DeepChek®

NGS Library Preparation + Adapters

V2 *(RUO)*

24
48
96

User Guide

Version 1 – Revision 2

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 116BX bundled with 124BX

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Document control

Date	Device version	IFU version	Description of change
2022/07/12	B	1.2	• Modification of the reaction mix tables for a better understanding • Addition of the 3 appendices

Contents

Application

The **DeepChek® NGS Library Preparation Assay V2** 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 Illumina platforms.

The **DeepChek® NGS Library Preparation Assay V2** 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

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.

Indication of use

The **DeepChek® NGS Library Preparation Assay V2** 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 Illumina platforms such as iSeq100, MiniSeq, MiSeq and NextSeq550.

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 Assay V2** is provided in 3 formats: 24 reactions (REF 116B24), 48 reactions (REF 116B48), or 96 reactions (REF 116B96).

Reagent	Total volume	Color Cap	Storage
FAE Buffer	135 µL	Red	-25°C to -15°C
FAE Enzyme	270 µL	Pink	-25°C to -15°C
Ligation buffer	670 µL	Green	-25°C to -15°C
DNA ligase	135 µL	Blue	-25°C to -15°C
PCR Primer Mix	135 µL	Yellow	-25°C to -15°C
PCR Mix	670 µL	Brown	-25°C to -15°C

Table 1: Assay components for 24 reactions (ref 116B24)

Reagent	Total volume	Color Cap	Storage
FAE Buffer	270 µL	Red	-25°C to -15°C
FAE Enzyme	530 µL	Pink	-25°C to -15°C
Ligation buffer	1350 µL	Green	-25°C to -15°C
DNA ligase	270 µL	Blue	-25°C to -15°C
PCR Primer Mix	270 µL	Yellow	-25°C to -15°C
PCR Mix	1350 µL	Brown	-25°C to -15°C

Table 2: Assay components for 48 reactions (ref 116B48)

Reagent	Total volume	Color Cap	Storage
FAE Buffer	530 µL	Red	-25°C to -15°C
FAE Enzyme	1100 µL	Pink	-25°C to -15°C
Ligation buffer	2650 µL	Green	-25°C to -15°C
DNA ligase	530 µL	Blue	-25°C to -15°C
PCR Primer Mix	530 µL	Yellow	-25°C to -15°C
PCR Mix	2650 µL	Brown	-25°C to -15°C

Table 3: Assay components for 96 reactions (ref 116B96)

Note: Do not mix the reagents from different batches.

Materials

Required and provided

- DeepChek® Normalization document (RUO)
  - MS Excel document developed by ABL to proceed with normalization.
  - A version is tailored for each DeepChek® genotyping application (HIV, SARS-CoV-2 …)
  - Denaturation/Dilution MiSeq instrument Nano V2 kit 2x 150bp (when applicable)
  - Denaturation/Dilution MiSeq instrument V2 kit 2x250 bp (when applicable)
  - Pool dilution iSeq 100 Instrument (when applicable)

- DeepChek® ADAPTERS – Plate Format
  - DeepChek® ADAPTERS (24) V2 / (ABL, REF 124B24, RUO)
  - DeepChek® ADAPTERS (48) V2 / (ABL, REF 124B48, RUO)
  - DeepChek® ADAPTERS (96) V2 / (ABL, REF 124B96, RUO)

Figure 1: "Plate" format for a single and direct use for either 24, 48 or 96 samples

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 (Cat ABL, REF N411-02)
- 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).
- Nuclease-free aerosol-resistant sterile PCR pipet tips with hydrophobic filters.
- Adjustable pipettes & fitting filtered pipette tips.
- 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 thermos 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

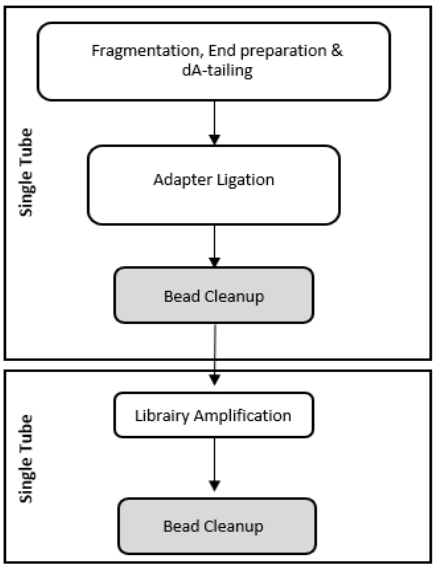


Figure 3: NGS library preparation workflow overview

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.
- Input DNA must be purified and quantified before processing by magnetic beads and fluorometric methods, respectively. A total of 25 ng in 8.75 µl of Dilution and Elution Buffer (or molecular grade water) is necessary for the library construction (equivalent to 3 ng/µL for the normalization). Proceed to the Quantification step using a Qubit instrument then proceed to the Normalization step.
- Perform dilution of each amplicon/target separately to 3 ng/µL using Molecular grade water DNase/RNase Free using the DeepChek® Normalization MS Excel document.
- For applicable solution, proceed to the pooling of all targets in one tube (make sure that all targets have been normalized at 3ng/µl 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) For applicable solutions, make sure to start the “Enzymatic Fragmentation” step using normalized-PCR-amplicon-pool at 3 ng/µL in order to obtain a total of 25 ng in 8.75 µl of Dilution.
2) For all other solutions, directly start the “Enzymatic Fragmentation” step from 25 ng in 8.75 µl of Diluent (equivalent to 3 ng/µL of the normalized PCR products).
3) Prepare a fragmentation reaction, End-Repair and A-tailing PCR reaction mix on ice according to **Table 4**. Prepare a volume of reaction greater (n+1) than that required for the total number of reactions to be performed.

Reaction Mix	Reagent	Volume
Reaction Mix	FAE Buffer	5 µL
	FAE Enzyme	10 µL
	Water Nuclease Free	26.25 µL
Input material	dsDNA	8.75 µL
Final Volume		**50 µL**

Table 4: Reaction components for fragmentation reaction, End Repair and A-tailing

4) Manually mix up and down gently the reaction mix, spin down. Keep mix on ice and proceed to next step.
5) Incubate in a thermocycler pre-cooled to 4°C and programmed according to **Table 5**.

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

Table 5: Fragmentation, End Repair and A-tailing Program

6) Transfer reactions to ice and proceed *immediately* to the next step.

Library construction

1) Prepare the reaction mix according to **Table 6**. Prepare a volume of mix greater (n+1) than that required for the total number of reactions to be performed.

Reaction Mix	Reagent	Volume
Reaction Mix	Ligation buffer	25 µL
	DNA Ligase	5 µL
	Adapter stock (1-96) *	1.25 µL
	Water Nuclease Free	18.75 µL
	Fragmented repaired detailed DNA	50 µL
Final Volume		**100 µL**

Table 6: Reaction components for Adapter Ligation

(*): DeepChek® ADAPTERS (24) V2/ (ABL, REF 124B24, RUO), DeepChek® ADAPTERS (48) V2 / (ABL, REF 124B48, RUO), DeepChek® ADAPTERS (96) V2 / (ABL, REF 124B96, RUO)

Note: adapters are in "Plate" format. Individual adapters are available in each well of the plate for direct use.

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 7**. Use a heated lid for this step.

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

Table 7: Adapter Ligation Program

4) Perform a 0.6X SPRI cleanup according to **Table 8**.

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

Table 8: Reaction components for SPRI cleanup

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. Gently and slowly mix up and down the beads and samples 12 times then incubate for 3-5 min or until the liquid is clear.
d) Carefully remove and discard the supernatant.
e) Remove the PCR plate from the magnetic stand, add 200 µl of **freshly prepared** 80% ethanol.
f) Incubate the PCR plate on the magnetic stand at room temperature for 30 sec.
g) Place the PCR plate on the magnetic stand, carefully remove and discard the supernatant.
h) Remove the PCR plate from the magnetic stand and add 200 µL of **freshly prepared** 80% ethanol. Gently and slowly mix up and down the beads and samples 4 to 5 times to resuspend then incubate at room temperature for 30 seconds
i) Place the PCR plate on the magnetic stand and incubate for 2 minutes.
j) With the PCR plate still on the magnetic stand, carefully remove and discard the clear supernatant without disturbing the beads.
k) Dry the beads at room temperature for 3-5 min, or until all the ethanol has evaporated. DO NOT OVER-DRY THE BEADS.
l) Remove the PCR plate from the magnetic stand.
m) Resuspend the beads in 22 µl of elution buffer or molecular grade DNase/RNase free water.
n) Incubate the PCR plate at room temperature for 2 min to elute DNA off the beads.
o) Place the PCR plate on a magnetic stand to capture the beads. Incubate for 3-5 min or until the liquid is clear.
p) Transfer 20 µl of the clear supernatant to a new PCR plate.
q) 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 9**. Prepare a volume of mix greater (n+1) than that required for the total number of reactions to be performed.

Reaction Mix	Reagent	Volume
Reaction Mix	PCR Mix	25 µL
	PCR Primer Mix	5 µL
	Adapter-ligated library	20 µL
Final Volume		**50 µL**

Table 9: Reaction components for PCR

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 10**. Use a heated lid for this step.

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

Table 10: PCR Program

4) Store the tubes at 4°C or -20°C for up to 72 hours or proceed directly to the next step.
5) Perform a 0.9X SPRI cleanup according to **Table 11**.

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

Table 11: Reaction components for SPRI cleanup

6) Mix thoroughly by vortexing and spin down briefly.
7) Incubate at room temperature for 5 min to bind DNA to the beads.
8) Place the PCR plate on a magnetic stand to capture the beads. Gently and slowly mix up and down the beads and samples 12 times then Incubate for 3-5 min or until the liquid is clear.
9) Carefully remove and discard the supernatant.
10) Remove the PCR plate from the magnetic stand and add 200 µL of **freshly prepared** 80% ethanol. Gently and slowly mix up and down the beads and samples 4 to 5 times to resuspend then incubate at room temperature for 30 seconds
11) Place the PCR plate on the magnetic stand and incubate for 2 minutes.
12) Repeat steps 10 to 11 for a total of 2 washed
13) With the PCR plate still on the magnetic stand, carefully remove and discard the clear supernatant without disturbing the beads.
14) Dry the beads at room temperature for 3-5 min, or until all the ethanol has evaporated. DO NOT OVER-DRY THE BEADS.
15) Remove the PCR plate from the magnetic stand.
16) Resuspend the beads in 32 µl of elution buffer or molecular grade DNase/RNase free water.
17) Incubate the PCR plate at room temperature for 2 min to elute DNA off the beads.
18) Place the PCR plate on a magnetic stand to capture the beads. Incubate for 3-5 min or until the liquid is clear.
19) Transfer 30 µl of the clear supernatant to the new PCR plate.
20) 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 qPCR or Qubit quantification. Report the Qubit Value on the 2nd sheet of the DeepChek® Normalization MS Excel document for the dilution and normalization steps at 2ng/µL.

3) Library Pooling

Proceed to the final pooling of all libraries at 2ng/µL into a single tube. Please from this step, we recommend using LowBind tube.

4) Final Dilution and Pooling with PhiX control

User shall then follow the recommended final dilution steps according to the sequencing instrument of use

Denaturation/Dilution MiSeq instrument (Nano V2 kit 2x 150bp vs V2 kit 2x250 bp)

Pool dilution iSeq 100 instruments

NGS run

After library preparation, the samples are ready for the NGS run kit processing using one of the following Illumina references:

- MS-103-1003 | MiSeq Reagent Nano Kit, v2 (500 cycles) or
- FC-420-1003 | Mid Output kit Reagents (2x150) or
- 20021533 | iSeq 100 i1 Reagent (2x150) or
- 20024908 | NextSeq 500/550 High Output Kit v2.5 (300 Cycles)

Data analysis

NGS files containing nucleotide sequences are analyzed by the DeepChek® software. User shall then follow the DeepChek software procedure to complete the data analysis and reporting processes.

Limitations

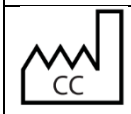
- For research use only. Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of disease.
- The kit is to be used by personnel specially instructed and trained in PCR.
- Strict compliance with the IFU is required for optimal results.
- Attention should be paid to expiration dates printed on the box and labels of all components. Do not use expired components.

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: https://ablsa.odoo.com/fr_FR/issue; 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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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 1.2

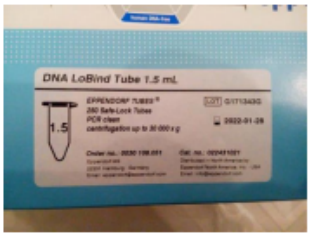
Effective date: 12th July 2022

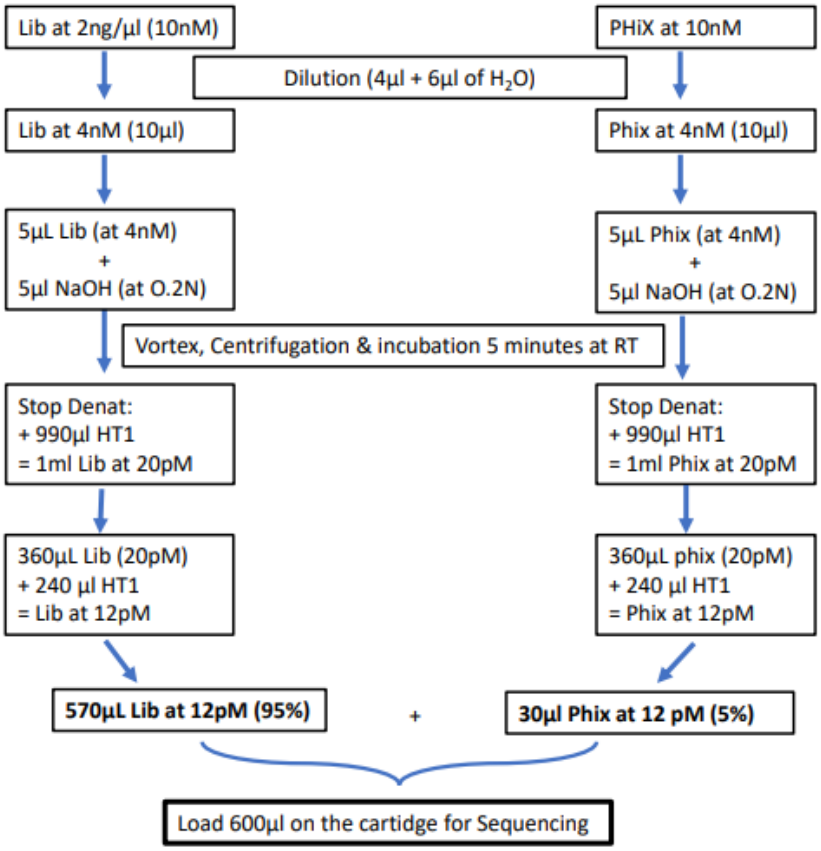
Appendix 1

Denaturation/Dilution (MiSeq V2)

$$2ng = \frac{2\frac{ng}{\mu L}(cubit\ concentration)}{660\ (molecule)\ X\ 300\ \ (bp\ average\ size)}\ X\ 10^6 = 10.10\ nM \approx 10\ nM$$

Note: It is recommended to use DNA Lobind tubes these dilutions





Appendix 2

Denaturation/Dilution (MiSeq Nano V2)

$$2ng = \frac{2\frac{ng}{\mu L}(cubit\ concentration)}{660\ (molecule)\ X\ 300\ \ (bp\ average\ size)}\ X\ 10^6 = 10.10\ nM \approx 10\ nM$$

Lib at 2ng/µl (10nM)

PHiX at 10nM

Dilution (4µl + 6µl of H_2O)

Lib at 4nM (10µl)

Phix at 4nM (10µl)

5µL Lib (at 4nM)
+
5µl NaOH (at O.2N)

5µL Phix (at 4nM)
+
5µl NaOH (at O.2N)

Vortex, Centrifugation & incubation 5 minutes at RT

Stop Denat:
+ 990µl HT1
= 1ml Lib at 20pM

Stop Denat:
+ 990µl HT1
= 1ml Phix at 20pM

360µL Lib (20pM)
+ 240 µl HT1
= Lib at 12pM

360µL phix (20pM)
+ 240 µl HT1
= Phix at 12pM

570µL Lib at 12pM (95%) + **30µl Phix at 12 pM (5%)**

Load 600µl on the cartidge for Sequencing

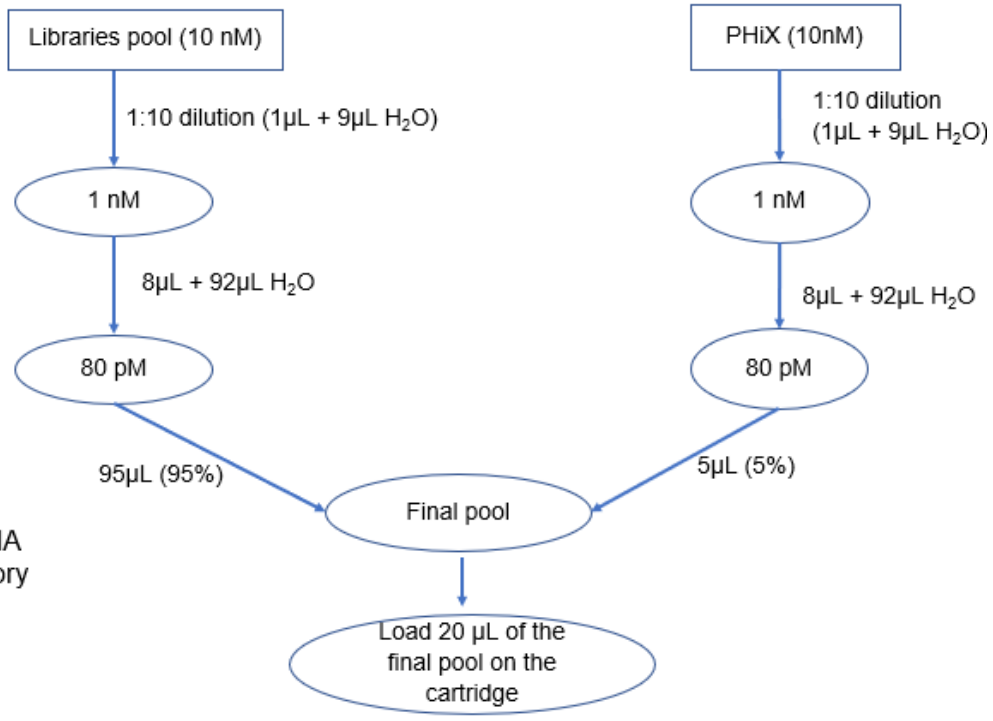
Note: the use DNA Lobind is mendatory

Appendix 3

Pool dilution (iSeq 100)

$$2ng = \frac{2\frac{ng}{\mu L}(cubit\ concentration)}{660\ (molecule)\ X\ 300\ \ (bp\ average\ size)}\ X\ 10^6 = 10.10\ nM \approx 10\ nM$$

Pooled libraries at 2ng/µL ≈ 10nM



Note: the use DNA Lobind is mendatory