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SHORT PROTOCOL No. 05 I March 2015

Automated Illumina® TruSeq® Nano DNA 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 100 or 200 ng DNA (depending on the insert size) with the Illumina TruSeq Nano DNA kit. As part of the manual preparation of the reagents the kit provided sample purification beads need to be diluted – please refer to the kit user guide for further details.
The overall hands on time is less than 1.5 hours, the total run time on the ep*Motion* 5075 TMX for 24 samples is approximately 10 hours.

After Covaris® fragmentation of the sample DNA, the entire library construction is automated on the ep*Motion* – the workflow is divided into three sub-methods. Intermediate products from the individual sub-methods can be stored at -20 °C for up to 7 days, according to Illumina's 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
> Reservoir rack
> 3x RR Module TC, Eppendorf Safe-Lock Tubes
> PCR Cycler, e.g. Eppendorf Mastercycler® Pro S
> Alpaqua® LE Magnet plate (low elution volume magnet, Alpaqua order no.: A000350)

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, semi skirted
> Eppendorf twin.tec PCR plates, 96-well, skirted (for the Index Adapters)
> Eppendorf Safe-Lock Tubes 1.5 mL
> Eppendorf Safe-Lock Tubes 2.0 mL
> ep*Motion* Reservoir 30 mL
> Eppendorf 400 mL Reservoir
> 80 % Ethanol
> mineral oil, PCR/molecular biology grade (Sigma-Aldrich®, order no. M5904-500ML)
> Illumina TruSeq Nano DNA kit

Methods

Method Name	approx. Runtime (24 samples)
XXYYZZ-01-TSNanoDNA.dws	4hrs
XXYYZZ-02-TSNanoDNA.dws	4hrs
XXYYZZ-03-TSNanoDNA.dws	2hrs, including external PCR

(XX = year, YY = month, ZZ = day)

Before the samples can be processed on the ep*Motion*, the DNA has to be fragmented via directed ultrasound methods (Covaris) – please refer to the kit user guide for the respective parameters. Depending on the insert size to be sequenced the kit provided sample purification beads need to be diluted – please refer to the kit user guide for details.

This approach is programmed to provide as much automation as possible; a maximum of 24 samples can be processed in iterations 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 three ep*Motion* methods (or sub-methods, see above). 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 according user guide. The third sub-method requires a user intervention to perform off-deck PCR amplification. To reduce dead volumes, all Illumina Kit reagents have to be provided in either 1.5 mL or 2 mL Tubes, only Sample Purification Beads and Ethanol for the washes 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 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 let them 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 twin.tec 96-well semi-skirted PCR plates, for heat incubation steps above 37 °C, samples are overlaid with oil to avoid evaporation and allow temperature incubations on the ep*Motion*. The PCR amplification needs to be carried out in an external PCR cycler, which requires about 20-30 minutes, depending on the device and the number of cycles (default are 8 cycles).
The methods were developed on the ep*Motion* 5075 TMX, but can also be transferred to the ep*Motion* 5075t.

Important: The output plate containing the samples that need to be processed in the subsequent sub-method will always be placed on the C2 position (Temp2) programmed to 10 °C at the end of the individual methods. Final libraries will be found in columns 10-12 of the plate labeled PCR. The total volume is 30 µL per library:

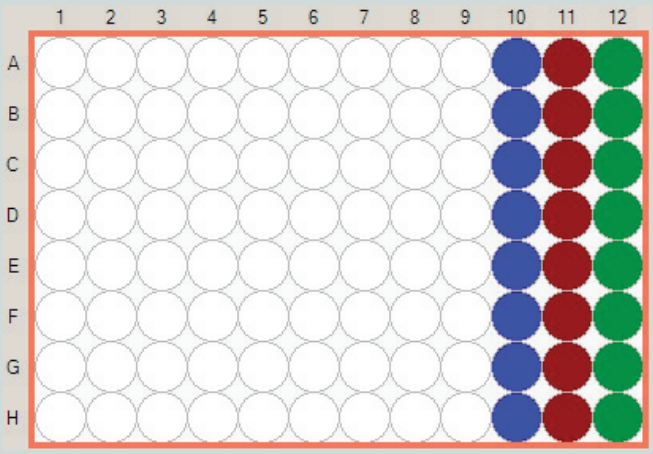

Figure 1: Location of the final libraries in the plate labeled PCR after completion of the entire procedure.

Sub-method 01

Start with 100 or 200 ng Covaris sheared DNA in a volume of 50 µL per sample. 8/16 or 24 samples have to be provided in the first three columns (Wells A1-H3) of an Eppendorf twin.tec 96-well semi skirted PCR plate (CSP-CEP), placed on the TMX position. The method ends with end repaired and size selected DNA in the first three columns (A1-H3) of a second of Eppendorf twin.tec 96-well semi skirted PCR plate (ALP). This plate needs to be used in sub-method 02.

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	Reservoir rack with 3x RR Module Eppendorf Safe-Lock Tubes + 3x 30 mL Reservoir for reagents (pos. 4, 5 & 7)
C4	Alpaqua LE magnet plate

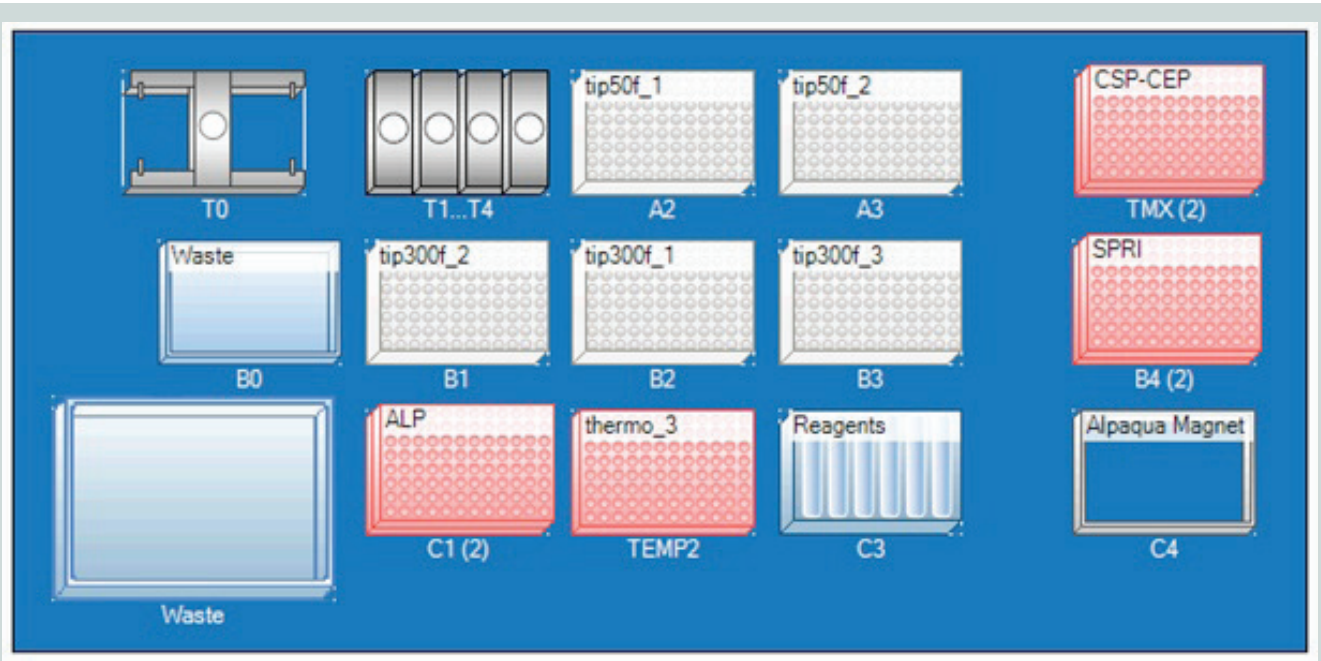

Figure 2: ep*Motion* worktable layout for method 1

Reservoir rack layout

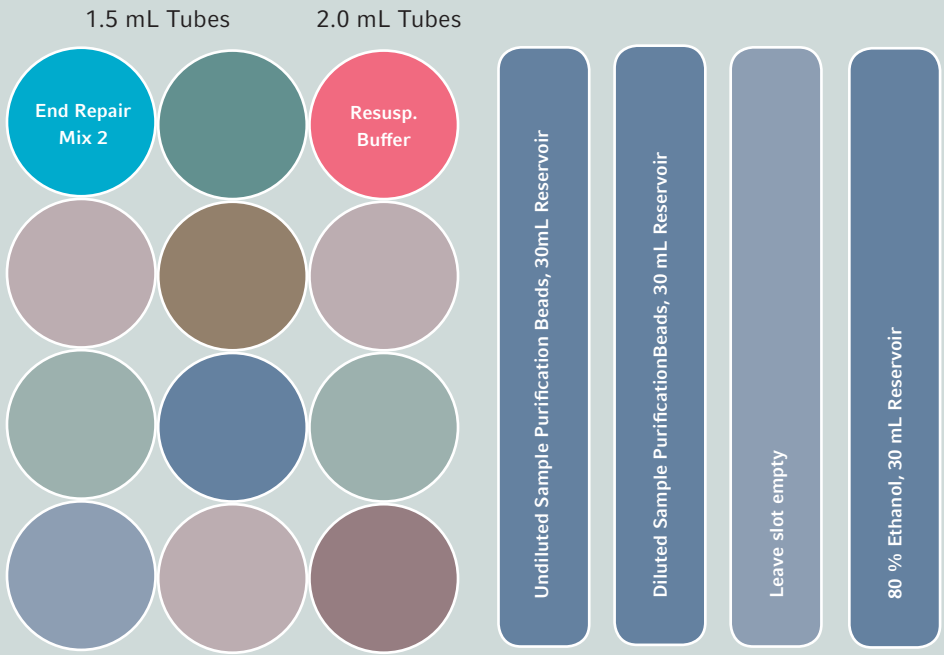

Figure 3: Reservoir rack layout for method 1

Sub-method 02
Start with the PCR plate labeled ALP containing the Samples from sub-method 01 in Positions A1 – H3, placed in position B4. The method ends with A-tailed and Index Adapter ligated clean DNA in a PCR plate labeled PCR. Depending on the sample number, sequencing setup, pooling scheme etc. the number, combination and labware of the Index Adapters (position B3) needs to be modified → also review/adjust step 25 + the worktable in the method. If the default setup is being used, a user intervention to refill 50 µL tips might be required.

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	skirted PCR plate with Index Adapters → review method programming
B4	Thermoadapter PCR 96 + PCR plate with DNA (ALP) from sub-method 01
C1	Thermoadapter PCR 96 + empty PCR plate (labeled PCR)
C2 (Temp)	Thermoadapter PCR 96
C3	Reservoir rack with 3x RR Module Eppendorf Safe-Lock Tubes + 2x 30 mL Reservoir (pos. 5 & 7)
C4	Alpaqua LE magnet plate

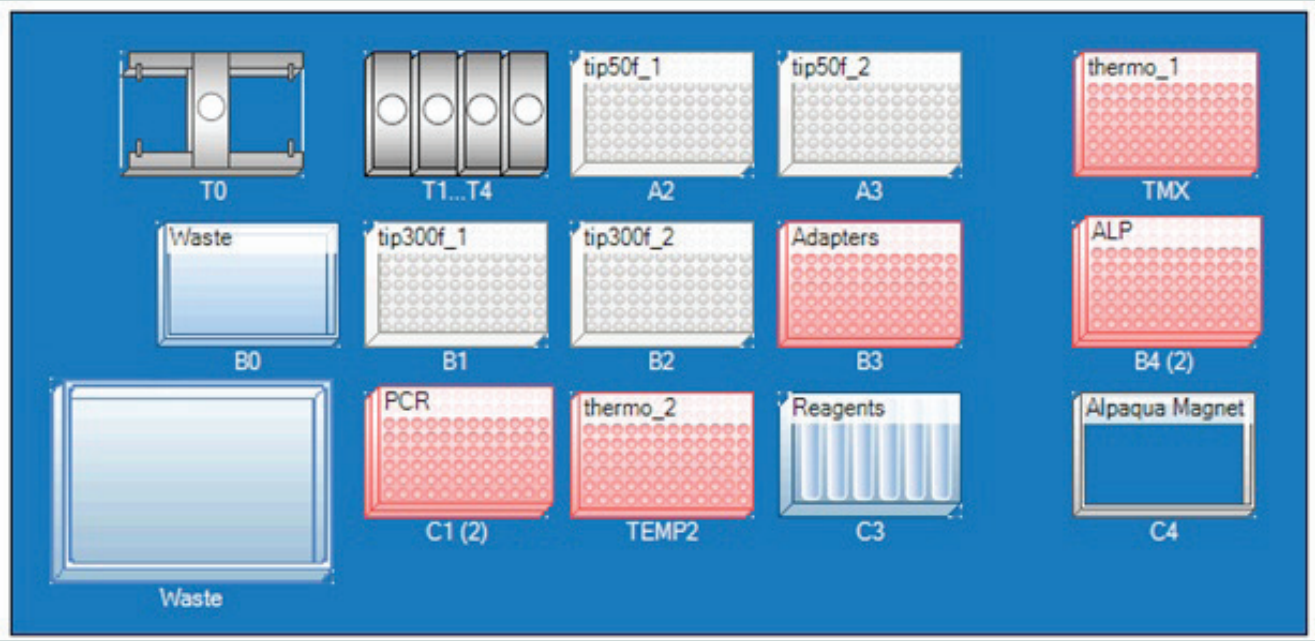

Figure 4: ep*Motion* worktable layout for method 2

Reservoir rack layout

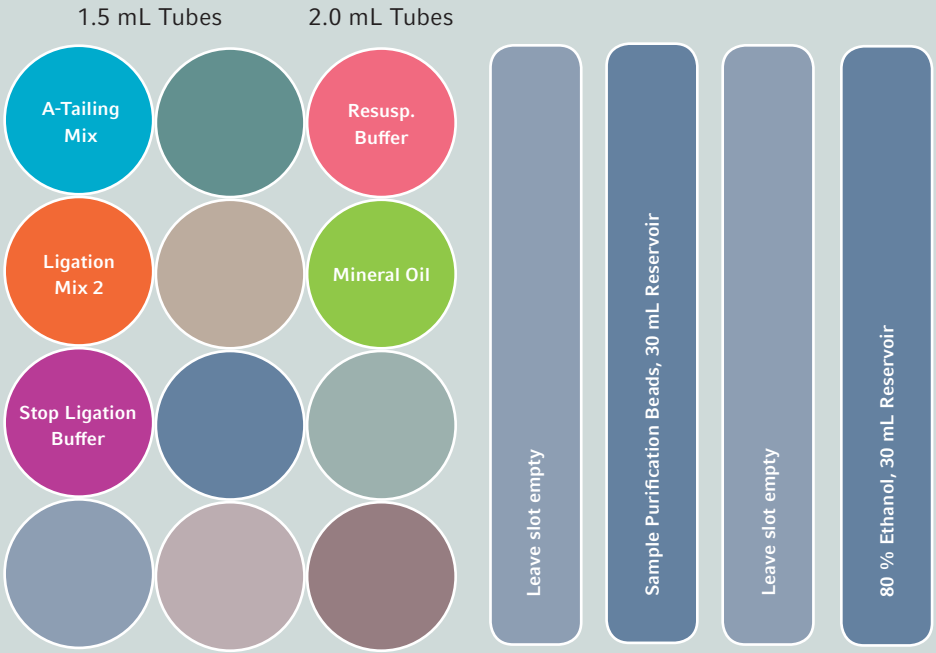

Figure 5: Reservoir rack layout for method 2

Sub-method 03
Start with 25 µL A-tailed and Index Adapter ligated samples in plate labeled PCR from sub-method 02 in TMX 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. Reopen the plate after PCR and return to the TMX position prior to continuing the method.

Worktable layout

Position	Item
A2	50 µL Filtertips
A3	empty
A4 (TMX)	Thermoadapter PCR 96 + PCR plate (PCR) with samples from sub-method 02
B0	400 mL tub for liquid waste
B1	300 µL Filtertips
B2	300 µL Filtertips
B3	empty
B4	Thermoadapter PCR 96
C1	Thermoadapter PCR 96
C2 (Temp)	Thermoadapter PCR 96
C3	Reservoir rack with 3x RR Module Eppebndorf Safe-Lock Tubes + 2x 30 mL Reservoir (pos. 5 & 7)
C4	Alpaqua LE magnet plate

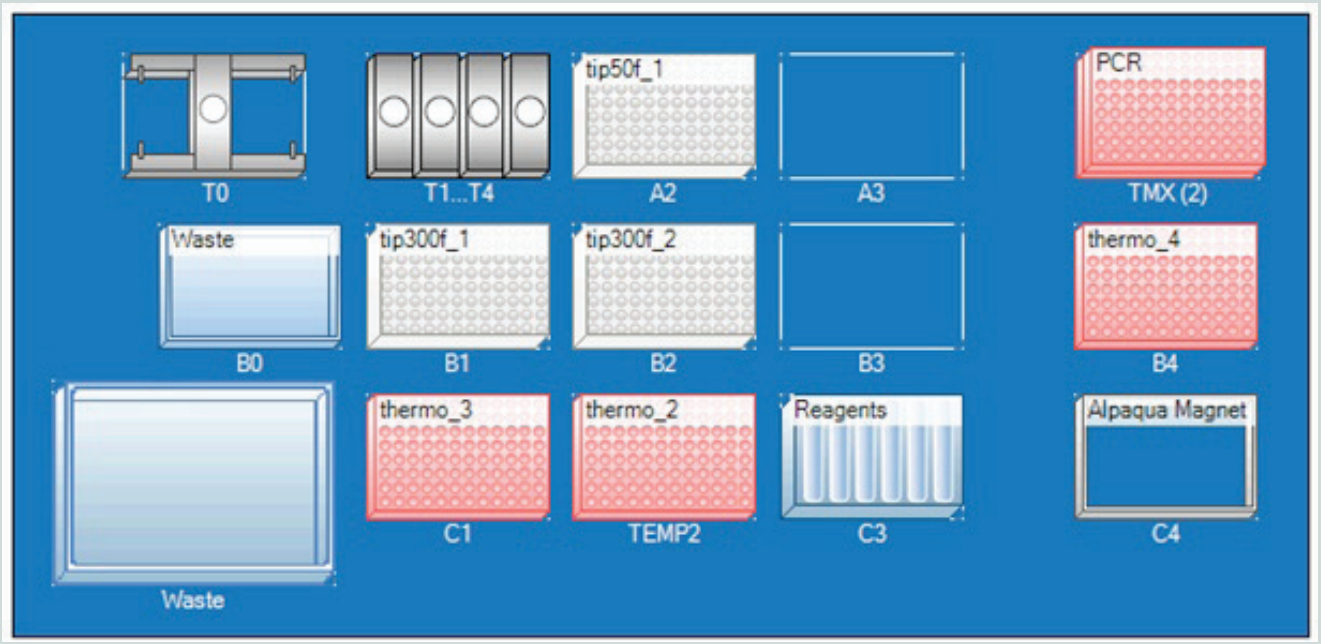

Figure 6: ep*Motion* worktable layout for method 3

Reservoir rack layout

Figure 7: Reservoir rack layout for method 3

Once all three sub-methods are completed, the final libraries (30 µL each) are in columns 10-12 of the plate labeled PCR in position C2 (Temp) at 10 °C.

Results

Typical results from the automated TruSeq Nano DNA library preparation are:

100 ng input DNA: 14.5 ng/µL, total yield (30 µL) = 435 ng
200 ng input DNA: 25.8 ng/µL, total yield (30 µL) = 774 ng

Paired end run (2x101+7) in rapid mode on a HiSeq® system:
passed filter reads: >95 %
passed filter reads aligned >94 %
reads >=Q30: >94 %

Ordering information

Description	Order no. international
ep*Motion*® 5075t	5075 000.302
Thermal module	5075 757.001
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	5075 787.008
Reservoir rack	5075 754.002
Reservoir rack Module TC, Eppendorf Safe–Lock	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, semi-skirted	0030 128.575
Eppendorf twin.tec® PCR Plate 96, skirted	0030 128.648
Eppendorf Safe-Lock Tubes, 1.5 mL	0030 120.086
Eppendorf Safe-Lock Tubes, 2.0 mL	0030 120.094

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