
    - ColdBlock (BCTubeBlock_500ulSarstedtTubes)
    - ModRes (BCI_QtrQtr)
    - EtOH (AgilentReservoir)
    - Deck Setup: Reagents
5. IndexPlate
    - IndexPlate (BioRad_HSP9601)
    - Deck Setup: IndexPlate
6. Samples
    - RNAInput (BioRad_HSP9601)
    - Deck Setup: Samples
7. Deck Setup: Lids

Final deck setup

Setup notes: To Make 3' Ligation Master Mix (Lig3):
115.7µl of 3' Adapter
115.7µl of RI
115.7µl of 3' Ligase
231.5µl of 3' Buffer
1,157.3µl of 2x miRNA Ligation Activator
0.0µl of Nuclease-free Water

1,736.0µl Total Volume

To Make 5' Ligation Master Mix (Lig5):
1,716.0µl of Nuclease-free Water
228.8µl of 5' Buffer
114.4µl of RI
114.4µl of 5' Ligase
114.4µl of 5' Adapter

2,288.0µl Total Volume

Configure a BCTubeBlock_500ulSarstedtTubes to match the display below.

	1	2	3	4	5	6
A	Lig3 434.0µL	Lig5 572.0µL	Ini 240.8µL	RT 516.8µL	Amp 627.2µL	Amp 627.2µL
B	Lig3 434.0µL	Lig5 572.0µL		RT 516.8µL	Amp 627.2µL	Amp 627.2µL
C	Lig3 434.0µL	Lig5 572.0µL		RT 516.8µL	Amp 627.2µL	
D	Lig3 434.0µL	Lig5 572.0µL		RT 516.8µL	Amp 627.2µL	

Figure 7: Guided Labware Setup for QIAseq® miRNA UDI Library Kit on the Biomek i7 Hybrid Liquid Handler

Biomek i7 Hybrid Liquid Handler automated method for QIAseq® miRNA UDI Library Kit

Automation runs were performed using 24 technical replicates of Human XpressRef Universal Total RNA (Qiagen, 338112) at 100 ng and 10 ng. Following library preparation, the libraries were analyzed using High Sensitivity D1000 ScreenTapes (Agilent, 5067-5584, 5067-5585) on the Agilent TapeStation Instrument for library size, concentration, and quality.

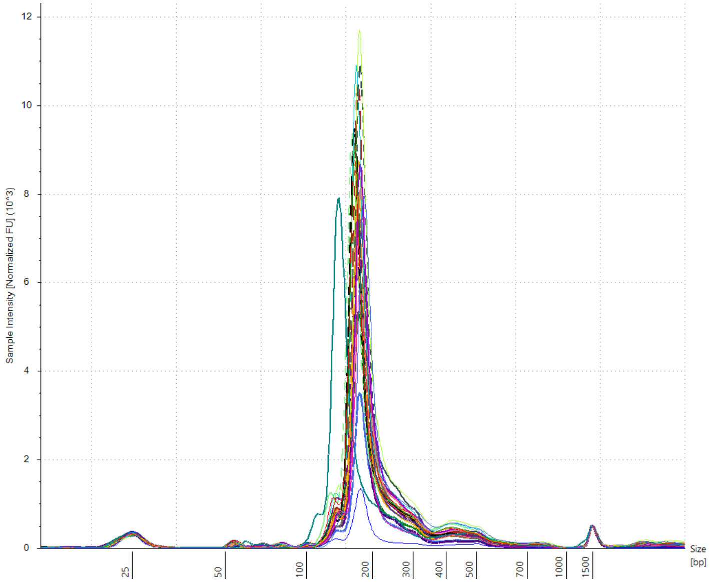


Figure 8: XpressRef 100ng libraries from QIAseq® miRNA UDI Library Kit on the Biomek i7 Hybrid Liquid Handler

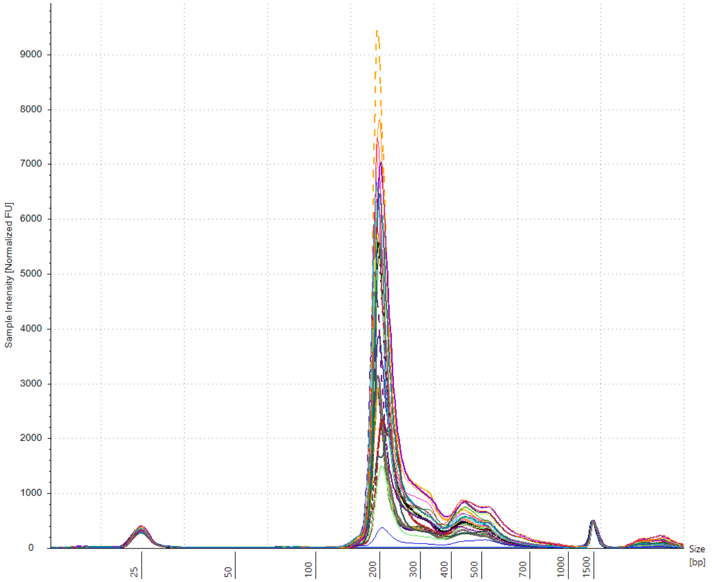


Figure 9: XpressRef 10ng libraries from QIAseq® miRNA UDI Library Kit on the Biomek i7 Hybrid Liquid Handler

Biomek i7 Hybrid Liquid Handler automated method for QIAseq® miRNA UDI Library Kit

High Sensitivity DNA analysis (Agilent TapeStation) of 24 technical replicates of Human XpressRef Universal Total RNA (Qiagen, 338112) was performed. The 100 ng libraries demonstrated an average library size of 177bp and average concentration of 6685 pg/µL. The 10 ng libraries demonstrated an average size of 201bp and average concentration of 4689 pg/µL.

Sample Type	Average Size (bp)	Average Concentration (pg/µL)
XpressRef (100 ng)	177	6685
XpressRef (10 ng)	201	4689

Table 3: Average size and concentration of 100ng and 10ng QIAseq® miRNA UDI libraries from the Biomek i7 Hybrid automated method.

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