SHORT PROTOCOL No. 17 I August 2016

Automated KAPA HyperPlus Library Preparation with the ep*Motion*® 5075t

Introduction

This protocol describes the system configuration and pre-programmed methods for automated construction of up to 48 (in multiples of eight) sequencing libraries from 1-200 ng of input DNA with the KAPA HyperPlus Library Preparation Kit (for Illumina® systems). Higher input is not officially validated but should be feasible with reduced PCR cycles.

The overall hands-on time is less than 15 minutes, and the total run time (including bead-based size selection but not the PCR amplification) is around 4 hours.

Bead-based size selection is indispensable in many NGS library construction procedures to maximize sequencing quality. However, people may elect to perform this step before the PCR amplification to allow more efficient enrichment of fragments of desired length, or after the PCR amplification when size selection may cause loss of rare sequences, or omit it when the workflow is optimized (i.e. good fragmentation and minimized residual primer dimers). To permit this flexibility, the procedure is divided into three sub-methods on the ep*Motion* 5075t.

Materials and Methods

Required equipment

> ep*Motion* 5075t
> Additional Thermal module (Position C2)
> Gripper
> TS 50 pipetting tool
> TM 50-8 pipetting tool
> TM 300-8 pipetting tool
> 2x Thermoadapter for PCR plates, 96-well
> 1x Thermoblock OC for PCR plates, 96-well
> 1x ReservoirRack
> 1x Reservoir rack Module TC for 1.5/2.0 mL Safe-Lock Tubes
> 1x Reservoir rack Module TC for 5.0 mL Safe-Lock Tubes
> 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-well, semi-skirted
> Eppendorf twin.tec PCR plates, 96-well, skirted (for the Index Adapters)
> Eppendorf Tubes® 1.5 mL, Safe-Lock
> Eppendorf Tubes 5.0 mL, Safe-Lock
> ep*Motion* Reservoir 30 mL
> Eppendorf 400 mL Reservoir
> 80 % Ethanol
> Elution Buffer (10mM Tris-HCl)
> Mineral oil, PCR/molecular biology grade (Sigma-Aldrich®, order no. M5904-500ML)
> KAPA HyperPlus Library Preparation Kit (KAPA, order no. KK8514)

Methods

Method Name	approx. Runtime (24 samples)
1-KAPAHyperPlus	160 min
2-KAPAHyperPlus	60 min
3-KAPAHyperPlus	45 min

The KAPA HyperPlus kit adopts an enzyme-based fragmentation strategy. Fragment size is related to the fragmentation time. Please refer to the kit manufacturer's manual for recommendations. The method provided sets the fragmentation time to 35 minutes as default.

Additionally, all incubation steps are performed on the on-deck TMX module with thermoshaker function and thermal module. To compensate for the fast heat loss on open surfaces, both the incubation temperature and time are given a small increase to match the intended result. Mineral oil is used to overcome evaporation when reaction mixes are incubated at high temperature (>37 °C).

The ligation time is also subject to user's modification. 15 minutes is set as the default, assuming optimized molar ratio between the adapter and sample.

Although size selection is not a mandatory step, it is recommended to incorporate this step in the workflow to exclude the unwanted fragments that will compete for flow cell capacity. A 0.6X-0.8X scheme is adopted by the provided method. To get different effect of size exclusion, other ratios may be used. However, related steps must be modified in sub-method 02.

This protocol is programmed to ensure the maximum walk-away time, therefore it is limited to process 48 samples. Processing 96 samples requires additional tips that exceed the deck capacity when running sub-method 01. The entire protocol is divided into three steps as shown below, each stops at a "safe stopping point" as defined by the kit manufacturer. Each master mix is required to be prepared manually to avoid leaving the stock reagents at room temperature (on deck) throughout the automated run.

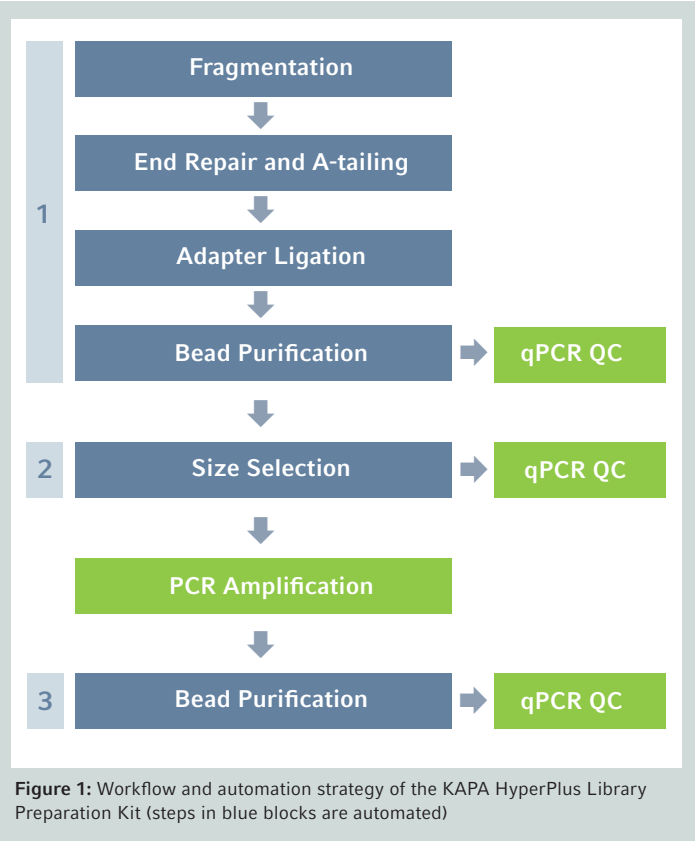

Figure 1: Workflow and automation strategy of the KAPA HyperPlus Library Preparation Kit (steps in blue blocks are automated)

Sub-method 01

Start with purified DNA in 35 µL reagent that contains no EDTA. DNA samples should be supplied in an Eppendorf twin.tec® 96-well semi-skirted PCR plate. Adapters should be supplied in an Eppendorf twin.tec 96-well full-skirted PCR plate. 10 µL of each adapter (corresponding to each sample in the "Work Plate") is recommended to overcome the dead volume of each well and evaporation loss.

5-10 % excess should be planned for master mixes supplied in the Reservoir Rack ("Reagents") to compensate for liquid loss due to adsorption to the tips and tubes. For reagents supplied in the 30 mL tubs, just pour in with extra and the system will identify the volume by optical scanning.

Worktable Layout

Position	Item
A2	50 µL Filtertips
A3	50 µL Filtertips
TMX	Thermoadapter PCR 96 OC
B0	400 mL tub for liquid waste
B1	50 µL Filtertips
B2	300 µL Filtertips
B3	300 µL Filtertips
B4	300 µL Filtertips

Position	Item
C1	Alpaqua MAGNUM FLX *Enhanced* Universal Magnet Plate
TEMP2	Thermoadapter PCR 96 + semi-skirted plate with samples (Work Plate)
C3	ReservoirRack (Reagents)
C4	Full-skirted plate with adapters (Adapters)
C5	Thermoadapter PCR 96 + empty semi-skirted plate (Elution Plate)

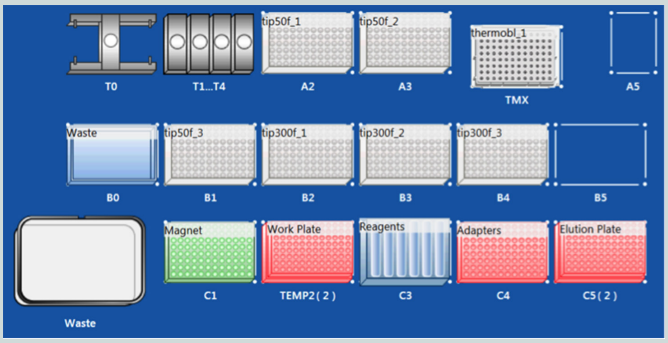

Figure 2: ep*Motion* worktable layout for sub-method 01

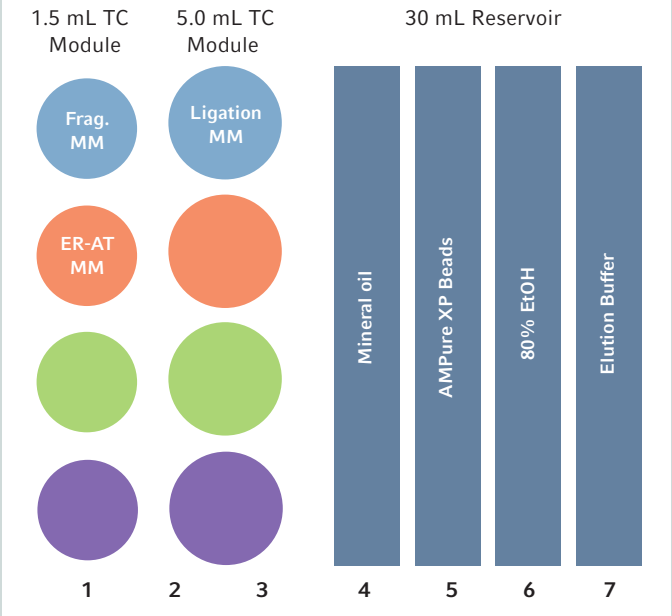

Figure 3: Reservoir rack layout for sub-method 01

Sub-method 02

Start with 50 µL purified sample stored in the "Work Plate" ("Elution Plate" from Sub-method 01).

Worktable layout

Position	Item
A2	50 µL Filtertips
A3	50 µL Filtertips
TMX	Thermoblock PCR 96 OC
B0	400 mL tub for liquid waste
B1	50 µL Filtertips
B2	300 µL Filtertips
B3	300 µL Filtertips
B4	300 µL Filtertips

Position	Item
C1	Alpaqua MAGNUM FLX Enhanced Universal Magnet Plate
TEMP2	Thermoadapter PCR 96 + semi-skirted plate with samples (Work Plate)
C3	ReservoirRack (Reagents)
C5	Thermoadapter PCR 96 + empty semi-skirted plate (Elution Plate)

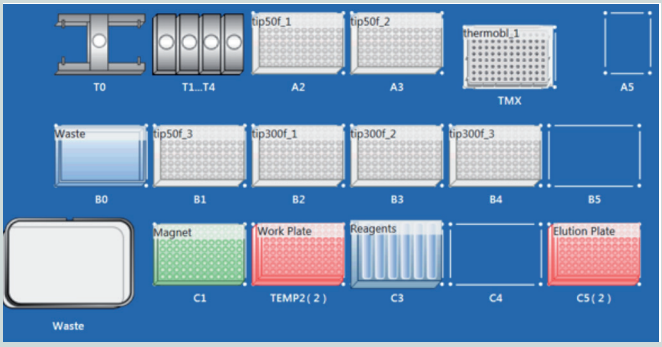

Figure 4: ep*Motion* worktable layout for sub-method 02

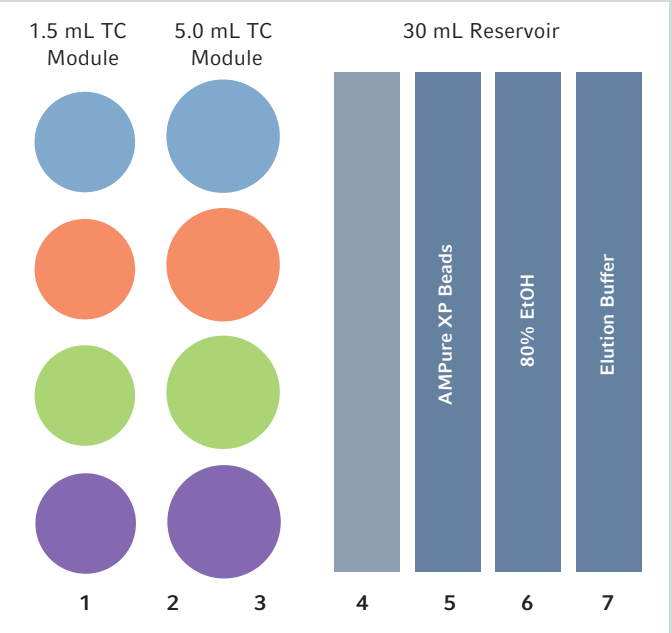

Figure 5: Reservoir rack layout for sub-method 02

Sub-method 03

Start with 50 µL amplified library stored in the "Work Plate" ("Elution Plate" from Sub-method 02).

Worktable layout

Position	Item
A2	50 µL Filtertips
A3	50 µL Filtertips
TMX	Thermoblock PCR 96 OC
B0	400 mL tub for liquid waste
B2	300 µL Filtertips
B3	300 µL Filtertips
B4	300 µL Filtertips

Position	Item
C1	Alpaqua MAGNUM FLX Enhanced Universal Magnet Plate
TEMP2	Thermoadapter PCR 96 + semi-skirted plate with samples (Work Plate)
C3	ReservoirRack (Reagents)
C5	Thermoadapter PCR 96 + empty semi-skirted plate (Elution Plate)

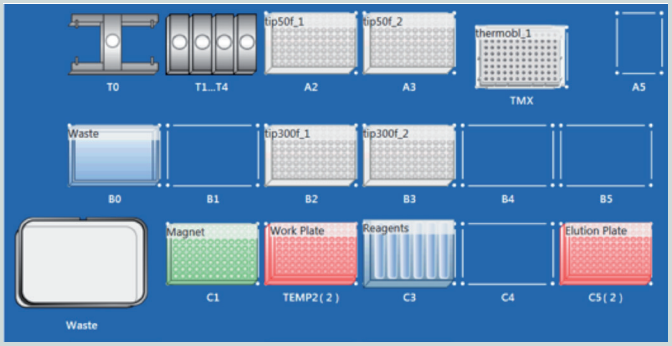

Figure 6: ep*Motion* worktable layout for sub-method 03

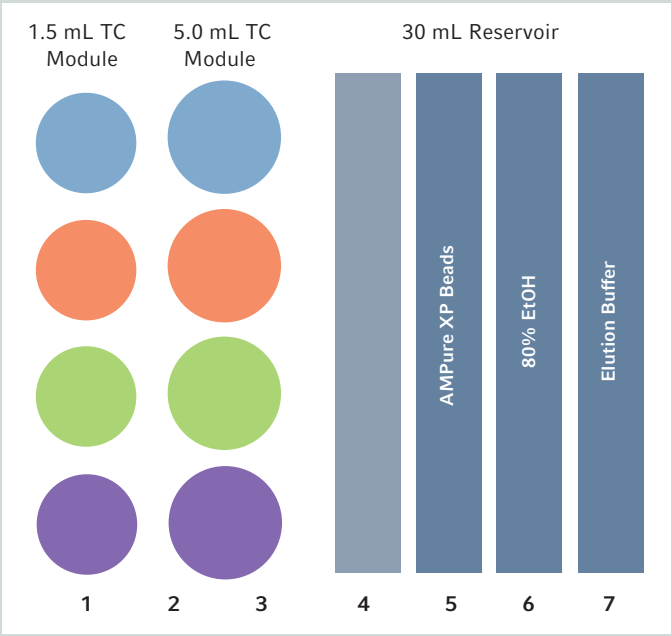

Figure 7: Reservoir rack layout for sub-method 03

Results

Typically, a BioAnalyzer® or equivalent device (TapeStation® or Fragment Analyzer) is used to analyze the final library for quality assessment before sequencing. The electropherogram may show varied size distribution depending on the fragmentation time and whether size selection is performed. The following data show library examples from a 2100 BioAnalyzer with the High-Sensitivity DNA chip. All samples were diluted 5-fold.

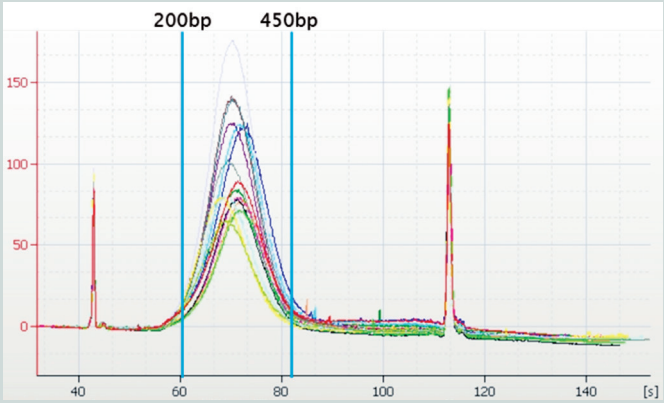

Figure 8: Electropherogram of sequencing-ready libraries. All DNA samples were fragmented for 35 minutes and subjected to 0.6X-0.8X size selection before the PCR amplification step.

Ordering information

Description	Order no. international
ep*Motion*® 5075t	5075 000.302
Thermal module	5075 757.001
TS 50 Dispensing Tool	5280 000.010
TM50-8 Dispensing Tool	5280 000.215
TM300-8 Dispensing Tool	5280 000.231
Gripper	5282 000.018
Thermoblock PCR 96 OC	5075 751.666
Thermoadapter PCR 96 (2x)	5075 787.008
Reservoir rack	5075 754.002
Reservoir Rack Module TC Safe –Lock	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, 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, 5.0 mL	0030 108.310

For more information about Roche sequencing solutions, please visit: **sequencing.roche.com**

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