SHORT PROTOCOL No. 15 I August 2016

Automated Illumina® TruSeq® DNA PCR-Free library construction with the ep*Motion*® 5075t/TMX

Introduction

This protocol describes the configuration and preprogrammed methods for the automated construction of 8/16 or 24 sequencing ready libraries from 1 µg or 2 µg DNA input (depending on the insert size) with the Illumina TruSeq DNA PCR-Free Sample Preparation kit. 1 µg input is recommended for the 350 bp insert size workflow and 2 µg for the 550 bp insert size workflow. The overall hands-on time is less than 1.5 hours. The total run time of the entire procedure is ~5.5 hours for 24 samples. It allows the construction of sequencing-ready libraries in just one day.

The library automation on the ep*Motion* starts after the Covaris® fragmentation of the DNA sample. The workflow is divided into two methods to allow best possible walk-away time. If necessary, intermediate products from the individual methods can be stored at -15 °C to -25 °C for up to 7 days, according to Illumina kit user guide.

Material and Methods

Required equipment

> ep*Motion* 5075 TMX or 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
> 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
> 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 DNA PCR-Free Sample Prep kit

Methods

Method Name	approx. Runtime (24 samples)
1-TSDNAPCRFree-V01.dws	2.5 hrs
2-TSDNAPCRFree-V01.dws	3 hrs

Before processing the samples on the ep*Motion*, the DNA must be sheared via directed ultrasound (Covaris). Please refer to the kit user guide for the respective parameters. Depending on the insert size to be sequenced, the Sample Purification Beads solution (provided in the kit) needs to be diluted as described in the kit user guide on the reagent preparation.

The kit includes different reagents containing DNA fragments (End repair Control, A-tailing Control and Ligation Control) to be used as controls for enzymatic activities. The use of these controls is optional. These control reagents can be replaced in the respective tube with the same volume of Resuspension Buffer present in the kit.

This approach is programmed to provide as much automation as possible; a maximum of 24 samples can be processed in iteration of 8 (8, 16 or 24). Other sample numbers are not supported due to the use of the 8-channel tools. The entire workflow is divided into two ep*Motion* methods. Each of the methods ends at a "Safe Stopping Point", allowing storage of the intermediate products at -25 °C to -15 °C for up to 7 days, as stated in the kit user guide. To reduce dead volumes, some Illumina kit reagents – in particular the enzyme mixes – must be provided in 1.5 mL Tubes. The remaining reagents, diluted and undiluted Sample Purification Beads, mineral oil, Resuspension Buffer, and Ethanol (80 %) are programmed 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. During the procedure no cooling of the reagents is required.

All steps of the procedure are performed in Eppendorf LoBind, semi-skirted Eppendorf twin.tec PCR plates 96. For incubation steps above 37 °C, samples are overlaid with oil to allow temperature incubations on the ep*Motion* without evaporation. The methods were developed on the ep*Motion* 5075 TMX model, but can also be transferred to the newer ep*Motion* 5075t without any adjustment.

Important: The output plate containing the samples of each method will be placed on the C2 position (Temp) set to 10 °C at the end of the individual methods. Final libraries will be found in columns 1-3 of the plate labeled CAP. The volume is 20 µL.

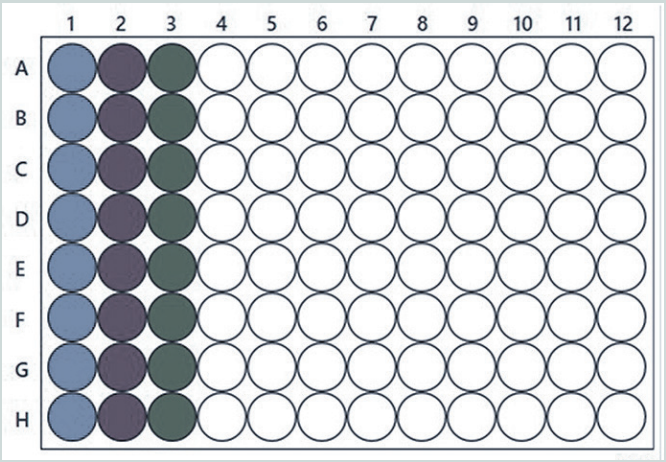

Figure 1: Position of the final libraries in the plate labeled CAP after completion of the entire procedure (Method 1 followed by method 2)

Method 1

To start, transfer 50 µL per sample (8/16 or 24 samples) of Covaris sheared DNA into the first three columns (Wells A1-H3) of an Eppendorf twin.tec semi skirted Eppendorf LoBind PCR plate 96 labeled CSP-CEP (process plate). 1 µg input is recommended for the 350 bp insert size workflow and 2 µg for the 550 bp insert size workflow. Place the process plate on the TMX position. This method ends with end repaired and size selected DNA in the first three columns (A1-H3) of a second Eppendorf twin.tec semi skirted Eppendorf LoBind PCR plate 96 (ALP). This plate must be used in method 2.

Worktable Layout

Position	Item
A2	50 µL Filtertips
A3	50 µL Filtertips
A4 (TMX)	Thermoadapter PCR 96 + PCR plate with DNA samples (labeled CSP-CEP)
B0	400 mL tub for liquid waste
B1	300 µL Filtertips
B2	300 µL Filtertips
B3	300 µL Filtertips
B4	Thermoadapter PCR 96 + empty PCR plate (labeled SPRI)
C1	Thermoadapter PCR 96 + empty PCR plate (labeled ALP)
C2 (Temp)	Thermoadapter PCR 96
C3	ReservoirRack with 1x RR Module Safe Lock + 4x 30 mL Reservoir for reagents
C4	Alpaqua MAGNUM FLX Enhanced Universal Magnet Plate

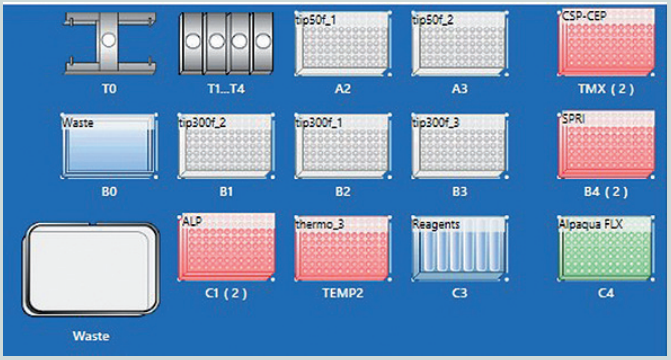

Figure 2: ep*Motion* worktable layout for method 1

ReservoirRack layout

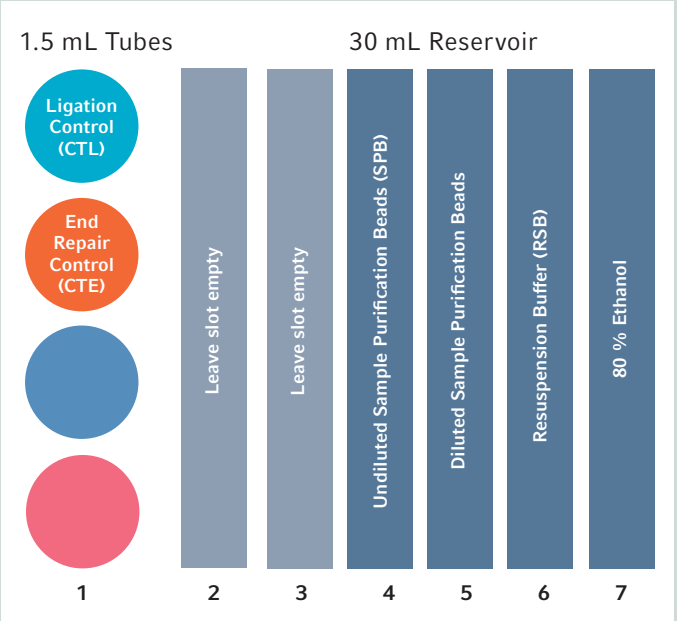

Figure 3: Reservoir rack layout for method 1

Attention: If kit included controls are not going to be used, use plain Resuspension Buffer in the according reagent position.

Method 2

Start with the PCR plate labeled ALP containing the samples from method 1 in wells A1 – H3, placed on position C2 of the ep*Motion* worktable. The Index Adapters must be transferred into a skirted Eppendorf twin.tec PCR plate. It is highly recommended to add an overage of at least 50 % to the requested Index Adapter volume per well to compensate for evaporation. As example, if the Index Adapters volume requested is 6 µL, it is recommended to put 9 µL of this solution per well. Depending on the sample number, sequencing setup, pooling scheme, etc., the number, combination and labware of the Index Adapters (position B4) need to be modified. According to the parameters needed, review/adjust command 29 of method 2. For stability reasons, keep the Ligation Mix 2 at -20 °C in the freezer at the start of the method. A user intervention is added to place it on the ep*Motion* just before use. After its transfer, another user intervention is added to place it back at -20 °C.

Once both methods are completed, final libraries (20 µL each) are in columns 1-3 of the plate labeled CAP on position C2 (Temp) at 10 °C.

Worktable layout

Position	Item
A2	50 µL Filtertips
A3	50 µL Filtertips
A4 (TMX)	Thermoadapter PCR 96
B0	400 mL tub for liquid waste
B1	300 µL Filtertips
B2	300 µL Filtertips
B3	50 µL Filtertips
B4	skirted PCR plate with Index Adapters → review method programming
C1	Thermoadapter PCR 96 + empty PCR plate (labeled CAP)
C2 (Temp)	Thermoadapter PCR 96 + PCR plate with DNA (ALP) from method 01
C3	ReservoirRack with 2x RR Module Safe Lock + 4x 30 mL Reservoir
C4	Alpaqua MAGNUM FLX Enhanced Universal Magnet Plate

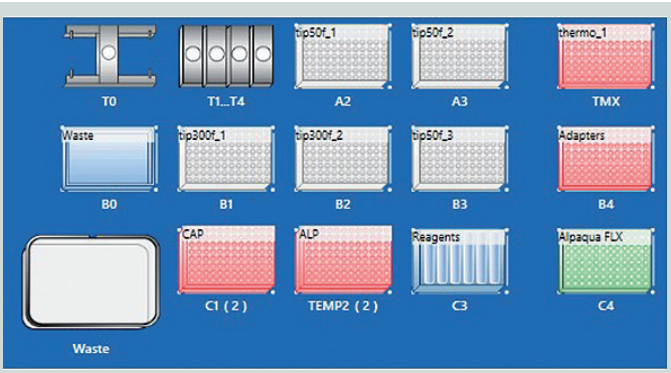

Figure 4: ep*Motion* worktable layout for method 2

ReservoirRack layout

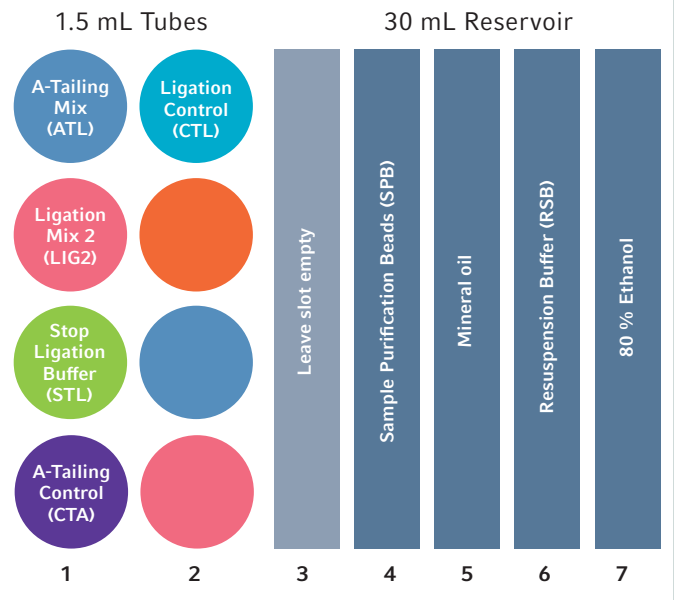

Figure 5: Reservoir rack layout for method 2

Attention: If kit included controls are not going to be used, use Resuspension Buffer in the according reagent positions.

Results

Typically the final libraries will be quality controlled on an Agilent® Technologies 2100 BioAnalyzer® or similar to assess the fragment size distribution. An example of a typical BioAnalyzer result is shown in figure 6.

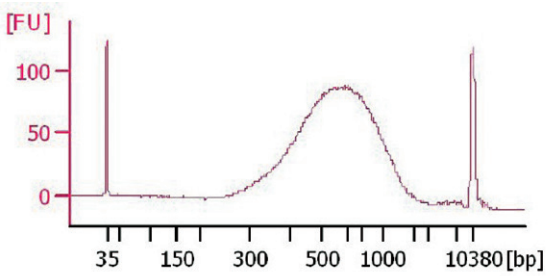

Figure 6: Example of a 2100 BioAnalyzer electropherogram of a TruSeq DNA PCR-Free library with 350 bp insert size. The final library was diluted 5 times before analysis with the High Sensitivity DNA Kit.
Final quantification of TruSeq DNA PCR-Free Sample Prep libraries is recommended to be done via qPCR.

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
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 Protein LoBind Tubes, 2.0 mL	0030 108.132

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