DeepChek®

NGS Library Preparation + UDI Adapters (MGI)

(RUO)

24
96

User Guide

Version 1 – Revision 0

For Research Use Only (RUO). Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of disease.

REF **203A24** (GTIN: 05407007961214) bundled with **204A24** (GTIN: 05407007961177)
203A96 (GTIN: 05407007961221) bundled with **204A96** (GTIN: 05407007961184)

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Document control

Date	Device version	IFU version	Description of change
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Contents

Application

The **DeepChek® NGS Library Preparation** is a collection of optimized reagents designed to convert an input DNA into indexed libraries for Next Generation Sequencing. The output libraries are compatible with different MGI platforms.

The **DeepChek® NGS Library Preparation** is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of Next Generation Sequencing.

Special conditions for use statements

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Indication of use

The **DeepChek® NGS Library Preparation** is suited for low and high-throughput NGS library construction workflows that require DNA fragmentation, end repair, A-tailing, adapter ligation and library amplification. It is designed for library construction from a wide range of sample types, and are compatible with complex, genomic DNA; low-complexity samples such as small viral genomes, plasmids, cDNA and long amplicons; and low-quality DNA such as FFPE samples. Libraries generated by this procedure are used for Next Generation Sequencing on different MGI platforms such as E25, G99, and G400.

Principles of the assay

The assay workflow combines enzymatic and bead clean-up steps. All the enzymatic reactions are performed in one single tube. First the reaction starts with the fragmentation using designed fragmentation enzyme to produce dsDNA fragments. Blunt ended dsDNA fragments produced further undergo an end repair and A-tailing to produce end-repaired 5'-phosphorylated, 3'-dA-tailed dsDNA fragments. At the end of this step adapter ligation reaction during which dsDNA adapters with 3'-dTTP overhangs are ligated to 3'-dA-tailed molecules is performed. Constructed libraries are cleaned up with magnetic beads before amplification which employs high-fidelity, low-bias PCR to amplify library fragments carrying appropriate adapter sequences on both ends. Finally, another beads cleanup is performed on amplified library products.

Assay components

The **DeepChek® NGS Library Preparation** is provided in 2 formats: 24 reactions (REF 203A24) or 96 reactions (REF 203A96).

Table 1: Assay components for 24 reactions (ref 203A24)

Label	Volume for 24 Rxn. (nb tube x volume)	Color cap	Storage
FEA Buffer MGI	1 x 165 µL	Red	-25°C to -15°C
FEA Enzyme MGI	1 x 330 µL	Pink	-25°C to -15°C
Ligation buffer MGI	1 x 815 µL	Green	-25°C to -15°C
DNA Ligase Buffer MGI	1 x 165 µL	Blue	-25°C to -15°C
Amplification Mix MGI	1 x 815 µL	Brown	-25°C to -15°C
Neutralization Buffer	1 x 165 µL	Clear	25°C to -15°C
Control DNA	1 x 10 µL	Black	25°C to -15°C

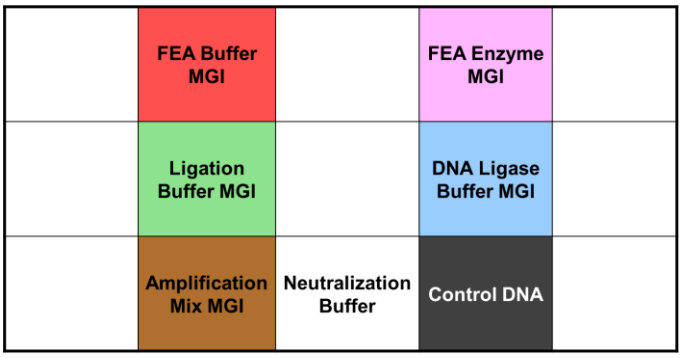

*Figure 1: Disposition of the assay components for the **reference 203A24***

Table 2: Assay components for 96 reactions (ref 203A96)

Reagent	Volume for 96 Rxn. (nb tube x volume)	Color Cap	Storage
FEA Buffer MGI	1 x 540 µL	Red	-25°C to -15°C
FEA Enzyme MGI	1 x 1090 µL	Pink	-25°C to -15°C
Ligation buffer MGI	2 x 1350 µL	Green	-25°C to -15°C
DNA ligase buffer MGI	1 x 540 µL	Blue	-25°C to -15°C
Amplification Mix MGI	2 x 1350 µL	Brown	-25°C to -15°C
Neutralization Buffer	1 x 540 µL	Clear	-25°C to -15°C
Control DNA	1 x 10 µL	Black	-25°C to -15°C

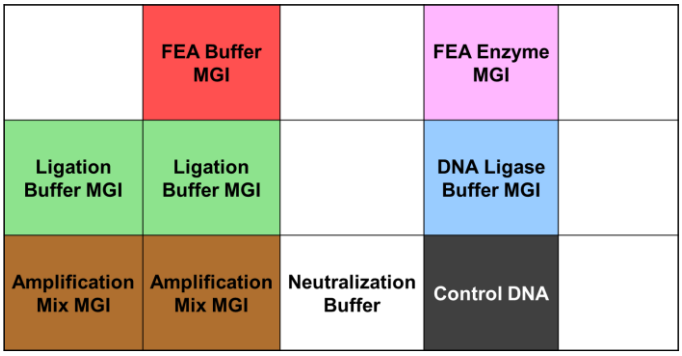

*Figure 2: Disposition of the assay components for the **reference 203A96***

Note: Do not mix the reagents from different batches.

Materials

Required and provided

- DeepChek® Normalization document (RUO) for MGI
  - MS Excel document developed by ABL to proceed with normalization.

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  - A version is tailored for each DeepChek® genotyping application (HIV, SARS-CoV-2 …)

- The **DeepChek® UDI Adapters (MGI)** is provided in plate format, containing 24 adapters (REF 204A24) or 96 adapters (REF 204A96).

Table 3: Assay components for 24 adapters (ref 204A24)

Reagent	Volume / well	Storage
Adapters (1 – 24)	5 µL	-25°C to -15°C

Table 4: Assay components for 24 adapters (ref 204A96)

Reagent	Volume / well	Storage
Adapters (1 – 96)	5 µL	-25°C to -15°C

For the disposition of adapters into the plate, please refer to the mapping label present on the kit.

Required but not provided

- PCR instrument e.g., ThermoFisher Scientific Proflex PCR System and associated specific material or any thermal cycler with enough ramp rate of ≥ 1°C/s.
- DeepChek® NGS Clean-up beads (ABL, REF N411-03 / N411-04, RUO)Benchtop centrifuge with rotor for 0.5 mL/1.5 mL reaction tubes (capable of attaining 10,000 rpm).
- Benchtop vortex mixer.
- Microliter pipets dedicated to PCR (0.1-2.5 µL; 1-10 or 1-20 µL; 20-200 µL; 1000 µL).
- Pipetting Robot (optional).
- Adjustable pipettes and nuclease-free aerosol-resistant sterile PCR pipet tips with hydrophobic filters.
- Appropriate PPE & workspaces for working with potentially infectious samples.
- Surface decontaminants such as DNAZap (Life Technologies), DNA Away (Thermo Fisher Scientific), RNAse Away (Thermo Fisher Scientific), 10% bleach.
- Nuclease-free dH2O.
- 0.5 mL or 1.5 mL RNase- and DNase-free PCR tubes.
- Ice/Icebox or even cooling blocks.
- 96 well plate cooler (optional).
- 96 well PCR plates.
- Plate seals.
- Plate centrifuge.
- 0.2 mL thin walled 8 tube & domed cap.

Reagent storage and handling

- The reagents of this kit are shipped with dry ice and should be maintained and stored immediately upon receipt between -25°C to -15°C to avoid compromising cold chain integrity.
- Before use, always thaw and mix kit components, except enzymes.
- Keep all enzymes components and master mixes on ice during handling and preparation.
- Expiration date: please refer to the label on the kit box.

Library preparation workflow overview

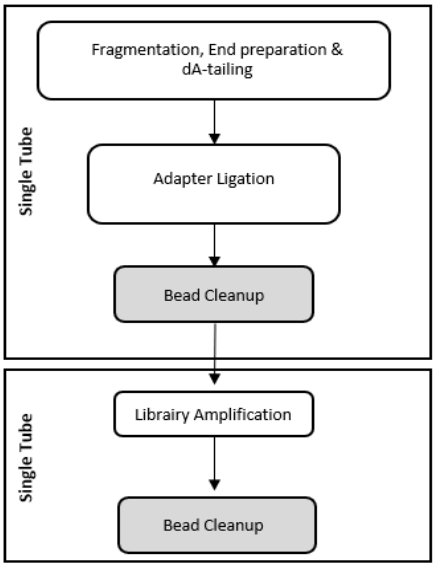


Figure 3: NGS library preparation workflow overview

Library preparation protocol

IMPORTANT POINTS BEFORE STARTING

- Use PCR product purified and eluted in water or 10 mM Tris-HCl (pH 8.0-8.5). **Buffer must not contain EDTA which can affect enzymatic fragmentation.**
- If the solvent contains EDTA, the corresponding amount of neutralization buffer can be added according to the final concentration of EDTA in the fragmentation solution, in order to neutralize the EDTA.

EDTA final concentration of fragmentation solution	The volume of neutralization buffer
1 mM	5 µL
0.8 mM	4 µL
0.6 mM	3 µL
0.5 mM	2.5 µL
0.4 mM	2 µL
0.2 mM	1 µL
0.1 mM	0.5 µL
< 0.1 mM	0 µL

Too much neutralization buffer can cause overreaction during fragmentation.

- Before starting the library preparation, input DNA must be purified and quantified by magnetic beads and fluorometric methods, respectively. A total of 50 ng DNA in molecular grade water is necessary for the library construction. Proceed to the Quantification step using a Qubit instrument then proceed to the Normalization step.
- Perform dilution of each amplicon/target separately to 50 ng in 35µL (corresponds to 1.4 ng / µL) using molecular grade water DNase/RNase Free using the DeepChek® Normalization MS Excel document for MGI.

- If applicable, proceed to the pooling of all targets of the same sample in one tube make sure that all targets have been normalized to 50 ng before pooling) (example: **HIV RT PR INT**, proceed to the pooling of all **3 targets** in one tubes)
- Avoid multiple freeze-thaw cycles of samples and reagents. Make single use aliquot.
- Include negative control (replace the template nucleic acid by nuclease-free water) in reaction mixes to detect possible contamination.

Enzymatic fragmentation

1) Make sure to start the "Enzymatic Fragmentation" step using 50 ng DNA in 35 µl molecular grade water. If applicable, start with normalized PCR amplicon pool (50 ng in 35 µL in molecular grade water).

2) Prepare a fragmentation reaction, End-Repair and A-tailing PCR reaction mix on ice according to **Table 5**. Prepare a volume of reaction greater (n+1) than that required for the total number of reactions to be performed.

Table 5: Reaction components for fragmentation reaction, End Repair and A-tailing

Reaction Mix	Reagent	Volume
Reaction Mix	FEA Buffer MGI	5 µL
	FEA Enzyme MGI	10 µL
Input material	dsDNA	35 µL
Final Volume		**50 µL**

3) Manually mix up and down gently the reaction mix, spin down. Keep mix on ice and proceed to next step.

4) Incubate in a thermocycler pre-cooled to 4°C and programmed according to **Table 6**.

Table 6: Fragmentation, End Repair and A-tailing Program

Time	Temperature
Hot Lid of 105°C	ON
12 min	37°C
30 min	65°C
∞	4°C

5) Transfer reactions to ice and proceed *immediately* to the next step. This is not a Safe Stopping Point.

Library construction

1) Prepare the reaction mix according to **Table 7**. Prepare a volume of mix greater (n+1) than that required for the total number of reactions to be performed.

Table 7: Reaction components for Adapter Ligation

Reaction Mix	Reagent	Volume
Reaction Mix	Ligation buffer MGI	25 µL
	DNA Ligase MGI	5 µL
	Adapter DNBSEQ*	5 µL
	Water Nuclease Free	15 µL
	Fragmented repaired detailed DNA	50 µL
Final volume		**100 µL**

(*) For 50 ng of DNA input dilute the Adapter DNBSEQ (1:2).

Note 1: Adapter DNBSEQ is in a single tube format.

Note 2: The quality and amount of adapters directly affect the preparation efficiency and library quality. If the adapter input is excessive, it may lead to residual adapters or adapter dimers. If the adapter input is too little, it may affect ligation efficiency and reduce library yield.

2) Manually mix up and down the reaction mix, spin down. Keep the mix on ice and proceed to the next step.
3) Incubate in a thermocycler programmed according to **Table 8**. Use a heated lid for this step.

Table 8: Adapter Ligation Program

Time	Temperature
Hot lid of 105°C	ON
15 min	20°C
∞	4°C

4) Perform beads cleanup according to **Table 9**.

Table 9: Reaction components for beads cleanup

Reagent	Volume
Adapter ligation reaction product	100 µL
DNA Magnetic Beads	60 µL
Final Volume	**160 µL**

a) Mix thoroughly by vortexing and spin down briefly.
b) Incubate at room temperature for 5 min to bind DNA to the beads.
c) Place the PCR plate on a magnetic stand to capture the beads. Then, incubate for 3-5 min or until the liquid is clear.
d) With the plate still on the magnetic stand, carefully remove and discard the supernatant without disturbing the beads.
e) With the plate on the magnetic stand, add 200 µL of **freshly prepared** 80% ethanol. Do not mix.
f) Incubate the PCR plate on the magnetic stand at room temperature for 30 sec.

g) Carefully remove and discard the supernatant without disturbing the beads.
h) Repeat steps e) to g) once.
i) Dry the beads at room temperature for 3-5 min, or until all the ethanol has evaporated. DO NOT OVER-DRY THE BEADS.
j) Remove the PCR plate from the magnetic stand.
k) Resuspend the beads in 22 µL of elution buffer or molecular grade DNase/RNase free water.
l) Incubate the PCR plate at room temperature for 2 min to elute DNA off the beads.
m) Place the PCR plate back on a magnetic stand to capture the beads. Incubate for 2 min or until the liquid is clear.
n) Transfer 20 µL of the clear supernatant to a new PCR plate.
o) Proceed to the next step or store purified adapter-ligated libraries at 4°C for 1 week, or at -20°C.

Library Amplification

1) Prepare the PCR mix according to **Table 10**. Prepare a volume of mix greater (n+1) than that required for the total number of reactions to be performed.

Table 10: Reaction components for PCR

Reaction Mix	Reagent	Volume
Reaction Mix	Amplification Mix MGI	25 µL
	UDI Adapters (MGI)*	5 µL
	Adapter-ligated library	20 µL
Final Volume		**50 µL**

(*): DeepChek® UDI ADAPTERS (MGI) (24) / (ABL, REF 204A24, RUO); DeepChek® UDI ADAPTERS (MGI) (96) / (ABL, REF 204A96, RUO).

Note: UDI Adapters (MGI) (Unique Dual Index / 10 bp) are in "Plate" format. Individual adapters are available in each well of the plate for direct use.

2) Mix thoroughly and spin down. Keep the mix on ice and proceed to the next step.
3) Incubate in a thermocycler programmed according to **Table 11**. Use a heated lid for this step.

Table 11: PCR Program

Time	Temperature	Cycles
3 min	95°C	1
20 sec	98°C	8
15 sec	60°C	
30 sec	72°C	
5 min	72°C	1
∞	4°C	1

4) Store the tubes at 4°C or -20°C for up to 72 hours or proceed directly to the next step.

Purification

1. Perform beads cleanup according to **Table 12**.

Table 12: Reaction components for beads cleanup

Reagent	Volume
Library amplification product	50 µL
DNA Magnetic Beads	45 µL
Final Volume	**95 µL**

a) Mix thoroughly by vortexing and spin down briefly.
b) Incubate at room temperature for 5 min to bind DNA to the beads.
c) Place the PCR plate on a magnetic stand to capture the beads. Then, incubate for 3-5 min or until the liquid is clear.
d) With the plate still on the magnetic stand, carefully remove and discard the supernatant without disturbing the beads.
e) With the plate on the magnetic stand, add 200 µL of **freshly prepared** 80% ethanol. Do not mix.
f) Incubate the PCR plate on the magnetic stand at room temperature for 30 sec.
g) Carefully remove and discard the supernatant without disturbing the beads.
h) Repeat steps e) to g) once.
i) Dry the beads at room temperature for 3-5 min, or until all the ethanol has evaporated. DO NOT OVER-DRY THE BEADS.
j) Remove the PCR plate from the magnetic stand.
k) Resuspend the beads in 32 µL of elution buffer or molecular grade DNase/RNase free water.
l) Incubate the PCR plate at room temperature for 2 min to elute DNA off the beads.
m) Place the PCR plate on a magnetic stand to capture the beads. Incubate for 2 min or until the liquid is clear.
n) Transfer 30 µL of the clear supernatant to the new PCR plate.
o) Store purified adapter-ligated libraries at 4°C for 1 week, or at -20°C for long-term storage.

Library quality control

1) Electrophoretic profile (optional)

Proceed to quality control with Agilent High Sensitivity chip or similar product (for instance Agarose gel 0.8-2%).

2) Library quantification

Proceed to Library quantification and Normalization. Use Qubit quantification.

Next Generation Sequencing

After library preparation, use MGIEasy Circularization Kit (MGI).

NGS data analysis

NGS files containing nucleotide sequences are analyzed by the DeepChek® software or a generic bioinformatics tool. User shall then follow the software user guide to complete the data analysis and reporting processes.

Limitations

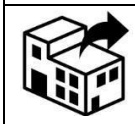
- For research use only. Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of disease.
- The kit is to be used by personnel specifically instructed and trained in NGS techniques.
- Strict compliance with the IFU is required for optimal results.
- Attention should be paid to expiration dates printed on the box and labels of all components. Do not use expired components.
- DeepChek® Normalization MS Excel is considered as a computerized system and it could be mandatory to validate it according to lab procedures. This file is intended to assess the library preparations, the provided information is merely for informational purposes. It is not intended to replace your own professional expertise. Should you decide to act upon any information on this document, you do so at your own risk. While the information of this document has been verified to the best of our abilities, we cannot guarantee that there is no mistake or errors. We reserve the right to change this document at any given time, of which you will be promptly updated.

Product quality control

- In accordance with ABL's Quality Management System, each lot of the assay is tested against predetermined specifications to ensure consistent product quality.
- Certificates of Analysis are available upon request.

Symbols

The following symbols may appear on the packaging and labeling:

 <N>	Contains reagents enough for <N> reactions		Consult instructions for use
	Caution		Manufacturer
	Catalog number		Serial Number
	Use by		Temperature limitation
	Distributor	**R*n***	R is for revision of the Instructions for Use (IFU) and n is the revision number
	Country of manufacture with a date of manufacture		

Contact Information

For technical assistance and more information, please see our Technical Support Center at Online: support-diag.ablsa.com; Email: support-diag@ablsa.com; Call +339 7017 0300 Or contact your ABL Field-Application Specialist or your local distributor. For up-to-date licensing information or product-specific disclaimers, see the respective ABL Assay User Guide. ABL User Guides are available at **www.ablsa.com/ifu** or can be requested from ABL Technical Services or your local distributor.

Manufacturer and distributors

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52-54 avenue du X Septembre
2550 Luxembourg, Luxembourg

AdvancedDx Biological Laboratories USA Inc.
5-7 Perry Way, Unit 15
Newburyport, MA 01950, USA

The customer is responsible for compliance with regulatory requirements that pertain to their procedures and uses of the medical device. The information in this guide is subject to change without notice.

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Version 1.0

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