

Flexible Library Preparation for Your Cancer Research Lab – Automate AmpliSeq[™] for Illumina[®] Kits on the ep*Motion*[®]

A targeted cancer research solution

Abstract

Due to the growing number of relevant genes and variants, next generation sequencing (NGS) has become a standard technique in the field of cancer research and molecular pathology. The AmpliSeq for Illumina library preparation panels are used to sequence DNA or RNA samples which have been isolated from a broad range of

primary samples. These could be blood, cell culture, fresh frozen tissue and also challenging samples such as formalin-fixed paraffin-embedded (FFPE) tissue. Highlighted by a few selected workflows, we illustrate here the ease of automating the preparation of NGS libraries on the ep*Motion* 5075t liquid handling workstation.

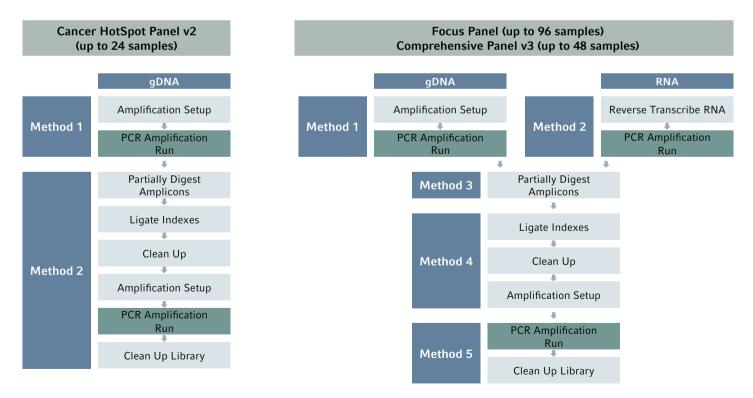


Figure 1: Overview of automated library preparation for the AmpliSeq for Illumina Cancer HotSpot Panel v2, the Focus Panel and the Comprehensive Panel v3 kits. The automated workflow is structured into submethods (blue boxes) that end at safe stopping points. The green boxes indicate PCR steps using the Eppendorf Mastercycler® X50. The epMotion performs the library preparation procedure starting with either genomic DNA or RNA. The number of samples per run (indicated) is variable to meet the user's daily requirements.



Table 1: Consumption of epT.I.P.S.® Motion and LoBind twin.tec 96 plates to process 24 reactions per run. The automated methods are capable of processing variable numbers of samples in less than one day. The hands-on time for setting up the individual sub-methods is roughly 10 minutes.

Step (24 reactions)	Cancer HotSpot Panel v2		Focus Panel			Comprehensive Panel v3			
	Tip Consumption	Plate Consumption	Time (min.)	Tip Consumption	Plate Consumption	Time (min.)	Tip Consumption	Plate Consumption	Time (min.)
Method 1	50 μl: 24 300 μl: 0	1	11	50 μl: 12 300 μl: 0	1	6	50 μl: 35 300 μl: 0	2	8
Method 2	50 μl: 121 300 μl: 250	0	170	50 μl: 25 300 μl: 0	1	54	50 μl: 64 300 μl: 0	2	59
Method 3	-	-	-	50 μl: 44 300 μl: 0	0	51	50 μl: 68 300 μl: 0	0	57
Method 4	-	-	-	50 μl: 80 300 μl: 96	0	82	50 μl: 80 300 μl: 96	0	82
Method 5	-	-	-	50 μl: 48 300 μl: 128	2	50	50 μl: 48 300 μl: 128	1	50
Total	50 μl: 145 300 μl: 250	1	181	50 μl: 209 300 μl: 224	4	243	50 μl: 295 300 μl: 224	5	256

Sequencing performance of the automated library preparation using gDNA standards

To demonstrate the performance of the automated methods on the ep*Motion* we performed a library preparation with 8 replicate library preparations of a gDNA standard (Coriell Institute) which were sequenced on the Illumina MiSeq® with 2×150bp reads. All 8 replicates show the genotypes which are expected with this standard gDNA extract (table 2), resulting in a 100% call rate concordant to reference data. The sequencing coverage across all amplicons of the Cancer HotSpot Panel v2 is shown in figure 2.

Table 2: Sequencing data from 8 replicate libraries, which were automatically prepared from a gDNA standard using the ep*Motion* 5075t and the AmpliSeq for Illumina Cancer HotSpot Panel v2.

	ep <i>Motion</i> replicate							
	1	2	3	4	5	6	7	8
Number of single nucleotide variant	9	9	9	9	9	9	9	9
Number of multi nucleotide variant	1	1	1	1	1	1	1	1
Number of indel variants	1	1	1	1	1	1	1	1
Rate	100%	100%	100%	100%	100%	100%	100%	100%

eppendorf

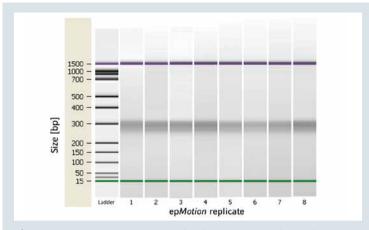


Figure 2: Fragment length analysis of the 8 post-enriched library preparations using the BioAnalyser® 2100.

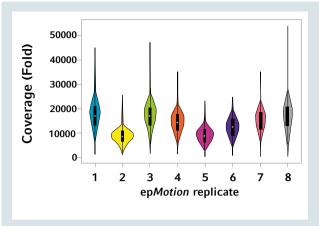


Figure 3: Distribution of sequencing coverage of the amplicons which are targeted with the AmpliSeq for Illumina Cancer HotSpot Panel v2.

Table 3: Minimum reagent requirements for a run with 24 reactions. For the Focus panel and Comprehensive v3 panel, the volumes shown are split among 12 samples running the RNA and DNA version of the protocol. For the Cancer HotSpot Panel v2 the calculation is based on a run with 24 reactions for a DNA pool each. The volumes provided are sufficient for a run with 24 reactions on the ep*Motion*. However, the volumes could become limited if a kit is used in multiple runs.

Ampliseq for Illumin	Cancer Hotspot Panel v2	Focus Panel	Comprehensive Panel v3	
Kit name/Manufacturer	Reagent	24 reactions (DNA)	24 reactions (DNA + RNA)	24 reactions (DNA + RNA)
Ampliseq cDNA Synthesis	10x AmpliSeq RT Enzyme Mix	-	13 μΙ	13 μΙ
for Illumina / Illumina / #0022654	5x AmpliSeq cDNA Reaction Mix	-	27 μl	27 μΙ
	5x HiFi Mix	98 μΙ	64 μΙ	120 μΙ
	FuPa Reagent	52 μΙ	64 μΙ	64 μΙ
AmpliSeq Library PLUS for Illumina	Switch Solution	100 μΙ	112 μΙ	112 μΙ
(96 rxns)/ Illumina / #200190102	DNA Ligase	52 μΙ	64 μΙ	64 μΙ
(24 rxns)/ Illumina / #200190101	10X Libray Amp Primers	122 μΙ	122 μΙ	122 μΙ
	1X Lib Amp Mix	1092.6 μΙ	1094 μΙ	1094 μΙ
	Low TE	754 μΙ	1327 μΙ	1327 μΙ
AmpliSeq Cancer HotSpot Panel v2 for Illumina / Illumina / # 20019161	5X AmpliSeq Cancer HotSpot v2 Panel	98 μΙ	-	-
AmpliSeq Focus Panel for	5X AmpliSeq Focus DNA Panel Pool 1	-	50 μΙ	-
Illumina / Illumina / #20019164	5X AmpliSeq Focus RNA Panel Pool 1	-	50 μΙ	-
	2X AmpliSeq Comprehensive Panel v3 DNA Panel Pool 2	-	-	64 μΙ
AmpliSeq Comprehensive Panel v3 for	2X AmpliSeq Comprehensive Panel v3 DNA Panel Pool 2	-	-	64 μΙ
Illumina / Illumina / #20019109	5X AmpliSeq Comprehensive Panel v3 RNA Panel Pool 1	-	-	28 μΙ
	5X AmpliSeq Comprehensive Panel v3 RNA Panel Pool 1	-	-	28 μΙ
AmpliSeq CD indexes set A for Illumina (96 indexes, 96 samples) / Illumina /#20019105		4 μl (x24)	4 μl (x24)	4 μl (x24)
Agencourt® AMPure XP bead/ Fisher Scientific® / NC9959336 or A63881		2965 μΙ	3730 μΙ	3730 μΙ
Vapor Lock® / Qiagen® / 981611		254 μΙ	380 μΙ	380 μΙ



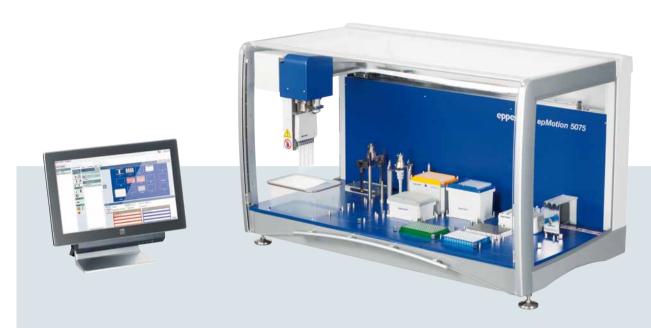


Figure 4: The Eppendorf epMotion is a multi-purpose liquid handling workstation, suitable for many laboratory procedures. The epMotion 5075t and other models in the family are ideal walk-away companions for labs that demand high efficiency, accuracy and automated workflows. The on-deck thermomixer and the incorporated thermal module provide incubation capability needed for the NGS library preparation. Eight dispensing tools covering 0.2-1000 µL range are available in both single- and 8-channel formats to meet different throughput and volume requirements. For labware transports between e.g. magnets or temperature controlled positions, a gripper is available.

Ordering information

Description	Order no. international	Order no. North America		
epMotion® 5075t NGS Solution	5075 000.962	5075000965		
The survey de side of DCD O/ (1.1)	F07F 707 000	0/0003100		
Thermoadapter PCR 96 (1x)	5075 787.008	960002199		

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