eppendorf

SHORT PROTOCOL No. 26

Automated Illumina® TruSight® Tumor 15 Library Preparation with the ep*Motion*® 5075t

Introduction

This protocol describes the system configuration and pre-programmed methods for automated construction of sequencing libraries from up to 24 samples (in multiples of eight) with at least 20 ng of DNA. The overall hands-on time is less than 15 minutes, and the total run time (not including PCR amplification) is 2 hours.

With simple modifications, the programs can be expanded to produce 48 libraries. However, due to the process involving splitting a sample into two parts to carry out the amplification separately, further expansion of sample number is not supported.

Material and Methods

Required equipment

> ep*Motion* 5075t
> Gripper
> TS50 dispensing tool
> TS300 dispensing tool
> TM300-8 dispensing tool
> Rack ILMN tubes
> 2x Thermoadapter for PCR plates, 96-well
> 1x Thermoblock OC for PCR plates, 96-well
> 1x ReservoirRack
> 1x 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 LoBind, semi-skirted
> Eppendorf Tubes® 1.5 mL, Safe-Lock
> epMotion Reservoir 30 mL
> Eppendorf 400 mL Reservoir
> 80% Ethanol
> Elution Buffer (10 mM Tris-HCl)
> Illumina TruSight Tumor 15 Kit (Illumina order no. OP-101-1002)

Methods

Method Name	Approximate run time for 24 samples (48 libraries)
1_TruSightTumor15	20 min
2_TruSightTumor15	40 min
3_TruSightTumor15	65 min

The Illumina TruSight Tumor 15 Kit provides coverage to 15 tumor-related genes. To increase amplification efficiency, each DNA sample is equally divided into two portions to undergo separate, parallel library preparations. Therefore, a 24-sample kit is capable of producing 48 libraries. To ensure the quality of the libraries, 20 ng DNA (isolated from FFPE samples) with minimum concentration of 2 ng/µL is desired.

The kit's reference guide (v04) splits the workflow into three main steps with thermal cycling and safe stopping points between them. Therefore, the full script package is comprised of three sub-methods. The following section describes how to properly set up each sub-method. To reconcile the discrepancy between the row-wise processing as described in the kit manual and column-wise handling by the robot, proper adjustments are made in the sub-methods.

Sub-Method 01

Start with up to 24 DNA samples (> 2 ng/µL) prepared in the "Sample Plate". DNA samples should be supplied column-wise in an Eppendorf 96-well twin.tec semi-skirted PCR plate. A minimum of 12 µL per sample is required to cover the protocol consumption and remaining volume. Mastermix A and B should be supplied in Eppendorf 1.5 mL Safe-Lock tubes. A 10% excess is recommended for each mastermix.

Worktable Layout

Position	Item
A2	50 µL Filtertips
TMX	Thermoblock PCR 96 OC + semi-skirted PCR plate (Work Plate)
B4	Rack ILMN tubes
B5	Thermoadapter PCR 96 + empty semi-skirted plate

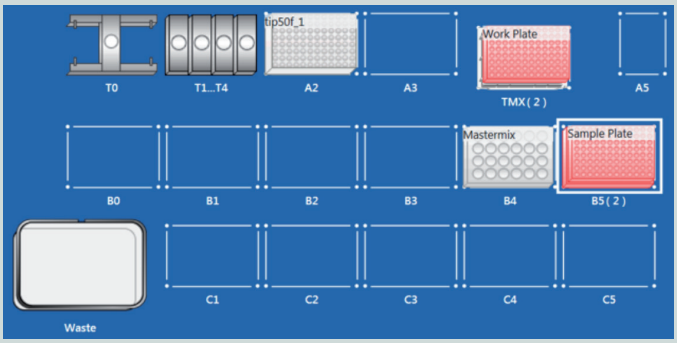

Figure 1: ep*Motion* worktable layout for sub-method 01

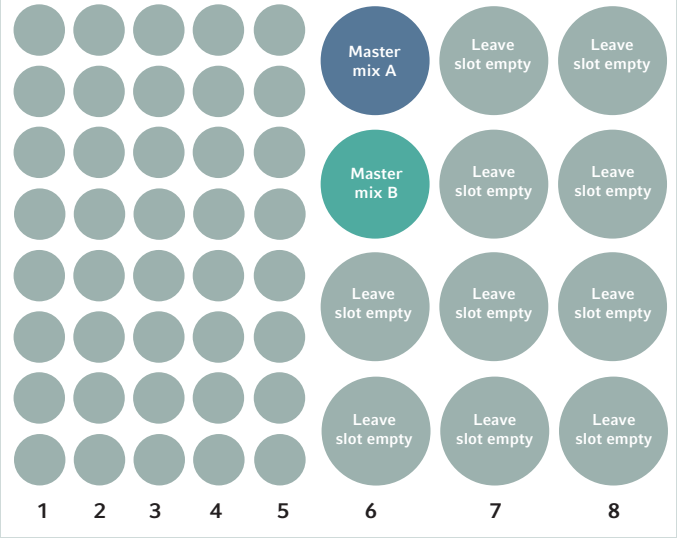

Figure 2: Rack ILMN tube layout for sub-method 01

Sub-Method 02

Start with up to 24 (in 48 wells) target-enriched samples in the "Work Plate" (the same "Work Plate" as in sub-method 01). Each sample pair is placed in adjacent wells of the same row (e.g. A1 and A2). Remove the caps from the kit-supplied i7 and i5 index tubes and place them according to Figure 4. Both i5 indexes should always be supplied. For less samples, reduce the number of i7 indexes column-wise from the right side. TAM (TruSight Tumor Amplification Mix) should be supplied in an Eppendorf 1.5 mL Safe-Lock tube with 10% excess.

Worktable Layout

Position	Item
A2	50 µL Filtertips
A3	50 µL Filtertips
TMX	Thermoblock PCR 96 OC + semi-skirted PCR plate (Work Plate)
B4	Rack ILMN tubes

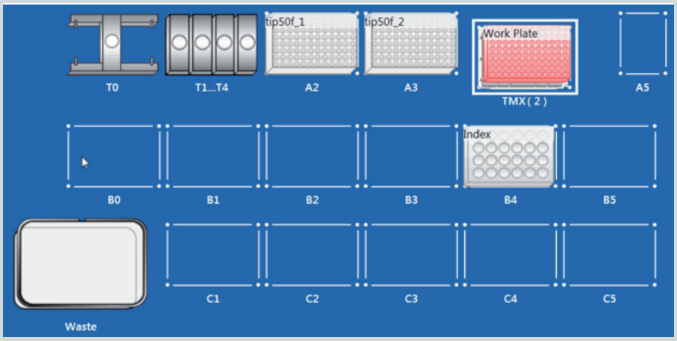

Figure 3: ep*Motion* worktable layout for sub-method 02

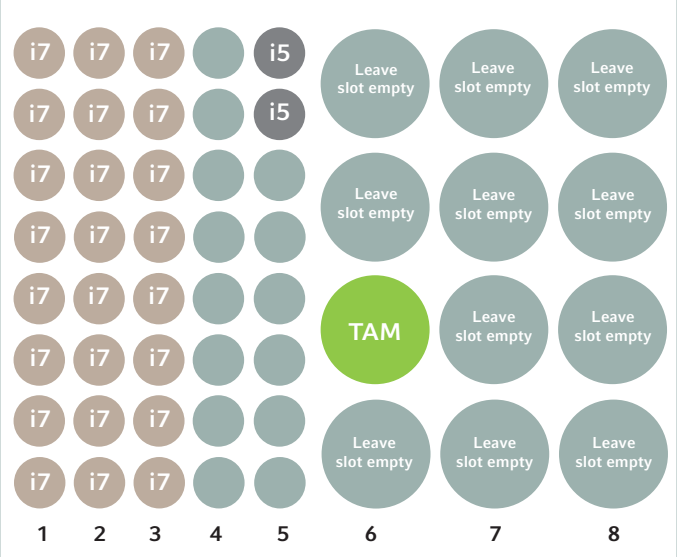

Figure 4: Rack ILMN tube layout for sub-method 02

Sub-Method 03

Start with up to 24 (in 48 wells) indexed samples in the "Work Plate" (the same "Work Plate" as in sub-method 01/02). All reagents in the ReservoirRack (Figure 6) should be supplied in 30 mL tubs with at least 10% excess. Make sure SPB (Sample Purification Beads) are fully re-suspended before transferring to the tub.

Worktable Layout

Position	Item
A2	50 µL Filtertips
A3	300 µL Filtertips
TMX	Thermoblock PCR 96 OC
B0	400 mL tub for liquid waste
B2	300 µL Filtertips
B3	300 µL Filtertips
C1	Alpaqua MAGNUM FLX Enhanced Universal Magnet
C2	Thermoadapter PCR 96 + semi-skirted plate with samples (Work Plate)
C3	ReservoirRack (Reagents)
C5	Thermoadapter PCR 96 + empty semi-skirted plate

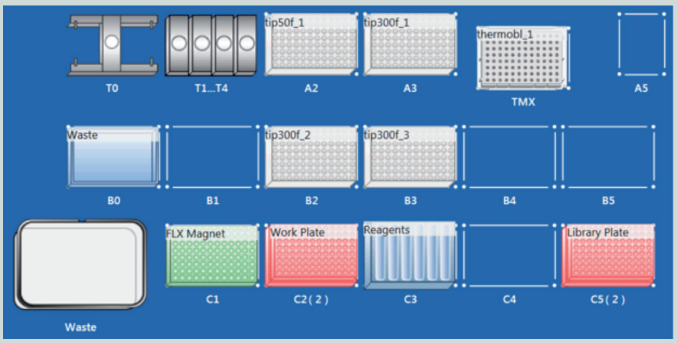

Figure 5: ep*Motion* worktable layout for sub-method 03

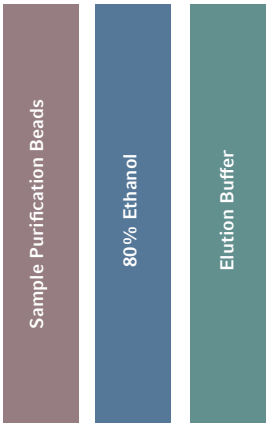

Figure 6: ReservoirRack layout for sub-method 03

Ordering information

Description	Order no. international
ep*Motion*® 5075t	5075 000.302
TS 50 Dispensing Tool	5280 000.010
TM300-8 Dispensing Tool	5280 000.231
TS300 Dispensing Tool	5280 000.037
Gripper	5282 000.018
Thermoblock PCR 96 OC	5075 751.666
Thermoadapter PCR 96	5075 787.008
ReservoirRack	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

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Eppendorf AG · 22331 Hamburg · Germany
eppendorf@eppendorf.com · www.eppendorf.com

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