

Flexible Library Preparation for Your Cancer Research Lab – Automate AmpliSeq™ for Illumina® Kits on the epMotion®

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 epMotion 5075t liquid handling workstation.

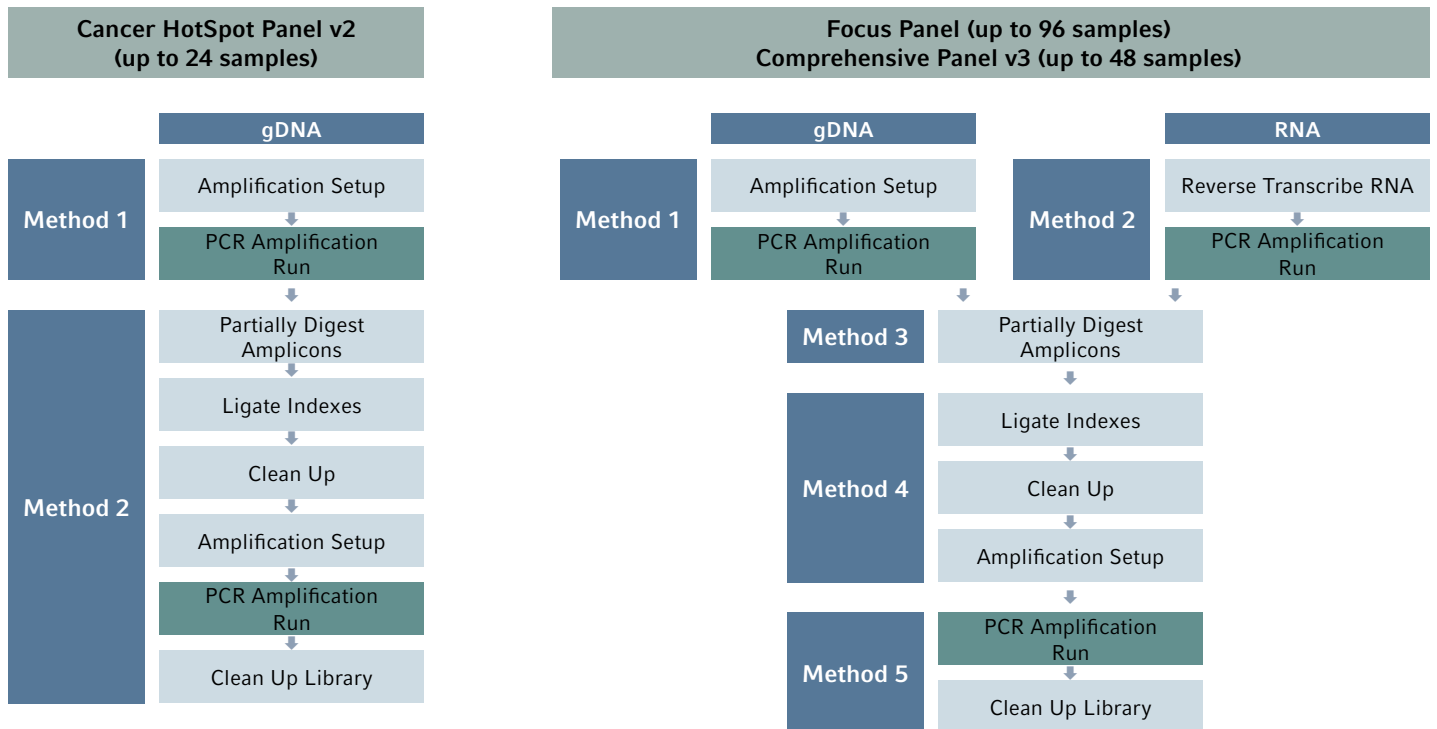


Figure 1: Overview of automated library preparation for the AmpliSeq for Illumina Cancer HotSpot Panel v2, the Focus Panel and the Comprehensive Panel v3 kits. The automated workflow is structured into submethods (blue boxes) that end at safe stopping points. The green boxes indicate PCR steps using 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.

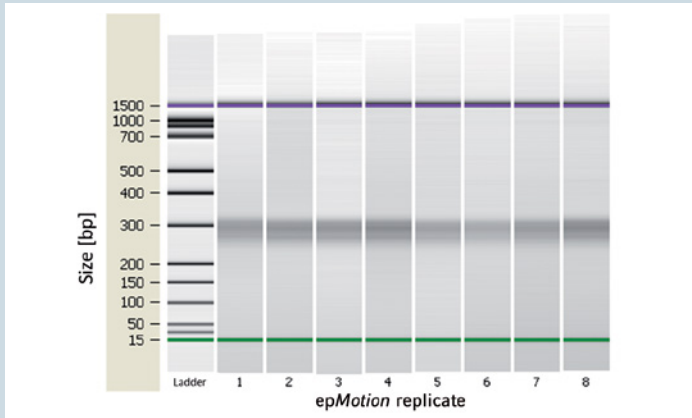


Figure 2: Fragment length analysis of the 8 post-enriched library preparations using the BioAnalyser® 2100.

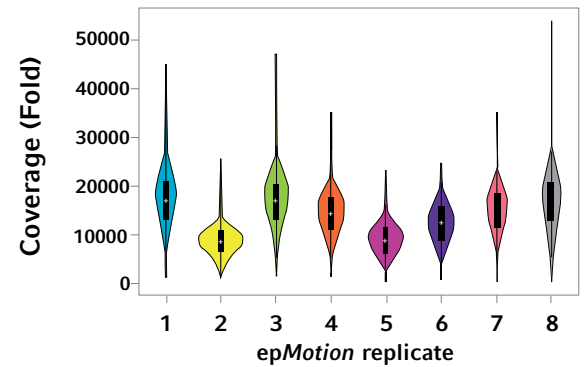


Figure 3: Distribution of sequencing coverage of the amplicons which are targeted with the AmpliSeq for Illumina Cancer HotSpot Panel v2.

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 epMotion. However, the volumes could become limited if a kit is used in multiple runs.

Ampliseq for Illumina		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 for Illumina / Illumina / #0022654	10x AmpliSeq RT Enzyme Mix	-	13 µl	13 µl
	5x AmpliSeq cDNA Reaction Mix	-	27 µl	27 µl
Ampliseq Library PLUS for Illumina (96 rxns)/ Illumina / #200190102 (24 rxns)/ Illumina / #200190101	5x HiFi Mix	98 µl	64 µl	120 µl
	FuPa Reagent	52 µl	64 µl	64 µl
	Switch Solution	100 µl	112 µl	112 µl
	DNA Ligase	52 µl	64 µl	64 µl
	10X Libray Amp Primers	122 µl	122 µl	122 µl
	1X Lib Amp Mix	1092.6 µl	1094 µl	1094 µl
	Low TE	754 µl	1327 µl	1327 µl
Ampliseq Cancer HotSpot Panel v2 for Illumina / Illumina / # 20019161	5X AmpliSeq Cancer HotSpot v2 Panel	98 µl	-	-
Ampliseq Focus Panel for Illumina / Illumina / #20019164	5X AmpliSeq Focus DNA Panel Pool 1	-	50 µl	-
	5X AmpliSeq Focus RNA Panel Pool 1	-	50 µl	-
Ampliseq Comprehensive Panel v3 for Illumina / Illumina / #20019109	2X AmpliSeq Comprehensive Panel v3 DNA Panel Pool 2	-	-	64 µl
	2X AmpliSeq Comprehensive Panel v3 DNA Panel Pool 2	-	-	64 µl
	5X AmpliSeq Comprehensive Panel v3 RNA Panel Pool 1	-	-	28 µl
	5X AmpliSeq Comprehensive Panel v3 RNA Panel Pool 1	-	-	28 µl
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 µl	3730 µl	3730 µl
Vapor Lock® / Qiagen® / 981611		254 µl	380 µl	380 µl

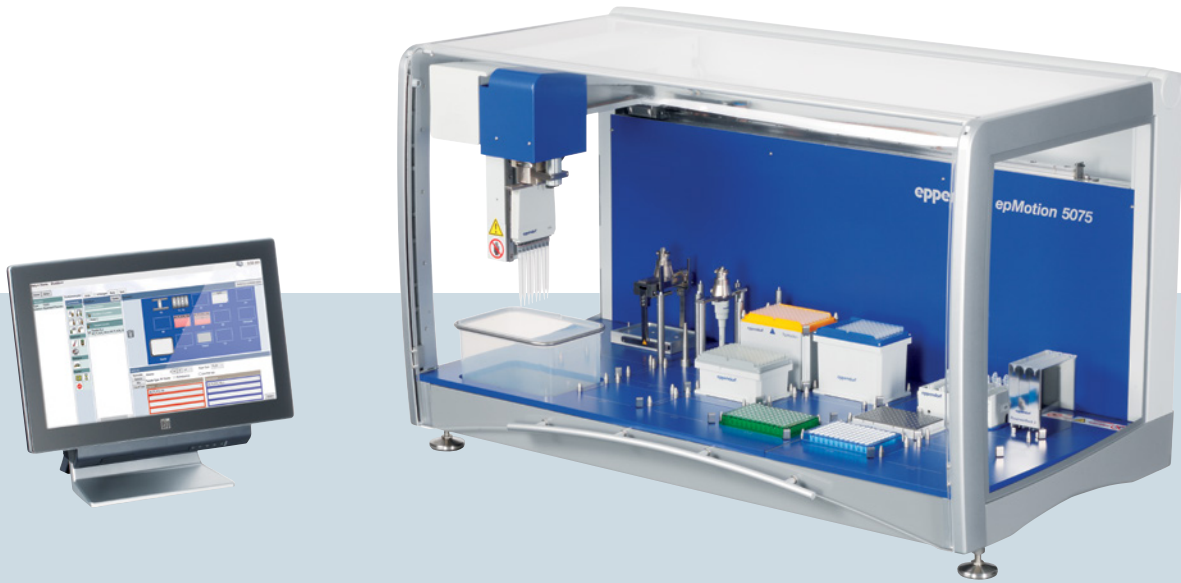


Figure 4: The Eppendorf epMotion is a multi-purpose liquid handling workstation, suitable for many laboratory procedures. The epMotion 5075t and other models in the family are ideal walk-away companions for labs that demand high efficiency, accuracy and automated workflows. The on-deck thermomixer and 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
Thermoadapter PCR 96 (1x)	5075 787.008	960002199

Your local distributor: www.eppendorf.com/contact
 Eppendorf AG · Barkausenweg 1 · 22339 Hamburg · Germany
eppendorf@eppendorf.com · www.eppendorf.com

www.eppendorf.com

Agencourt® is a registered trademark of Beckman Coulter; Inc., USA. Fisher Scientific® is a registered trademark of Fisher Scientific Company LLC, USA. Vapor Lock® is a registered trademark of Corbett Life Science Pty Ltd., Australia. Qiagen® is a registered trademark of Qiagen GmbH, Germany. Illumina® and MiSeq® are registered trademarks of Illumina, Inc., USA. Bioanalyzer® is a registered trademark of Agilent Technologies, Inc. AmpliSeq™ is a trademark of Thermo Fisher Scientific Inc., USA. Eppendorf®, the Eppendorf Brand Design, Mastercycler®, Eppendorfr twin.tec®, Eppendorf LoBind®, epMotion®, and epT.I.P.S.® are registered trademarks of Eppendorf AG, Germany. All rights reserved including graphics and images. Copyright © 2018 by Eppendorf AG