diagenode

A Hologic Company

iDeal Library Preparation kit

(incl. Index Primer Set 1)

Cat. No. C05010020 (24 rxns)

USER GUIDE

Version 2 03_2019

The iDeal Library Preparation Kit has been validated on IP-Star Compact Automated System. Two versions of protocol (manual and automated) are described in this manual.

Please read this manual carefully before starting your experiment

Contents

Kit materials

The iDeal Library Preparation Kit contains all reagents necessary for the preparation of 24 libraries. The kit contains also the indexes allowing for multiplexing up to 12 samples. The set of additional 12 indexes is available separately (Index Primer Set 2 (iDeal Library Preparation Kit x24), Diagenode, Cat. No. C05010021) allowing for multiplexing up to 24 samples.

Table 1. Reagents supplied with the iDeal Library Preparation Kit.

Description	Storage
iDeal Library End Repair/dA-Tailing Enzyme Mix (green)	-20°C
iDeal Library End Repair/dA-Tailing Buffer (green)	-20°C
iDeal Library Ligation Master Mix (red)	-20°C
iDeal Library Ligation Enhancer (red)	-20°C
iDeal Library PCR Master Mix (blue)	-20°C
iDeal Library Adaptor for Illumina (red)	-20°C
iDeal Library Uracil Excision Reagent (red)	-20°C
iDeal Library Universal PCR Primer for Illumina (blue)	-20°C

Table 2. List of indexes supplied with the iDeal Library Preparation Kit.

Product	Index Primer Sequence	Expected index Primer Sequence Read	Quantity	Storage
iDeal Library Index 1 Primer for Illumina	5′-CAAGCAGAAGACGGCATACGAGAT**CGTGAT**GTGA CTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3′	ATCACG	10 µl	-20°C
iDeal Library Index 2 Primer for Illumina	5′-CAAGCAGAAGACGGCATACGAGAT**ACATCG**GTGA CTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3′	CGATGT	10 µl	-20°C
iDeal Library Index 3 Primer for Illumina	5′-CAAGCAGAAGACGGCATACGAGAT**GCCTAA**GTGA CTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3′	TTAGGC	10 µl	-20°C
iDeal Library Index 4 Primer for Illumina	5′-CAAGCAGAAGACGGCATACGAGAT**TGGTCA**GTGA CTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3′	TGACCA	10 µl	-20°C
iDeal Library Index 5 Primer for Illumina	5′-CAAGCAGAAGACGGCATACGAGAT**CACTGT**GTGA CTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3′	ACAGTG	10 µl	-20°C
iDeal Library Index 6 Primer for Illumina	5′-CAAGCAGAAGACGGCATACGAGAT**ATTGGC**GTGA CTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3′	GCCAAT	10 µl	-20°C
iDeal Library Index 7 Primer for Illumina	5′-CAAGCAGAAGACGGCATACGAGAT**GATCTG**GTGA CTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3′	CAGATC	10 µl	-20°C
iDeal Library Index 8 Primer for Illumina	5′-CAAGCAGAAGACGGCATACGAGAT**TCAAGT**GTGA CTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3′	ACTTGA	10 µl	-20°C
iDeal Library Index 9 Primer for Illumina	5′-CAAGCAGAAGACGGCATACGAGAT**CTGATC**GTGA CTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3′	GATCAG	10 µl	-20°C
iDeal Library Index 10 Primer for Illumina	5′-CAAGCAGAAGACGGCATACGAGAT**AAGCTA**GTGA CTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3′	TAGCTT	10 µl	-20°C
iDeal Library Index 11 Primer for Illumina	5′-CAAGCAGAAGACGGCATACGAGATG**TAGCCG**TGAC TGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3′	GGCTAC	10 µl	-20°C
iDeal Library Index 12 Primer for Illumina	5′-CAAGCAGAAGACGGCATACGAGAT**TACAAG**GTGA CTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3′	CTTGTA	10 µl	-20°C

Where -s- indicates phosphorothioate bond.

***NOTE**: If fewer than 12 indexes are used in a lane for sequencing, it is recommended to use the following indexes:*

- *Pool of 2 samples: Index #6 and 12*
- *Pool of 3 samples: Index #4, 6 and 12*
- *Pool of 6 samples: Index #2, 4, 5, 6, 7 and 12*

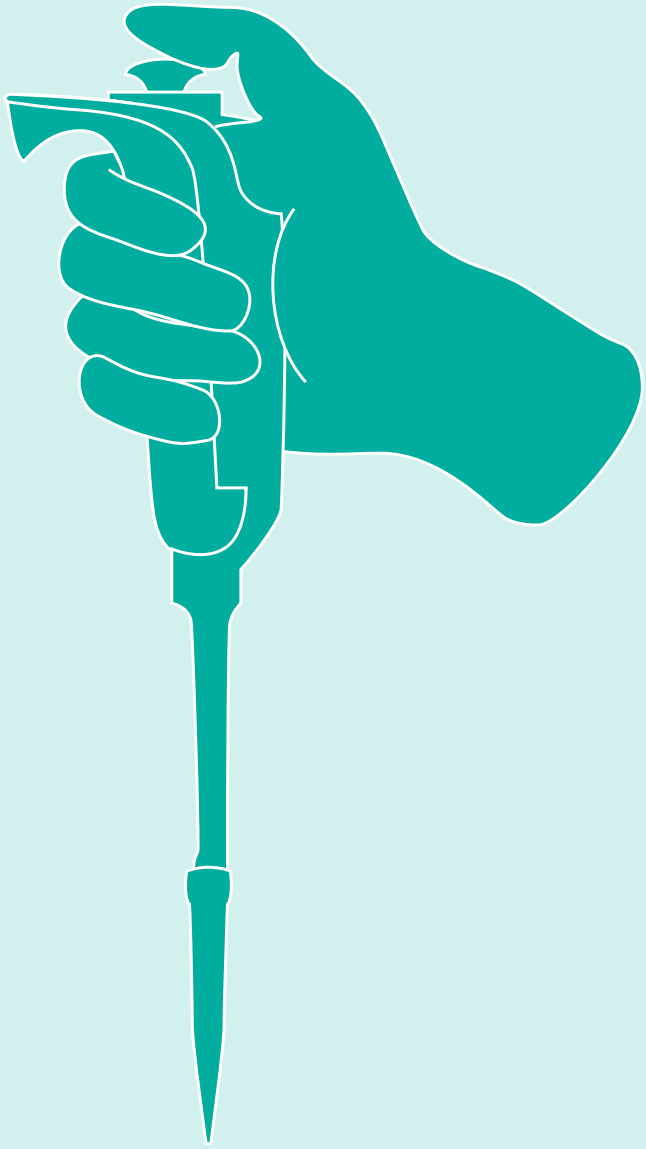
Required materials not provided

- 100% Ethanol
- Nuclease-free Water
- 10 mM Tris-HCl, pH 8.0 or 0.1X TE
- DNA LoBind Tubes (Eppendorf #022431021)
- AMPure® XP Beads (Beckman Coulter, Inc. #A63881)
- Magnetic rack -DiaMag 0.2 ml (Diagenode, Cat. No. B04000001)
- PCR Machine
- Optional: Index Primer Set 2 (iDeal Library Preparation Kit x24) (Diagenode, Cat. No. C05010021)

Table 3. List of indexes included in the kit Index Primer Set 2 (iDeal Library Preparation Kit x24).

Product	Index Primer Sequence	Expected index Primer Sequence Read	Quantity	Storage
iDeal Library Index 13 Primer for Illumina	5′-CAAGCAGAAGACGGCATACGAGATTG**TTGACT**GTG ACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3′	AGTCAA	10 µl	-20°C
iDeal Library Index 14 Primer for Illumina	5′-CAAGCAGAAGACGGCATACGAGATAC**GGAACT**GTG ACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3′	AGTTCC	10 µl	-20°C
iDeal Library Index 15 Primer for Illumina	5′-CAAGCAGAAGACGGCATACGAGATTC**TGACAT**GTG ACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3′	ATGTCA	10 µl	-20°C
iDeal Library Index 16 Primer for Illumina	5′-CAAGCAGAAGACGGCATACGAGATCG**GGACGG**GTG ACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3′	CCGTCC	10 µl	-20°C
iDeal Library Index 18 Primer for Illumina	5′-CAAGCAGAAGACGGCATACGAGATG**TGCGGA**CGT GACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3′	GTCCGC	10 µl	-20°C
iDeal Library Index 19 Primer for Illumina	5′-CAAGCAGAAGACGGCATACGAGATCG**TTTCAC**GTG ACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3	GTGAAA	10 µl	-20°C
iDeal Library Index 20 Primer for Illumina	5′-CAAGCAGAAGACGGCATACGAGATA**AGGCCA**CGT GACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3′	GTGGCC	10 µl	-20°C
iDeal Library Index 21 Primer for Illumina	5′-CAAGCAGAAGACGGCATACGAGATTC**CGAAAC**GTG ACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3′	GTTTCG	10 µl	-20°C
iDeal Library Index 22 Primer for Illumina	5′-CAAGCAGAAGACGGCATACGAGATTA**CGTACG**GTG ACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3′	CGTACG	10 µl	-20°C
iDeal Library Index 23 Primer for Illumina	5′-CAAGCAGAAGACGGCATACGAGATAT**CCACTC**GTG ACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3′	GAGTGG	10 µl	-20°C
iDeal Library Index 25 Primer for Illumina	5′-CAAGCAGAAGACGGCATACGAGATATA**TCAGTG**TGA CTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3′	ACTGAT	10 µl	-20°C
iDeal Library Index 27 Primer for Illumina	5′-CAAGCAGAAGACGGCATACGAGATAA**AGGAAT**GTG ACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3′	ATTCCT	10 µl	-20°C

PROTOCOL



MANUAL PROCESSING

STEP 1

End Preparation

Starting Material

5 ng –1 µg fragmented DNA.

1.1 Mix the following components in a sterile nuclease-free tube:

iDeal Library End Repair/dA-Tailing Enzyme Mix (green)	3 µl
iDeal Library End Repair/dA-Tailing Buffer (green)	6.5 µl
Fragmented DNA	55.5 µl*
TOTAL volume	**65 µl**

**If DNA Input is less than 55.5 µl, complete with water up to 55.5 µl.*

1.2 Mix by pipetting followed by a quick spin to collect all liquid from the sides of the tube.

1.3 Place in a thermocycler, with the heated lid on, and run the following program:

- 30 minutes at 20°C
- 30 minutes at 65°C
- Hold at 4°C

STEP 2

Adaptor Ligation

***NOTE**: If DNA input is < 100 ng, dilute the **iDeal Library Preparation Adaptor for Illumina** (provided at 15 µM) 1:10 in 10mM TRIS-HCl with 10 mM NaCl to a final concentration of 1.5 µM. Use immediately.*

2.1 Add the following components directly to the iDeal Library End Prep reaction mixture and mix well:

iDeal Library Ligation Master Mix (red)	15 µl
iDeal Library Adaptor for Illumina* (red)	2.5 µl
iDeal Library Ligation Enhancer (red)	1 µl
TOTAL volume	**83.5 µl**

2.2 Incubate at **20°C** for 15 minutes in a thermal cycler.

2.3 Add **3 µl of iDeal Library Uracil Excision Reagent** (red) to the ligation mixture.

2.4 Mix well and incubate at **37°C** for 15 minutes.

***NOTE**: If needed samples can be stored overnight at -20°C.*

OPTION A

Size Selection of adaptor-ligated DNA

Size selection is optional. If the starting material is less than 50 ng, size selection is not recommended. If you are not performing size selection, proceed to the next page and perform clean-up step prior to PCR amplification. The following size selection protocol is for libraries with 200 bp inserts only. For libraries with different size fragment inserts, refer to Table 4 for the appropriate volume of beads to be added. The size selection protocol is based on a starting volume of 100 µl.

Table 4: Recommended conditions for bead based size selection.

Approximate insert size		150 bp	200 bp	250 bp	300 - 400 bp	400 - 500 bp	500 - 700 bp
Total Library size (insert + adaptor)		270 bp	320 bp	400 bp	400 - 500 bp	500 - 600 bp	600 - 800 bp
Volume of ligation reaction		100 µl					
Volume of beads to add	1st Bead Selection	65 µl	55 µl	45 µl	40 µl	35 µl	30 µl
	2nd Bead Selection	25 µl	25 µl	25 µl	20 µl	15 µl	15 µl

***NOTE**: If neede the libraries can be stored at -20°C.*

2.5 Vortex **AMPure XP beads** to resuspend.

2.6 Add **13.5 µl dH_2O** to the ligation reaction for a 100 µl total volume.

2.7 Add **55 µl of resuspended AMPure XP beads** to the 100 µl ligation reaction. Mix well by pipetting up and down at least 10 times.

2.8 Incubate for 5 minutes at room temperature.

2.9 Quickly spin the tube and place the tube on the DiaMag02 - magnetic rack (Cat. No. B04000001) to separate the beads from the supernatant. After the solution is clear (about 5 minutes), carefully transfer the supernatant containing your DNA to a new tube (**Caution: do not discard the supernatant**). Discard the beads that contain the unwanted large fragments.

2.10 Add **25 µl resuspended AMPure XP beads** to the supernatant, mix well and incubate for 5 minutes at room temperature.

2.11 Quickly spin the tube and place it on the **magnetic rack** to separate the beads from the supernatant. After the solution is clear (about 5 minutes), carefully remove and discard the supernatant that contains unwanted DNA. Be careful not to disturb the beads that contain the desired DNA targets (**Caution: do not discard beads**).

2.12 Add **200 µl of 80% freshly prepared ethanol** to the tube while in the magnetic rack. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant.

2.13 Repeat Step 2.12 once for a total of two washes.

2.14 Air the dry beads for 5 minutes while the tube is on the **magnetic rack** with the lid open.

2.15 Elute the DNA target from the beads into **17 µl of 10 mM Tris-HCl or 0.1 X Te, pH 8.0**. Mix well on a vortex mixer or by pipetting up and down. Incubate for at least 2 minutes at room temperature. Quickly spin the tube and place it on a magnetic stand. After the solution is clear (about 5 minutes), transfer 15 µl to a new PCR tube for amplification.

***NOTE**: Be sure not to transfer any beads. Trace amounts of bead carry over may affect the optimal performance of the polymerase used in the iDeal Library PCR Master Mix in the subsequent PCR step.*

OPTION B

Alternatively, Clean-up of adaptor-ligated DNA without Size Selection

2.5 Vortex **AMPure XP beads** to resuspend.

2.6 Add **86.5 µl resuspended AMPure XP beads** to the ligation reaction. Mix well by pipetting up and down at least 10 times.

2.7 Incubate for 5 minutes at room temperature.

2.8 Quickly spin the tube and place it on the DiaMag02 - magnetic rack (Cat. No. B04000001) to separate beads from supernatant. After the solution is clear (about 5 minutes), carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets (**Caution: do not discard beads**).

2.9 Add **200 µl of 80% freshly prepared ethanol** to the tube while in the magnetic rack. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant.

2.10 Repeat Step 2.9 once, for a total of two washes.

2.11 Air the dry beads for 5 minutes while the tube is on the magnetic stand with the lid open.

2.12 Elute the DNA target from the beads by adding **17 µl of 10 mM Tris-HCl, pH 8.0 or 0.1X Te**.

2.13 **NOTE**: Be sure not to transfer any beads. Trace amounts of bead carry over may affect the optimal performance of the polymerase used in the iDeal library PCR Master Mix in the subsequent PCR step.

2.14 Mix well by pipetting up and down, or on a vortex mixer. Incubate for at least 2 minutes at room temperature.

2.15 Quickly spin the tube and place it on the magnetic stand.

2.16 After the solution is clear (about 5 minutes), transfer **15 µl** to a new PCR tube for amplification.

STEP 3

PCR amplification

3.1 Mix the following components in sterile strip tubes:

Adaptor Ligated DNA Fragments	15 µl
iDeal Library PCR Master Mix (blue)	25 µl
iDeal Library Index Primer* (blue)	5 µl
iDeal Universal PCR Primer* (blue)	5 µl
TOTAL volume	**50 µl**

** These primers (Index Primer Set 1) are included. Index Primer Set 2 may be purchased separately (Cat. No. C05010021).*

***NOTE**: If needed the samples can be stored at-20°C.*

PCR cycling conditions

3.2 Transfer tubes to a pre-programmed thermal cycler and incubate as follows.

Cycle Step	Temp	Time	Cycles
Initial Denaturation	98°C	30 seconds	1
Denaturation	98°C	10 seconds	4 – 12*
Annealing - Extension	65°C	75 seconds	
Final Extension	65°C	5 minutes	1
Hold	4°C	∞	

**Suggestion: 4 PCR cycles for 1 µg DNA input, 7 - 8 cycles for 50 ng, and 12 for 5 ng DNA input. Further optimization of PCR cycle number may be required.*

Clean-up of PCR amplification

3.3 Vortex **AMPure XP beads** to resuspend.

3.4 Add **45 µl of resuspended AMPure XP beads** to the PCR reactions (~50 µl). Mix well by pipetting up and down at least 10 times.

3.5 Incubate for 5 minutes at room temperature.

3.6 Quickly spin the tube and place it on DiaMag02 - magnetic rack to separate beads from supernatant. After the solution is clear (about 5 minutes), carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets (**Caution: do not discard beads**).

3.7 Add **200 µl of 80% ethanol** to the tubes while in the DiaMag02 - magnetic rack. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant.

3.8 Repeat Step 3.7 once.

3.9 Air dry the beads for 5 minutes while the tubesare on the magnetic stand with the lid open.

3.10 Elute DNA target from beads into **33 µl of 0.1X TE**. Mix well by pipetting up and down at least 10 times. Incubate for at least 2 minutes at room temperature. Quickly spin the tube and place it on an appropriate magnetic stand to separate beads from supernatant. After the solution is clear (about 5 minutes), carefully transfer 30 µl supernatant to a new PCR tube. Store libraries at –20°C.

3.11 Check the size distribution on an Agilent Bioanalyser High Sensitivity DNA Chip. The sample may need to be diluted before loading. A 5 fold dilution can be used.

AUTO PROTOCOL

AUTOMATED PROCESSING

Protocol

The "Ideal_Library_Preparation" protocol on the iP-Star® Compact is using the standard iDeal Library Preparation Kit from Diagenode. The iDeal Library Preparation kit allows the preparation of indexed libraries of genomic or ChIP DNA.

It provides flexibility to prepare 1 to 32 libraries in one run starting with 5 ng of DNA. The whole protocol takes approximately 1h30. It allows you to prepare up to 96 libraries per day with 3 runs. At the end, you recover ligated products ready for amplification.

IP-Star setup

1.1 Switch ON the **IP-Star Compact**.

1.2 Select "Protocols" icon and then click on "**Library prep**".

1.3 Under "**Library prep**", select "**Ideal_Library_Preparation**".

***NOTE**: If you plan to run between:*

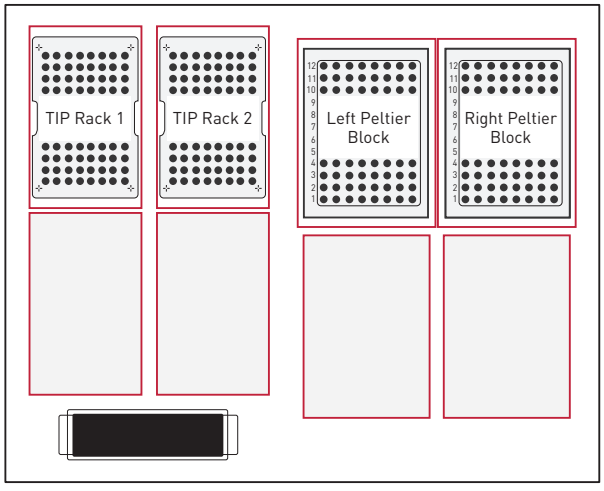
- *1 and 8 samples, chose "**Ideal_Library_Preparation_08**"*
- *9 and 16 samples, chose "**Ideal_Library_Preparation_16**"*
- *17 and 24 samples, chose "**Ideal_Library_Preparation_24**"*
- *25 and 32 samples, chose "**Ideal_Library_Preparation_32**"*

1.4 Setup the exact number of samples that you want to process.

***NOTE**: The **Left Peltier Block** is now cooling down to 4°C to keep the enzymes and reagents cold.*

1.5 Setup all the plastics on the platform according to the screen layout.

1.6 Fill **TIP Rack 1** (and 2 if processing more than 8 samples) with tips according to the screen.



1.7 Fill **Left and Right Peltier Blocks** with 200 µl tube strips according to the screen.

STEP 1 & 2

End Preparation and Adaptor Ligation

***NOTE**: Allow the reagents from **iDeal Library Preparation Kit** to come at 4°C. Work on ice from this point.*

2.1 Prepare the following mixes.

• iDeal Library End Prep Mix:

Number of samples	1	8	16	24	32
iDeal Library End Repair/dA-Tailing Enzyme Mix (green)	3 µl	24 µl	48 µl	72 µl	96 µl
iDeal Library End Repair/dA-Tailing Buffer (green)	6.5 µl	52 µl	104 µl	156 µl	208 µl
TOTAL	9.5 µl	76 µl	152 µl	204 µl	280 µl

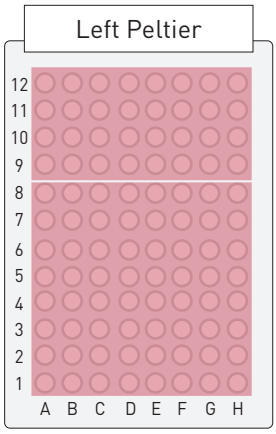
***NOTE**: **55.5 µl of DNA** will be added later for each sample.*

• iDeal Library Adaptor Ligation Mix:

Number of samples	1	8	16	24	32
iDeal Library Ligation Master Mix (red)	15 µl	120 µl	240 µl	360 µl	480 µl
iDeal Library Ligation Enhancer (red)	1 µl	8 µl	16 µl	24 µl	32 µl
TOTAL	16 µl	128 µl	256 µl	238 µl	512 µl

2.2 Fill the strips of the **Left Peltier Block** according to the screen layout with the following reagents:

- **Ligation Mix** – 16 µl per well
- **iDeal Library Adaptor for Illumina** (red) – 2.5 µl per well
- **iDeal Library Uracil Excision Reagent** (red) – 3 µl per well



PCR tube

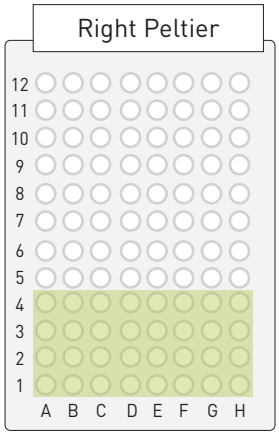
Well 9-12
- Uracil Excision Reagent 3 µl

Well 5-8
- Adaptor for Illumina 2.5 µl

Well 1-4
Adaptor Ligation Mix 16 µl
- Ligation Master Mix 15 µl
- Ligation Enhancer 1 µl

2.3 Fill the strips of the **Right Peltier Block** according to the screen layout with:

- **iDeal Library End Prep Mix** - 9.5 µl per well
- **DNA sample** – 55.5 µl per well



PCR tube

Well 1-4
iDeal Library End Prep 65 µl
- Fragmented DNA 55.5 µl
- End Repair/dA-Tailing Enzyme Mix 3 µl
- End Repair/dA-Tailing Buffer 6.5 µl

Samples 25-32
Samples 17-24
Samples 9-16
Samples 1-8

2.4 Close the door and Run.

OPTION A

Size Selection of adaptor-ligated DNA

Size selection is optional. If the starting material is less than 50 ng, size selection is not recommended. If you are not performing size selection, proceed to the clean-up step prior to PCR amplification.

> ***NOTE**: Use the IP-Star and room temperature AMPure XP beads for the size selection.*

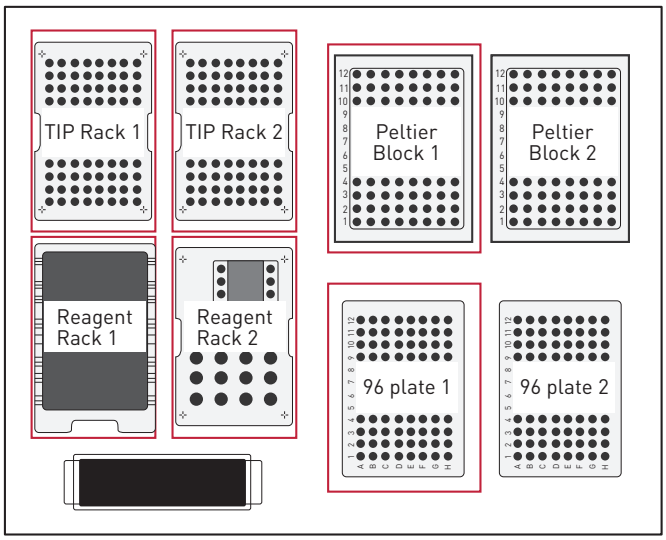
2.5 Switch on the IP-Star and select "**Protocols**" icon and then "**Library prep**" category.

2.6 Select "**AMPure_XP_Size_Selection_08**" if you plan to run between 1 and 8 samples, or "**AMPure_XP_Size_Selection_16**" if you plan to run between 9 and 16 samples.

2.7 Setup the exact number of samples that you want to process by pressing the black box.

> ***NOTE**: The Peltier Block 1 is now cooling down to 4°C to keep your samples cold.*

2.8 Setup all the plastics on the platform according to the screen layout



- Fill **TIP Rack 1** (and 2 if processing more than 8 samples) with tips according to the screen.
- Fill **Reagent Rack 1 & 2** with reagent containers according to the screen.
- Fill **96 plate 1** with a 96 well microplate.
- Fill **Peltier Block 1** with 200 µl tube strips according to the screen.

2.9 Fill the robot with all reagents.

- Add **80 µl of ChIP-seq grade water** to each sample to have a final volume of 100 µl. Put your samples in lane 1 (and 2 if processing more than 8 samples) of the **Peltier Block 1**.
- Distribute the AMPure XP beads according to the required size following the recommendations from the table :

AMPure XP	Final size
110 µl	250 bp
100 µl	280 bp
90 µl	325 bp
80 µl	400 bp
70 µl	500 bp

We recommend to use the size selection protocol for the final library size of 325 bp (insert + adaptor).

***NOTE**: Resuspend the beads with pipetting up and down several times before dispense them.*

- Fill the container of the **Reagent Rack 1** with freshly prepared 80% Ethanol according to the screen.
- Fill the container of **Reagent Rack 2** with Resuspension Buffer according to the screen.
- Check the proper insertion of the racks and the consumables.

2.10 Close the door and press "Run" to start.

2.11 After the run, recover your samples on the upper row of the Left Peltier Block. The final volume is 20 µl for each sample.

2.12 Press "OK" and "Back" until the homepage appears on the screen. Press "Shutdown" and wait until the screen is black before switching off the IP-Star.

***NOTE**: Remove all the plastics from the platform, empty the waste shuttle and clean the inner side of the IP-Star with 70% ethanol.*

OPTION B

Alternatively, Clean-up of adaptor ligated DNA without size selection

***NOTE**: Use the IP-Star and room temperature AMPure XP beads for the clean-up.*

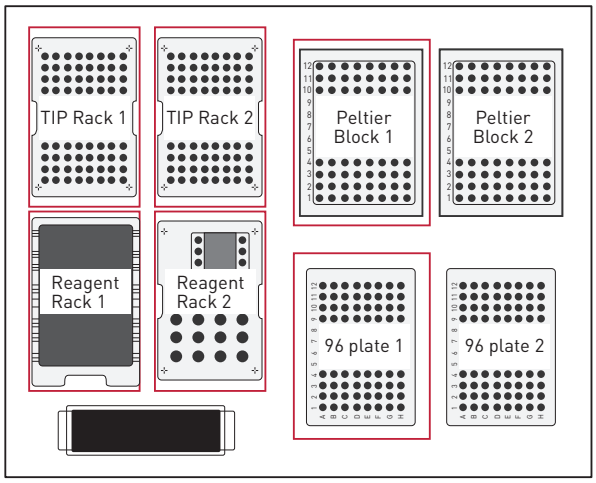
2.5 Select "**Protocols**" icon and then "**IPure**" category.

2.6 Select "**AMPure_XP_Purification_08**" if you plan to run between 1 and 8 samples, or "**AMPure_XP_Purification_16**" if you plan to run between 9 and 16 samples.

2.7 Setup the exact number of samples that you want to process by pressing the black box.

***NOTE**: The Peltier Block is now cooling down to 4°C to keep your samples cold.*

2.8 Setup all the plastics on the platform according to the screen layout.



- Fill **TIP Rack 1** (and 2 if processing more than 8 samples) with tips according to the screen.
- Fill **Reagent Rack 1 & 2** with reagent containers according to the screen.
- Fill **96 plate 1** with a 96 well microplate.
- Fill **Peltier Block 1** with 200 µl tube strips according to the screen.

2.9 Fill the robot with all reagents

- Put your samples in lane 1 (and 2 if processing more than 8 samples) of the **Peltier Block 1**.
- Distribute **86.5 µl** room temperature **AMPure XP beads** in each well of row 1 (and 5 if processing more than 8 samples) of the **96-well Microplate**.

***NOTE**: Resuspend the beads with pipetting up and down several times before dispense them.*

- Fill the container of the **Reagent Rack 1** with freshly prepared 80% Ethanol according to the screen.
- Fill the container of **Reagent Rack 2** with Resuspension Buffer according to the screen.
- Check the proper insertion of the racks and the consumables.

2.10 Close the door and press "Run" to start.

2.11 After the run, recover your samples on the upper row of the **Peltier Block 1**. The final volume is 20 µl per each sample.

2.12 Press "OK" and "Back" until the homepage appears on the screen. Press "Shutdown" and wait until the screen is black before switching off the IP-Star.

***NOTE**: Remove all the plastics from the IP-Star platform, empty the waste shuttle, and clean the inner side of the IP-Star with 70% ethanol.*

STEP 3

PCR amplification

3.1 Mix the following components in sterile strip tubes:

Adaptor Ligated DNA Fragments	15 µl
iDeal Library PCR Master Mix (blue)	25 µl
iDeal Library Index Primer* (blue)	5 µl
iDeal Universal PCR Primer* (blue)	5 µl
TOTAL volume	**50 µl**

** These primers (Index Primer Set 1) are included. Index Primer Set 2 may be purchased separately (Cat. No. C05010021).*

***NOTE**: If needed the samples can be stored at-20°C.*

PCR cycling conditions

3.2 Transfer tubes to a pre-programmed thermal cycler and incubate as follows.

Cycle Step	Temp	Time	Cycles
Initial Denaturation	98°C	30 seconds	1
Denaturation	98°C	10 seconds	4 – 12*
Annealing - Extension	65°C	75 seconds	
Final Extension	65°C	5 minutes	1
Hold	4°C	∞	

**Suggestion: 4 PCR cycles for 1 µg DNA input, 7 - 8 cycles for 50 ng, and 12 for 5 ng DNA input. Further optimization of PCR cycle number may be required.*

Clean-up of PCR amplification

***NOTE**: Use the IP-Star and room temperature AMPure XP beads for the clean-up.*

3.3 Select "**Protocols**" icon and then "**IPure**" category.

3.4 Select "**AMPure_XP_Purification_08**" if you plan to run between 1 and 8 samples, or "**AMPure_XP_Purification_16**" if you plan to run between 9 and 16 samples.

3.5 Setup the exact number of samples that you want to process by pressing the black box.

***NOTE**: The Peltier Block is now cooling down to 4°C to keep your samples cold.*

3.6 Setup all the plastics on the platform according to the screen layout.

- Fill **TIP Rack 1** (and 2 if processing more than 8 samples) with tips according to the screen.
- Fill **Reagent Rack 1 & 2** with reagent containers according to the screen.
- Fill **96 plate 1** with a 96 well microplate.
- Fill **Peltier Block 1** with 200 µl tube strips according to the screen.

3.7 Fill the robot with all reagents

- Put your samples in lane 1 (and 2 if processing more than 8 samples) of the **Peltier Block 1**.
- Distribute **45 µl** room temperature **AMPure XP beads** in each well of row 1 (and 5 if processing more than 8 samples) of the **96-well Microplate**.

***NOTE**: Resuspend the beads with pipetting up and down several times before dispense them.*

- Fill the container of the **Reagent Rack 1** with freshly prepared 80% Ethanol according to the screen.
- Fill the container of **Reagent Rack 2** with Resuspension Buffer according to the screen.

- Check the proper insertion of the racks and the consumables.

3.8 Close the door and press "Run" to start.

3.9 After the run, recover your samples on the upper row of the **Peltier Block 1**. The final volume is 20 µl per each sample.

3.10 Press "OK" and "Back" until the homepage appears on the screen. Press "Shutdown" and wait until the screen is black before switching off the IP-Star.

***NOTE**: Remove all the plastics from the IP-Star platform, empty the waste shuttle, and clean the inner side of the IP-Star with 70% ethanol.*

Related products

Product	Cat. No.
iDeal ChIP-Seq Kit for Histones	C01010051
Auto iDeal ChIP-seq Kit for Histones	C01010171
Bioruptor Pico	B01060010
IP-Star Compact Automated System	B03000002
DiaMag 0.2 ml - magnetic rack	B04000001
MagMeDIP-seq kit	C02010023
Auto MagMeDIP-seq kit	C02010016

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