eppendorf

SHORT PROTOCOL No. 24

Automated Illumina® TruSight® Rapid Capture Library Preparation with the ep*Motion*® 5075t

Introduction

This protocol describes the configuration and preprogrammed methods for the automated construction of 1 to 24 sequencing libraries from 50 ng of input DNA with the Illumina TruSight Rapid Capture kit. In the enrichment steps, the number of library pools that can be processed in parallel is 1 to 6.
The TruSight Rapid Capture DNA Sample Preparation protocol is the same as the Nextera® Rapid Capture Enrichment DNA Sample Prep protocol except for an increased number of cycles in the final PCR Amplification steps. The same reagents are used, but are designed to be used in conjunction with different oligo panels targeting specific regions of DNA – such as the Custom Selected Oligos (CSO) from the TruSight Cancer Sequencing Panel kit. It allows the construction of sequencing ready libraries in approximately three days. The workflow is divided into four methods on the ep*Motion* 5075t.

Material and Methods

Required equipment
> ep*Motion* 5075t
> TS50 pipetting tool
> TS300 pipetting tool
> TM300-8 pipetting tool
> Gripper
> 1× Thermoadapter for PCR plates, 96-well
> 1× Thermoblock OC for PCR plates, 96-well
> 1× ReservoirRack
> 1× Rack ILMN Tubes
> 1× 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, LoBind, semi-skirted
> Eppendorf Protein LoBind Tube 1.5 mL
> epMotion Reservoir 30 mL
> Eppendorf 400 mL Reservoir
> 80% Ethanol
> RNase free water
> Mineral oil, PCR/molecular biology grade (Sigma-Aldrich®, order no. M5904-500ML)
> TruSight Rapid Capture kit (Illumina, order no. FC-121-1101; FC-121-1102; FC-121-1103; FC-121-1104)
> TruSight Cancer Sequencing Panel kit (Illumina, order no. FC-121-02052)

Methods

Method Name	Approximate running time for 24 samples
1-TSRapidCapture-VXY.export4	1 hour 50 minutes
2-TSRapidCapture-VXY.export4	1 hour 10 minutes
	Approximate running time for 2 samples
3-TSRapidCapture -VXY.export4	2 hours 30 minutes
4-TSRapidCapture -VXY.export4	4 hours 10 minutes

The entire workflow is divided into four ep*Motion* methods. The steps of the protocol covered by the ep*Motion* are listed in figure 1.
A maximum of 24 samples can be processed for the steps of the protocol before the hybridizations (methods 1 and 2). All sample numbers between 1 and 24 are supported. From the first hybridization capture steps to the end of the protocol (methods 3 and 4), the maximum number of samples that can be processed is 6 as libraries are pooled.
The library quantification, pooling and the first hybridization setup are non automated steps. The hybridization and the PCR amplification programs are run on a thermal cycler outside the ep*Motion*.
To reduce dead volumes, most of the Illumina kit reagents must be provided in Eppendorf Protein LoBind Tubes. 5-10% excess volume should be added. 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. At the end of each method, it is recommended to keep the residual volume of each stock solution for future use in next runs except the Premix Elution solution that needs to be freshly prepared.
All steps of the procedure are performed in Eppendorf LoBind, semi-skirted 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.

Figure 1: Workflow and automated strategy of the TruSight Rapid Capture Library Preparation protocol. Blue steps are automated.

Method 1

Method 1 is programmed for a maximum of 24 samples (1-24 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 three columns (Wells A1-H3) of a twin.tec semi-skirted LoBind PCR plate 96 labeled "Input Samples".
For stability reasons, the Library Amp Mix (NLM) and the Index Adapters are not placed on the worktable at the start of the method. A user intervention is programmed to place it in the Rack ILMN tubes just before use.
The Index Adapters are pipetted directly from the Illumina stock tubes. Depending on the sample number, sequencing setup, pooling scheme etc., the number, combination and pattern of the Index Adapters in the Rack ILMN Tubes need to be modified.
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 columns 4 to 6 (A4-H6) of a second twin.tec semi-skirted LoBind PCR plate 96 labeled PCR Setup at position TMX.
This plate must be sealed, mixed and centrifuged before being placed in a thermal cycler to run the NLM AMP program.
This plate must be used in method 2 when the NLM AMP program is finished.

Worktable Layout

Position	Item
A2	50 µL Filtertips
A3	300 µL Filtertips
A4 (TMX	Thermoblock PCR 96 OC + semi-skirted PCR plate with samples (Input Samples)
B0	400 mL tub for liquid waste
B2	300 µL Filtertips
B4	Rack ILMN Tubes
C1	Alpaqua MAGNUM FLX Enhanced Universal Magnet Plate
C2 (Temp2)	Thermoadapter PCR 96 + empty semi-skirted PCR plate (PCR Setup)
C3	ReservoirRack with 4× 30 mL Reservoir for reagents

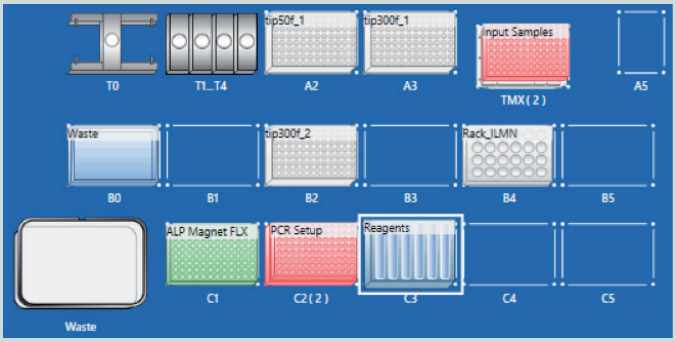

Figure 2: ep*Motion* worktable layout for method 01

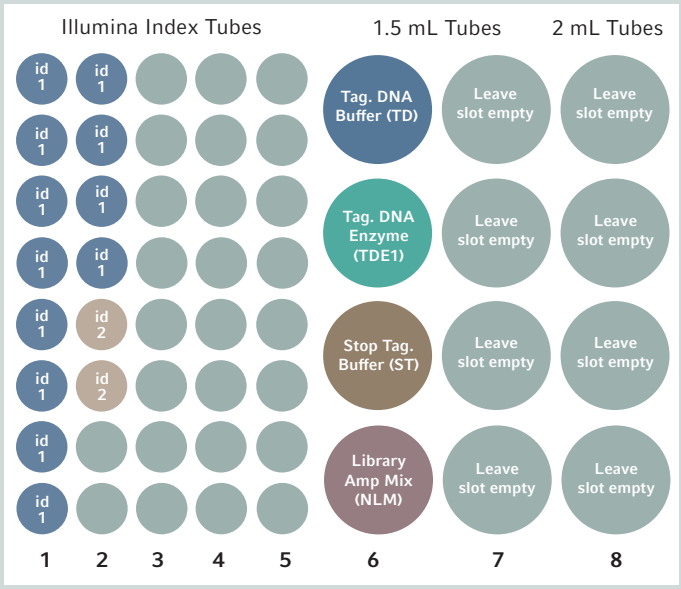

Figure 3: Rack ILMN layout for method 1

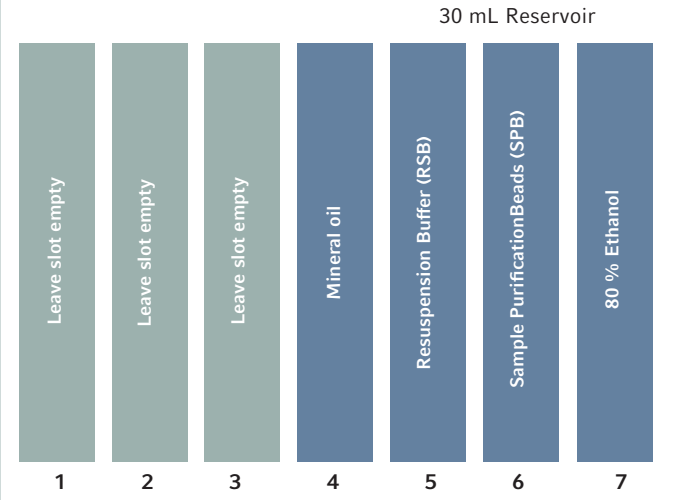

Figure 4: Reservoir rack layout for method 1

Method 2

Method 2 is programmed for a maximum of 24 samples (1-24 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 A4-H6, placed on position A4 (TMX) of the ep*Motion* worktable.

This method ends with the cleaned up amplified DNA in the columns 7 to 9 (A7-H9) of the same twin.tec semi-skirted LoBind PCR plate 96, at position TMX.

The quantification, the pooling, the hybridization setup and the hybridization of the libraries steps are not part of the automation – so please refer to the kit user guide for further details.

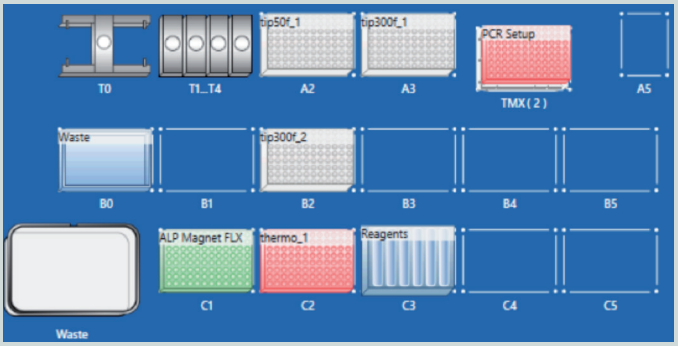

Figure 5: ep*Motion* worktable layout for method 2

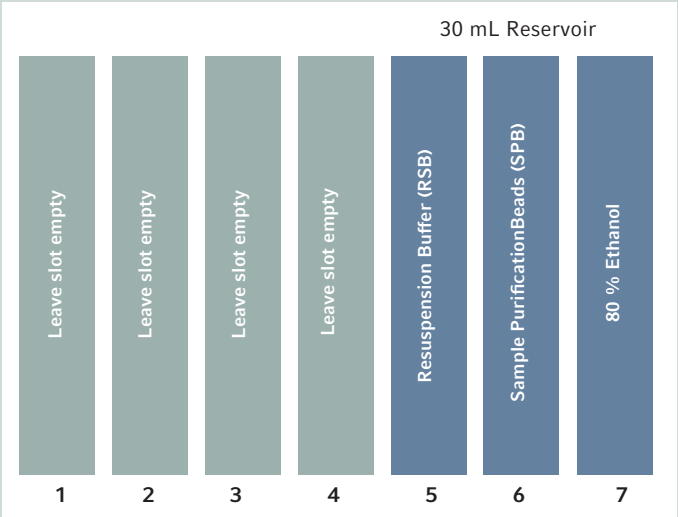

Figure 6: Reservoir rack layout for method 2

Worktable Layout

Position	Item
A2	50 µL Filtertips
A3	300 µL Filtertips
A4 (TMX	Thermoblock PCR 96 OC + semi-skirted PCR plate with samples (PCR Setup)
B0	400 mL tub for liquid waste
B2	300 µL Filtertips
C1	Alpaqua MAGNUM FLX Enhanced Universal Magnet Plate
C2 (Temp)	Thermoadapter PCR 96
C3	ReservoirRack (Reagents)

Method 3

Method 3 is programmed for a maximum of 6 samples (1-6 samples). It includes the first capture hybridized probes steps.

Start with 100 µL of up to 6 pools of DNA libraries in wells A1-F1 of a twin.tec semi-skirted LoBind PCR plate 96 labeled NEH1. Place the NEH1 plate on the Thermoblock PCR 96 OC present on B4 position.

It is recommended to prepare the required volume of the solutions listed in parenthesis (Elution Premix; Elute Target Buffer (ET2); Enrichment Hybridization Buffer (EHB); Resuspension Buffer (RSB) and the Custom Selected Oligos (CSO)) in individual Eppendorf Protein LoBind 1.5 mL and 2 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 Rack ILMN Tubes just before use. Please be aware, the Elution Premix is prepared freshly manually by mixing the Enrichment Elution Buffer (EE1) and 2N NaOH (HP3) reagents.

This method ends with the second hybridization setup. The library samples are in 6 wells (A1-F1) of a twin.tec semi-skirted LoBind PCR plate 96 labeled NEH2, at position C2 (TEMP2).

The NEH2 PCR Plate must be sealed, mixed and centrifuged before being placed in a thermal cycler to run the NRC HYB program.

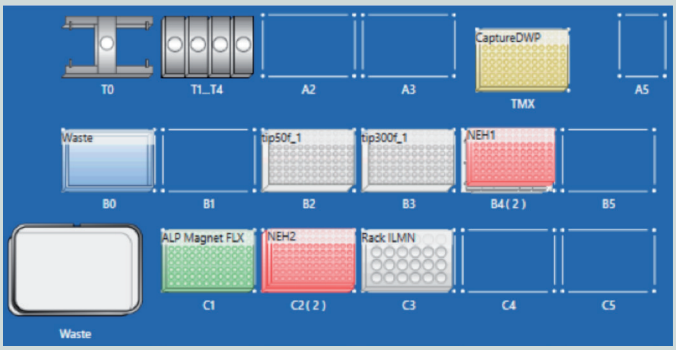

Figure 7: ep*Motion* worktable layout for method 3

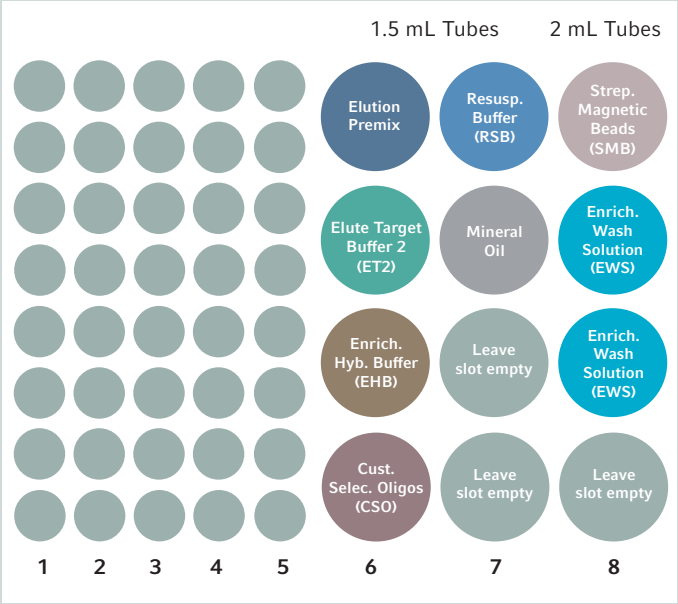

Figure 8: Rack ILMN Tubes layout for method 3

Worktable Layout

Position	Item
A4 (TMX	Empty Deepwell Plate 96/500 µL (CaptureDWP)
B0	400 mL tub for liquid waste
B2	50 µL Filtertips
B3	300 µL Filtertips
B4	Thermoblock PCR 96 OC + semi-skirted PCR plate with samples (NEH1)
C1	Alpaqua MAGNUM FLX Enhanced Universal Magnet Plate
C2 (Temp)	Thermoadapter PCR 96 + empty semi-skirted PCR plate (NEH2)
C3	Rack ILMN Tubes

Method 4

Method 4 is programmed for a maximum of 6 samples (1-6 samples). It includes the second round of hybridization capture, the cleanup of the captured libraries, the amplification and cleanup of the enriched libraries.

Start with the 100 µL of hybridized libraries coming from the thermal cycler in up to 6 wells (A1-F1) of a twin.tec semi-skirted LoBind PCR plate 96 labeled NHE2. Place NEH2 plate on the thermoblock placed at C2 position.

It is recommended to prepare the needed volume of the solutions listed in parenthesis (Elution Premix; Elute target Buffer (ET2); PCR Primer Cocktail (PPC); Enriched Amplification Mix (NEM); Sample purification Beads (SPB); Resuspension Buffer (RSB)) in a 1.5 mL and 2 mL Eppendorf Protein LoBind tube and keep it 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 manually by mixing the Enrichment Elution Buffer (EE1) and 2N NaOH (HP3) reagents.

A second user intervention occurs in the method where the PCR plate needs to be sealed and cycled in a PCR cycler to enrich the libraries using the NEM AMP10 or NEM AMP12 program. Reopen the plate after PCR and return to the TMX position prior to continuing the method. This method ends with 30 µL of the final post-enriched library samples in six wells (A12-F12) of a twin.tec semi-skirted LoBind PCR plate 96, labeled NEC1-NEA, at position TMX.

Worktable Layout

Position	Item
A4 (TMX	Empty Deepwell Plate 96/500 µL (labelled CaptureDWP2)
B0	400 mL tub for liquid waste
B2	50 µL Filtertips
B3	300 µL Filtertips
B4	Thermoblock PCR 96 OC + empty semi-skirted PCR plate (NEC1-NEA)
C1	Alpaqua MAGNUM FLX Enhanced Universal Magnet Plate
C2 (Temp)	Thermoadapter PCR 96 + empty semi-skirted PCR plate with samples (NEH2)
C3	Rack ILMN Tubes

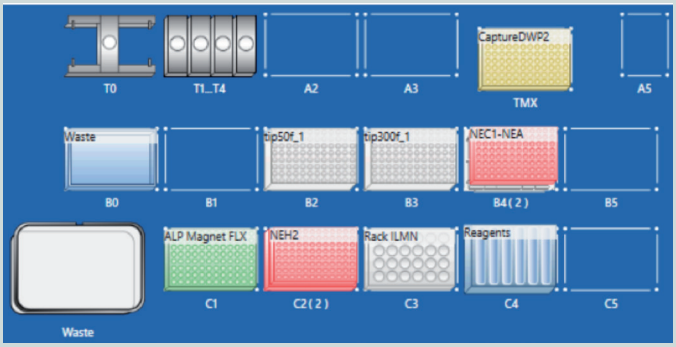

Figure 9: ep*Motion* worktable layout for method 4

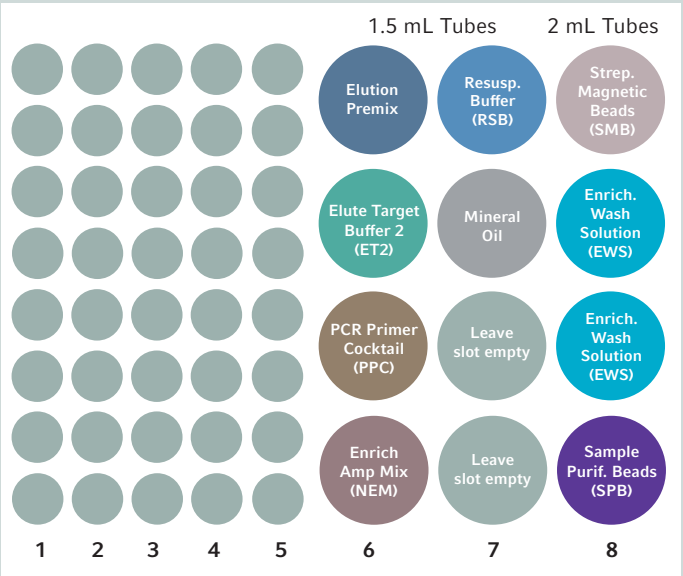

Figure 10: Rack ILMN Tubes layout for method 4

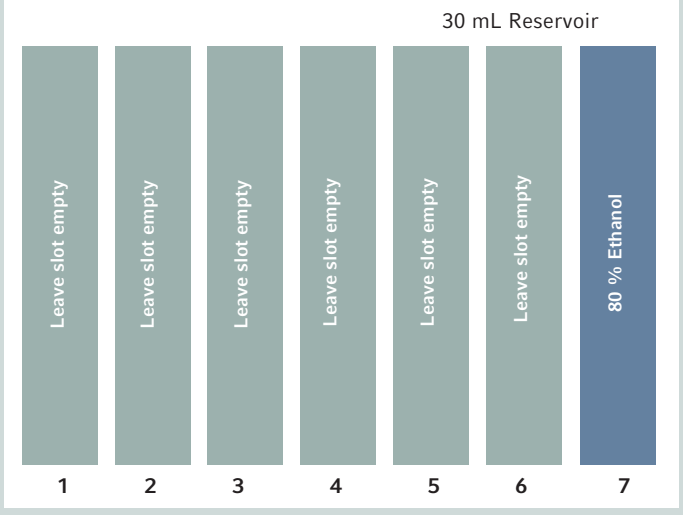

Figure 11: Reservoir rack layout for method 4

Results

Typically the libraries will be quality controlled at different steps of the process on an Agilent Technologies 2100 BioAnalyzer or similar. An example of a typical BioAnalyzer result using the automated protocol on the ep*Motion* of the post-enriched library distributions is shown in figure 11. A distribution of DNA fragments with a size range from ~200 bp to ~1 kbp is expected. Depending of the level of indexing, insert size distribution can vary slightly.

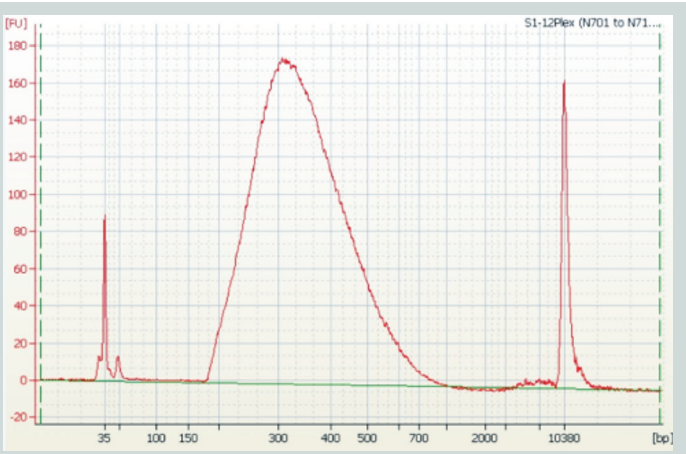

Figure 12: Example of a 2100 BioAnalyzer electropherogram of a 12-plex post-enriched library distribution using the TruSight Rapid Capture library prep kit in combination with the Illumina TruSight Cancer Sequencing Panel kit automated on the ep*Motion*. 1 µL of the post-enriched library was run on an Agilent BioAnalyzer using a High Sensitivity DNA chip.

Ordering information

Description	Order no. international
ep*Motion*® 5075t	5075 000.302
TS 50 Dispensing Tool	5280 000.010
TS 300 Dispensing Tool	5280 000.037
TM300-8 Dispensing Tool	5280 000.231
Gripper	5282 000.018
Thermoadapter PCR 96 (1×)	5075 787.008
Thermoblock PCR 96 OC (1×)	5075 751.666
Reservoir rack	5075 754.002
Rack ILMN Tubes	5075 751.747
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 LoBind, semi-skirted	0030 129.504
Deepwell Plate 96/500 µL	0030 501.101
Eppendorf Protein LoBind Tubes, 1.5 mL	0030 108.116
Eppendorf Protein LoBind Tubes, 2.0 mL	0030 108.132
Eppendorf PCR Foil, self adhesive	0030 127.790

Your local distributor: www.eppendorf.com/contact
Eppendorf AG · 22331 Hamburg · Germany
eppendorf@eppendorf.com · www.eppendorf.com

www.eppendorf.com/automation