SHORT PROTOCOL No. 16 I September 2016

Automated Illumina® TruSeq® Rapid Exome Library construction with the ep*Motion*® 5075t

Introduction

This protocol describes the configuration and preprogrammed methods for the automated construction of 8-48 sequencing libraries from 50 ng DNA input with the Illumina TruSeq Rapid Exome Library Preparation kit. It allows the construction of sequencing ready libraries in approximately three days. The total time varies depending on the number of samples, quality controls and quantifications performed during the protocol. The workflow is divided into five methods to allow best possible walk away time. If necessary, intermediate products from the individual methods can be stored according to the Illumina kit user guide.

Material and Methods

Required equipment

> ep*Motion* 5075t
> Additional Thermal module (Position C2)
> Gripper
> TS50 pipetting tool
> TS300 pipetting tool
> TM50-8 pipetting tool
> TM300-8 pipetting tool
> 4x Thermoadapter for PCR plates, 96-well
> ReservoirRack
> 2x Reservoir rack Module TC Safe-Lock Tubes
> 1x Reservoir rack Module TC for Eppendorf Tubes® 5.0 mL
> Alpaqua® MAGNUM FLX® Enhanced Universal Magnet Plate (Alpaqua order no. A000400)

Required consumables

> epT.I.P.S.® Motion 50 µL Filter
> epT.I.P.S. Motion 300 µL Filter
> Eppendorf twin.tec® PCR plates, 96-well, Eppendorf LoBind®, semi-skirted
> Eppendorf twin.tec PCR plates, 96-well, Eppendorf LoBind, skirted (for the Index Adapters)
> Eppendorf Protein LoBind Tube 1.5 mL
> Eppendorf DNA LoBind Tube 1.5 mL
> ep*Motion* Reservoir 30 mL
> Eppendorf 400 mL Reservoir
> 80 % Ethanol
> RNase free water
> Mineral oil, PCR/molecular biology grade (Sigma-Aldrich®, order no. M5904-500ML)
> Illumina TruSeq Rapid Exome Library Prep kit

Methods

Method Name	approx. Runtime (24 samples)
1-TSRapidExome-V01.export3	2 hours 15 minutes
2-TSRapidExome-V01.export3	1 hour
	(4 pools of 12 samples =12-plex)
3-TSRapidExome-V01.export3	6 hours
4-TSRapidExome-V01.export3	6 hours 40 minutes
5-TSRapidExome-V01.export3	1 hour 20 minutes

In close exchange with Illumina, the following modifications of the TruSeq Rapid Exome Library Prep protocol (TruSeq Rapid Exome Library Prep Reference Guide 1000000000751 v00) were introduced:

The first modifications are present in the tagmented DNA cleanup step. A single size selection step is performed instead of a double one. On page 12 of the TruSeq Rapid Exome Library prep Illumina instruction manual, the volume of step 2 is modified from 52 µL to 65 µL. It continues with step 3 and 4 as listed, steps 5 to 8 are removed and it follows with step 9 through completion of protocol.
The next modifications are linked to the hybridization steps. The Enrichment hybridization Buffer 1 (EHB1) volume in 1st and 2nd hybridization is increased from 7.5 µL to 15.2 µL. 15.2 µL are added to the Sample Purification Beads and 15 µL removed. The Enrichment hybridization Buffer 2 (EHB2) volume in 1st and 2nd hybridization is increased from 2.5 µL to 5 µL. The times of the 1st and 2nd hybridization step are increased to 120 minutes instead of 30 minutes.
The entire workflow is divided into five ep*Motion* methods. Each of the methods ends at a "Safe Stopping Point", allowing storage of the intermediate products as stated in the kit's user guide. The steps of the protocol covered by the ep*Motion* and the number of samples processed in each method are listed in table 1.
This approach is programmed to provide as much automation as possible; a maximum of 48 samples can be processed in iteration of 8 (8, 16, 24, 32, 40, or 48) for the steps of the protocol before the hybridizations (methods 1 and 2). Other sample numbers are not supported due to the use of the 8-channel tools up to this step. From the first hybridization step to the end of the protocol (methods 3, 4 and 5), the maximum number of samples that can be processed is 12 as

Table 1: TruSeq Rapid Exome Library Prep protocol steps performed by the ep*Motion* and maximum number of samples processed in each method

Protocol steps	ep*Motion* methods	Sample number
gDNA Quantification	Off deck	8 - 48
Tagmention, Clean up and First PCR Amplification Setup	Method 1	
First PCR Amplification	Off deck	
First Clean up	Method 2	
Library Quantification and Pool	Off deck	
First Hybridization and First Capture	Method 3	1 - 12
Second Hybridization, Capture and Clean up	Method 4	
Second PCR Setup	Method 5	
Second PCR Amplification	Off deck	
Second PCR Cleanup	Method 5	

libraries are pooled. In methods 3, 4 and 5, the sample number is flexible from 1 to 12 as the single channel tools are used. The library quantification and pooling are non-automated steps. The PCR amplification programs are run on a thermal cycler outside the ep*Motion.*
To reduce dead volumes, some Illumina kit reagents – in particular the enzyme mixes - must be provided in 1.5 mL tubes or 5.0 mL tubes. The reagents containing proteins such as enzymes are placed in Eppendorf Protein LoBind Tubes and the other reagents in Eppendorf DNA LoBind Tubes. The remaining reagents, Sample Purification Beads, mineral oil, Resuspension Buffer and Ethanol (80 %) are provided in 30 mL reservoirs to allow 8-channel pipetting. All liquid waste is collected in a 400 mL reservoir in Position B0. As most of the used volumes are very low, all reagents must be checked for absence of foam, air bubbles etc. to ensure best performance prior to starting the runs. For some of the reagents, the beads and the mineral oil, it is mandatory to equilibrate at room temperature to ensure proper function and pipetting behavior due to changes in viscosity.
All steps of the procedure are performed in Eppendorf twin.tec PCR Plates LoBind, semi-skirted. For incubation steps above 37 °C, samples are overlaid with oil to allow heating on the ep*Motion* without evaporation.
The amount of epT.I.P.S. Motion on the worktable corresponds to the number needed to process the maximum number of samples.
The output plate containing the samples of each method will be placed on the C2 position (Temp2) set to 10 °C at the end of the individual methods.

Method 1

Method 1 is programmed for a maximum of 48 samples (8- 48 samples). It includes the genomic DNA tagmentation, the tagmented DNA cleanup and amplification setup. To start, transfer 10 µL of 5 ng/µL DNA per sample into the first six columns (Wells A1-H6) of an Eppendorf twin.tec PCR Plate LoBind, semi-skirted labeled "Input Samples". Place the Input Samples plate on the C2 (Temp2) position.
The Index Adapters must be transferred into an Eppendorf twin.tec PCR Plate LoBind, semi-skirted. Depending on the sample number, sequencing setup, pooling scheme etc., the number, combination and pattern of the Index Adapters in the Adapter plate (position C4) need to be modified. The Adapter plate must be kept at 4 °C at the start of the method. A user intervention is added to place it on the ep*Motion* (position C4) just before use.
For stability reasons, prepare the required volume of the Library Amplification Mix (LAM) in a 1.5 mL tube and keep it at 4 °C at the start of the method. A user intervention is present to place it in the 1D position of the reservoir rack just before use.
Another user intervention allows taking 1 µL of the cleaned up, tagmented DNA to a new tube in order to run it on a fragment analysis system such as the Agilent® Technologies 2100 BioAnalyzer® as an intermediate QC checkpoint. This method ends with the tagmented DNA amplification mix in the first six columns (A1-H6) of a second Eppendorf twin.tec PCR Plate LoBind, semi-skirted labeled PCR Setup at position C2/Temp2. This plate must be sealed, mixed and centrifuged before being placed in a Thermocycler to run the LAM AMP program. This plate must be used in method 2 when the LAM AMP program is finished.
If you are stopping here, keep the plate sealed after amplification and store at 2 °C to 8 °C for up to 2 days. Alternatively, leave on the thermal cycler overnight.

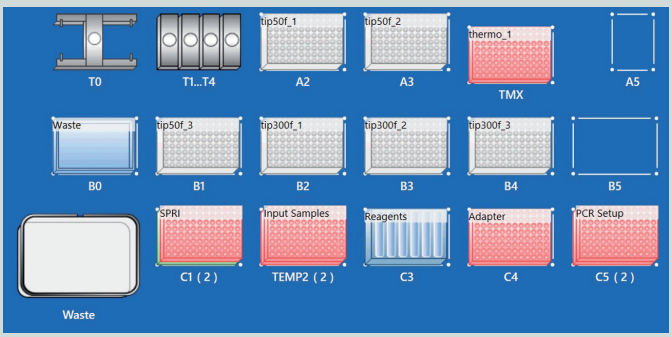

Figure 1: ep*Motion* worktable layout for method 1

Position	Item
A2	50 µL Filtertips
A3	50 µL Filtertips
A4 (TMX)	Thermoadapter PCR 96
B0	400 mL tub for liquid waste
B1	50 µL Filtertips
B2	300 µL Filtertips
B3	300 µL Filtertips
B4	300 µL Filtertips
C1	Alpaqua MAGNUM FLX Enhanced Universal Magnet Plate + empty PCR plate (labeled SPRI)
C2 (Temp2)	Thermoadapter PCR 96 + PCR plate with DNA samples (labeled Input Samples)
C3	ReservoirRack with 1x RR Module Safe Lock + 4x 30 mL Reservoir for reagents
C4	Skirted PCR plate with Index Adapters → review method programming
C5	Thermoadapter PCR 96 + empty PCR plate (labeled PCR Setup)

ReservoirRack Layout

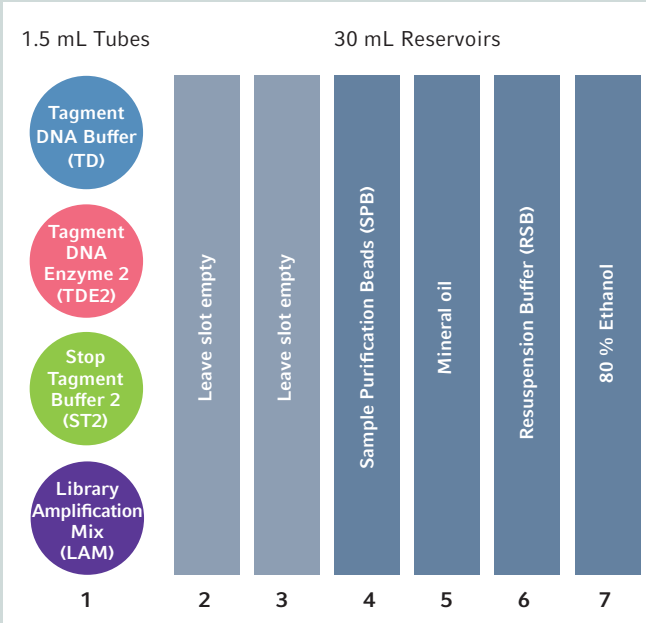

Figure 2: Reservoir rack layout for method 1

Method 2

Method 2 is programmed for a maximum of 48 samples (8-48 samples). It includes the amplified DNA cleanup.
Start with the PCR plate labeled PCR Setup containing the amplified tagmented DNA samples from method 1 in wells A1 – H6, placed on position A4 (TMX) of the ep*Motion* worktable.
This method ends with the cleaned up, amplified DNA in the first six columns (A1-H6) of a second Eppendorf twin.tec PCR Plate LoBind, semi-skirted labeled Hybridization, on position C2 (Temp2).
The libraries must then be quantified using a fluorometric method before pooling. The quantification and the pooling of the libraries steps are not part of the automation – so please refer to the kit user guide for further details.
Optionally, the library can be quality controlled on a fragment analyzer.
If you are stopping here, seal the plate and store at -25 °C to -15 °C for up to 14 days.

Worktable layout

Position	Item
A2	50 µL Filtertips
A3	50 µL Filtertips
A4 (TMX)	Thermoadapter PCR 96 + PCR plate with DNA (PCR Setup) from method 01
B0	400 mL tub for liquid waste
B2	300 µL Filtertips
B3	300 µL Filtertips
C1	Alpaqua MAGNUM FLX Enhanced Universal Magnet Plate
C2 (Temp2)	Thermoadapter PCR 96
C3	ReservoirRack with 3x 30 mL Reservoir
C4	Thermoadapter PCR 96 + empty PCR plate (labeled Hybridization)

Figure 3: ep*Motion* worktable layout for method 2

ReservoirRack Layout

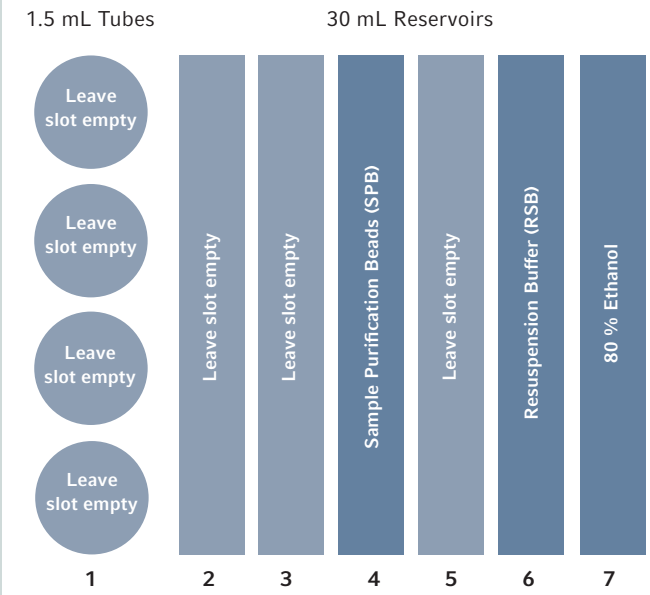

Figure 4: Reservoir rack layout for method 2

Method 3

Method 3 is programmed for 1-12 samples = pools, depending on the desired plexity for sequencing. It includes the first round of hybridization and capture steps.
Start with up to 12 pools of DNA libraries in wells A1-D2 of an Eppendorf twin.tec PCR Plate LoBind, semi-skirted labeled Hybridization. Place the Hybridization plate on the C2 (Temp2) position.
It is recommended to prepare the required volume of the solutions listed in parentheses (Streptavidin Magnetic beads (SMB); 2N NaOH (HP3); Enrichment Elution Buffer (EE1); Elute target Buffer (ET2); Enhanced Enrichment Wash Solution (EEW); Resuspension Buffer (RSB)) in individual 1.5 mL tubes and keep them at 4 °C at the start of the method. A user intervention is added to place it on the ep*Motion* in the reservoir rack just before use. Please be aware, the Elution Premix is prepared freshly by the ep*Motion* by mixing the Enrichment Elution Buffer (EE1) and 2N NaOH (HP3) reagents. In the default method, it is set for 12 samples. Depending on the sample number processed, the volume of the 2 components of the Elution Premix and the mixing volume must be modified each time this method is executed. The recommended volumes of EE1 and HP3 solutions and the mixing volume are listed in Table 2 according to the number of samples processed.

Table 2: Volumes of EE1 and HP3 solutions and mixing volumes recommended according to the number of samples processed

Sample number	HP3 volume (µL)	EE1 volume (µL)	Mix volume (µL)
1	5	86	70
2	5	86	70
3	5	86	70
4	6	108	70
5	7	130	100
6	8	152	100
7	9	174	100
8	10	196	100
9	11	218	150
10	13	240	150
11	14	262	200
12	15	284	200

This method ends with the first captured library samples in twelve wells (A3-D4) of an Eppendorf twin.tec PCR Plate LoBind, semi-skirted labeled Capt Hyb Probe, at position C2 (Temp2). This plate must be used in method 4. If you are stopping here, seal the plate and store at -25 °C to -15 °C for up to 7 days.

Worktable layout

Position	Item
A2	50 µL Filtertips
A3	50 µL Filtertips
A4 (TMX)	Thermoadapter PCR 96
B0	400 mL tub for liquid waste
B2	300 µL Filtertips
B3	300 µL Filtertips
C1	Alpaqua MAGNUM FLX Enhanced Universal Magnet Plate
C2 (Temp2)	Thermoadapter PCR 96 + PCR plate with the samples (Hybridization)
C3	ReservoirRack with 2x RR Module Safe Lock + 1x RR Module 5.0 mL + 3x 30 mL Reservoir
C4	Thermoadapter PCR 96 + empty PCR plate (labeled Capt Hyb Probe)

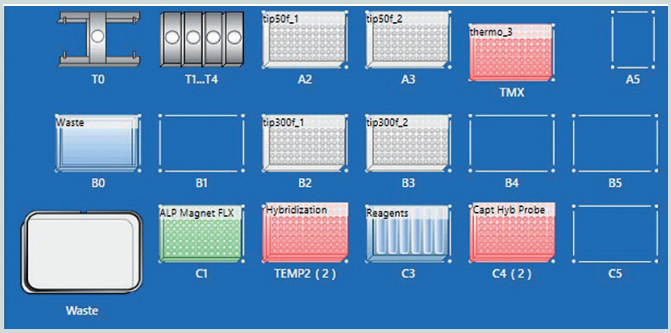

Figure 5: ep*Motion* worktable layout for method 3

ReservoirRack Layout

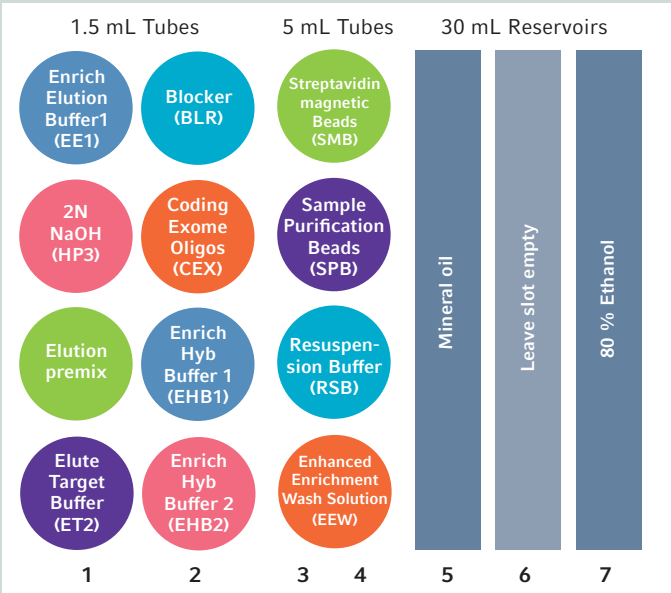

Figure 6: Reservoir rack layout for method 3

Method 4

Method 4 is programmed for a maximum of 12 samples (1-12 samples). It includes the second round of hybridization, capture and the cleanup of the captured libraries.
Start with the 30 µL of DNA library pools from method 3 in up to twelve wells (A3-D4) of an Eppendorf twin.tec PCR Plate LoBind, semi-skirted labeled Capt Hyb Probe. Place the Capt Hyb Probe plate on the C2 (Temp2) position.
It is recommended to prepare the needed volume of the solutions listed in parentheses (Streptavidin Magnetic beads (SMB); 2N NaOH (HP3); Enrichment Elution Buffer (EE1); Elute target Buffer (ET2); Enhanced Enrichment Wash Solution (EEW); Resuspension Buffer (RSB)) in a 1.5 mL tube and keep it at 4 °C at the start of the method. A user intervention is added to place it on the epMotion in the reservoir rack just before use.
Please be aware, the Elution Premix is prepared freshly by the ep*Motion* by mixing the Enrichment Elution Buffer (EE1) and 2N NaOH (HP3) reagents. In the default method, it is set for 12 samples. Depending on the sample number processed, the volume of the 2 components of the Elution Premix must be modified. The recommended volumes of EE1 and HP3 solutions and the mixing volume are listed in Table 2 according to the number of samples processed.
This method ends with the second capture library samples in twelve wells (A1-D2) of an Eppendorf twin.tec PCR Plate LoBind, semi-skirted labeled PCR, at position C2 (Temp2). This plate must be used in method 5.
If you are stopping here, seal the plate and store at -25 °C to -15 °C for up to 7 days.

Worktable layout

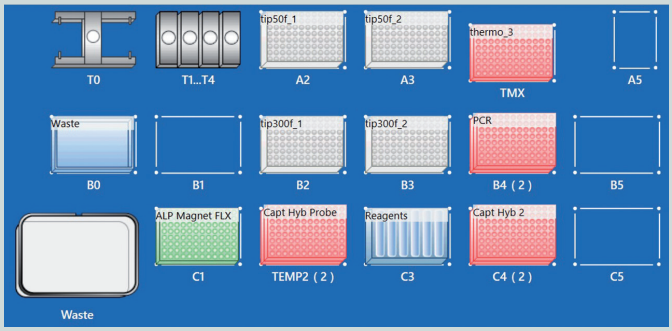

Figure 7: ep*Motion* worktable layout for method 4

Position	Item
A2	50 µL Filtertips
A3	50 µL Filtertips
A4 (TMX)	Thermoadapter PCR 96
B0	400 mL tub for liquid waste
B2	300 µL Filtertips
B3	300 µL Filtertips
B4	Thermoadapter PCR 96 + empty PCR plate (labeled PCR)
C1	Alpaqua MAGNUM FLX Enhanced Universal Magnet Plate
C2 (Temp2)	Thermoadapter PCR 96 + PCR plate with the samples (labeled Capt Hyb Probe)
C3	ReservoirRack with 2x RR Module Safe Lock + 1x RR Module 5.0 mL + 3x 30 mL Reservoir
C4	Thermoadapter PCR 96 + empty PCR plate (labeled Capt Hyb 2)

ReservoirRack Layout

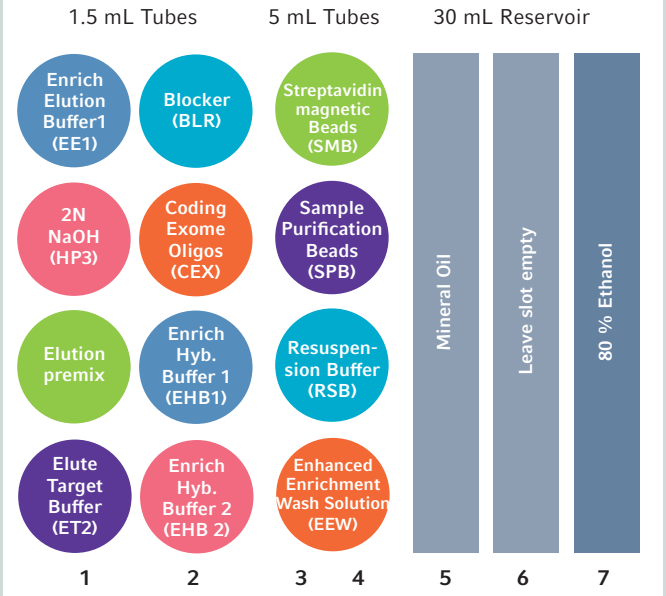

Figure 8: Reservoir rack layout for method 4

Method 5

Method 5 is programmed for a maximum of 12 samples (1-12 samples). It includes the enriched library amplification and cleanup steps.
Start with the 25 µL of DNA library sample from method 4 in twelve wells (A1-D2) of an Eppendorf twin.tec PCR Plate LoBind, semi-skirted labeled PCR. Place the PCR plate on the C2 (Temp2) position.
Only the PCR setup will be pipetted, which takes a few minutes, then a user intervention occurs where the PCR plate needs to be sealed and cycled in a PCR cycler to enrich the libraries using the AMP10 program. Reopen the plate after PCR and return to the C2 (Temp2) position prior to continuing the method.
This method ends with 30 µL of the final libraries in twelve wells (A1-D2) of a an Eppendorf twin.tec PCR Plate LoBind, semi-skirted labeled Amp Enr Lib, at position C2 (Temp2).
If you are stopping here, seal the plate and store at -25 °C to -15 °C for up to 7 days.

Worktable layout

Position	Item
A2	50 µL Filtertips
A4 (TMX)	Thermoadapter PCR 96
B0	400 mL tub for liquid waste
B2	300 µL Filtertips
C1	Alpaqua MAGNUM FLX Enhanced Universal Magnet Plate
C2 (Temp2)	Thermoadapter PCR 96 + PCR plate with the samples (PCR)
C3	ReservoirRack with 2x RR Module Safe Lock + 1x RR Module 5.0 mL + 4x 30 mL Reservoir
C4	Thermoadapter PCR 96 + empty PCR plate (labeled Amp Enr Lib)

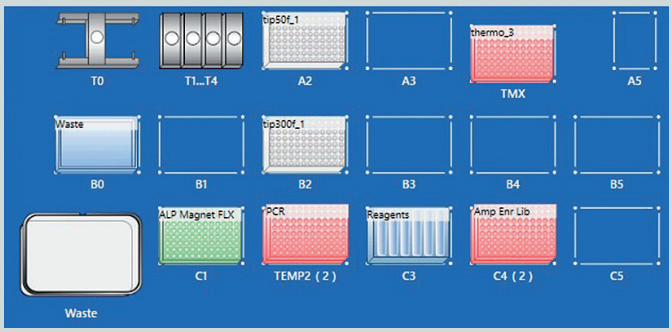

Figure 9: ep*Motion* worktable layout for method 5

ReservoirRack Layout

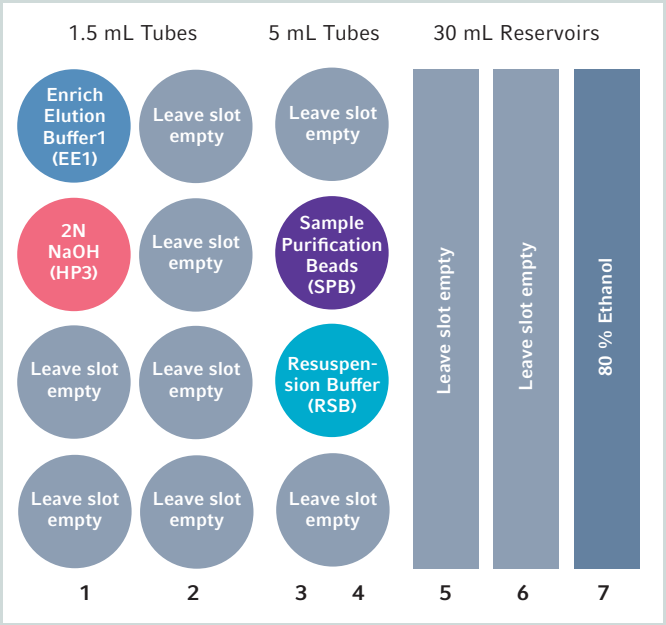

Figure 10: Reservoir rack layout for method 5

Final quantification of TruSeq Rapid Exome Sample Prep libraries is recommended to be done via qPCR or a fluorometric dsDNA assay.

Results

Typically the libraries will be quality controlled at different steps of the process on an Agilent Technologies 2100 BioAnalyzer or similar. Typical examples of BioAnalyzer results for post-tagmentation, post-PCR-pre-enriched and post-enriched libraries generated with the ep*Motion* are shown in figures 11-13.

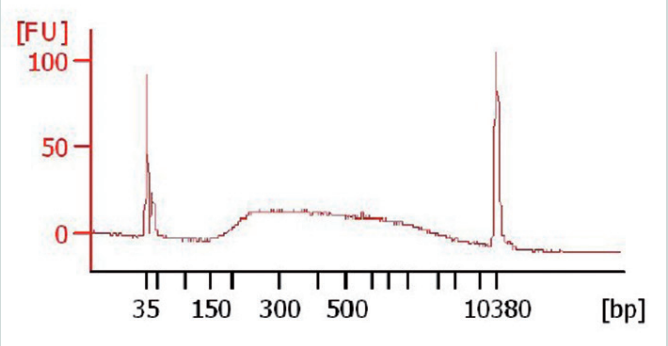

Figure 11: Example of a 2100 BioAnalyzer electropherogram of a post-tagmentation library distribution using the TruSeq Rapid Exome library prep kit automated on the ep*Motion*. 1 µL of the undiluted post-tagmentation library was analyzed with the High Sensitivity DNA Kit.

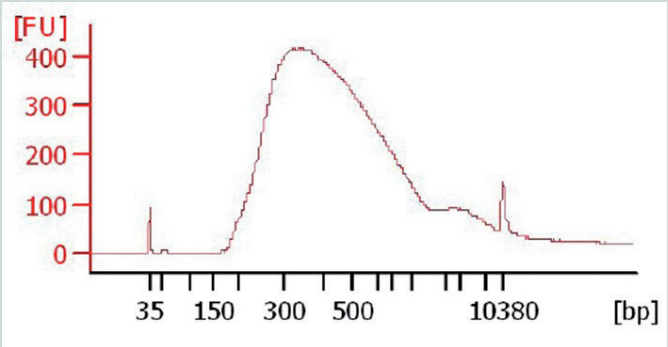

Figure 12: Example of a 2100 BioAnalyzer electropherogram of a post-PCR pre-enriched library distribution using the TruSeq Rapid Exome library prep kit automated on the ep*Motion*. The post-PCR pre-enriched library was diluted 10 fold before analysis with the High Sensitivity DNA Kit.

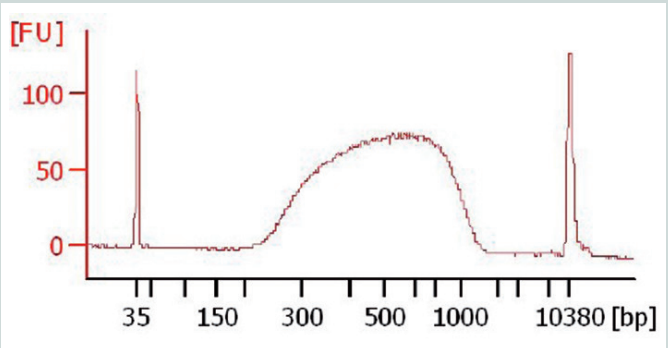

Figure 13: Example of a 2100 BioAnalyzer electropherogram of a post-enriched library distribution using the TruSeq Rapid Exome library prep kit automated on the ep*Motion*. The post-enriched library was diluted 10-fold before analysis with the High Sensitivity DNA Kit.

Ordering information

Description	Order no. international
ep*Motion*® 5075t	5075 000.302
Thermal module on position C2	5075 002.612
TS 50 Dispensing Tool	5280 000.010
TS 300 Dispensing Tool	5280 000.037
TM50-8 Dispensing Tool	5280 000.215
TM300-8 Dispensing Tool	5280 000.231
Gripper	5282 000.018
Thermoadapter PCR 96 (4x)	5075 787.008
Reservoir Rack	5075 754.002
Reservoir Rack Module TC Safe –Lock (2x)	5075 799.081
Reservoir Rack Module Eppendorf Tubes® 5.0 mL	5075 799.340
epT.I.P.S.® Motion, 50 µL, filtered	0030 014.413
epT.I.P.S.® Motion, 300 µL, filtered	0030 014.456
Reservoir 30 mL	0030 126.505
400 mL Reservoir	5075 751.364
Eppendorf twin.tec® PCR Plate 96 Eppendorf LoBind®, semi-skirted	0030 129.504
Eppendorf twin.tec® PCR Plate 96 Eppendorf LoBind®, skirted	0030 129.512
Eppendorf Protein LoBind Tubes, 1.5 mL	0030 108.116
Eppendorf DNA LoBind Tubes, 1.5 mL	0030 108.051
Eppendorf Protein LoBind Tubes, 5.0 mL	0030 108.302
Eppendorf DNA LoBind Tubes, 5.0 mL	0030 108.310
Eppendorf PCR Film	0030 127.781
Eppendorf Storage Foil	0030 127.889

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