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# DNBSEQ rRNA Depletion Kit

- Cat. No.: 940-001812-00 (16 RXN)
  940-001811-00 (96 RXN)
- Kit Version: V1.0

For Research Use Only

## About the user manual



## Manufacturer information

| | |
|---|---|
| **Manufacturer** | Complete Genomics, Inc. |
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## Revision history

| Manual Rev | Kit version | Date | Description |
|---|---|---|---|
| 1.0 | V1.0 | Dec. 2023 | Initial release |

*i* Please use the latest version of the manual and use it with the corresponding kit.

# Contents

# 1 Product overview

## 1.1 Introduction

DNBSEQ rRNA Depletion Kit depletes rRNA from human, mouse, and rat total RNA in 10 ng - 1 μg (including cytoplasmic 5 S rRNA, 5.8 S rRNA, 18 S rRNA, 28 S rRNA, mitochondrial ribosomal RNA 12 S rRNA, 16 S rRNA and 45 S pre-ribosomal RNA). The resulting mRNA and other non-coding RNA are suitable for downstream RNA analysis applications. This kit is compatible with both intact and degraded RNA (e.g., FFPE RNA). The library prepared by using this kit with RNA Library Prep Kit (e.g., DNBSEQ Fast RNA Library Prep Kit) is suitable for RNA quantitation, transcriptome or non-coding RNA research applications.

## 1.2 Intended use

This kit is applicable to total RNA samples from all human, mouse, and rat.

## 1.3 Components

DNBSEQ rRNA Depletion Kit comes in two specifications: 16 RXN and 96 RXN. For component details, refer to the following table.

**Table 1 DNBSEQ rRNA Depletion Kit (16 RXN) (Cat. No.: 940-001812-00 )**

| Item & Cat. No. | Component | Cap color | Spec & Quantity |
|---|---|---|---|
| DNBSEQ rRNA Depletion Kit Cat. No.: 940-001812-00 | rRNA Probe Mix | White | 32 μL/tube × 1 |
| | Hybridization Buffer | White | 80 μL/tube × 1 |
| | RNase H Buffer | Orange | 48 μL/tube × 1 |
| | RNase H | Orange | 32 μL/tube × 1 |

| Item & Cat. No. | Component | Cap color | Spec & Quantity |
|---|---|---|---|
| | DNase I Buffer | Blue | 280 µL/tube × 1 |
| | DNase I | Blue | 40 µL/tube × 1 |

**Table 2 DNBSEQ rRNA Depletion Kit (96 RXN) (Cat. No.: 940-001811-00 )**

| Item & Cat. No. | Component | Cap color | Spec & Quantity |
|---|---|---|---|
| DNBSEQ rRNA Depletion Kit Cat. No.: 940-001811-00 | rRNA Probe Mix | White | 192 µL/tube × 1 |
| | Hybridization Buffer | White | 480 µL/tube × 1 |
| | RNase H Buffer | Orange | 288 µL/tube × 1 |
| | RNase H | Orange | 192 µL/tube × 1 |
| | DNase I Buffer | Blue | 840 µL/tube × 2 |
| | DNase I | Blue | 240 µL/tube × 1 |

## 1.4 Storage and transportation

DNBSEQ rRNA Depletion Kit

- Storage temperature: -25 ℃ to -15 ℃
- Transportation temperature: -80 ℃ to -15 ℃

- Production date and expiration date: refer to the label.
- For dry ice shipments, ensure that there is enough dry ice remaining after transportation.
- With proper transport, storage, and use, all components can maintain complete activity within their shelf life.

## 1.5 User-supplied materials

Before the experiment, prepare the following equipment and materials. The "optional" material is determined by the actual requirement. For example, if DNase I Digestion is used for eliminating contaminating DNA in RNA sample, the digested product needs to be quantified. Therefore, you need to prepare "Qubit Fluorometer". If rRNA-depleted RNA sample needs the band distribution detection, you need to prepare "Agilent 2100 Bioanalyzer" and reagents.

**Table 3 Order information for CG products**

| Catalog number | Model | Name |
|---|---|---|
| 940-001518-00 | 16 RXN | DNBSEQ Fast RNA Library Prep Set |
| 940-001522-00 | 96 RXN, B | DNBSEQ Fast RNA Library Prep Set |
| 940-001836-00 | 96 RXN, C | DNBSEQ Fast RNA Library Prep Set |
| 940-001515-00 | 192 RXN | DNBSEQ Fast RNA Library Prep Set |

**Table 4 User-supplied equipment list**

| Equipment | Recommended brand |
|---|---|
| Vortex mixer | / |
| Desktop centrifuge | / |
| Pipettes | / |
| Thermocycler | Bio-Rad, or Thermal Cycler with thermal gradient capability |
| Magnetic rack DynaMag -2, or equivalent | Thermo Fisher Scientific, Cat. No.: 12321D |
| Qubit Fluorometer, or equivalent | Thermo Fisher, Cat. No.: Q33216 |
| Agilent 2100 Bioanalyzer, or equivalent | Agilent Technologies , Cat. No.: G2939AA |

**Table 5 Recommended reagent/consumable list**

| Reagent/consumable | Recommended brand |
|---|---|
| Nuclease Free (NF) water | Ambion, Cat. No.: AM9937, or equivalent |
| RNase Zap | Ambion, Cat. No.: AM9780 |
| RNA cleanup beads, Agencourt RNAClean XP 40 mL Kit | Beckman Coulter, Cat. No.: A63987, or equivalent |
| DNase I (optional) | NEB, Cat. No.: M0303S |
| 100% Ethanol (Analytical Grade) | / |
| Agilent RNA 6000 Pico Kit | Agilent Technologies, Cat. No.: 5067-1513 |
| Qubit RNA HS Assay Kit | Invitrogen, Cat. No.: Q32852 |
| RNase-free tips | / |
| 1.5 mL RNase-free non-stick microcentrifuge tubes | Ambion, Cat. No.: AM12450 |

| Reagent/consumable | Recommended brand |
|---|---|
| 0.2 mL RNase-free PCR tubes or 96-well plate | Axygen, Cat. No.: PCR-02-C or Axygen, Cat. No.: PCR-96M2-HS-C |
| Qubit Assay Tubes or 0.5 mL Thin-wall PCR Tubes | Invitrogen or Axygen, or equivalent |

## 1.6 Precautions and warnings

- This product is for research use only, not for use in vitro diagnosis. Read this manual carefully before use.
- Familiarize yourself with the precautions and operation methods of various instruments before performing the experiment.
- This manual aims to provide a standard protocol. Changes can be made for different applications, but changes must be tested prior to starting the protocol.
- Wear masks and gloves before preparing RNA samples. Spray and wipe the pipette, test tube rack, and bench with RNase Zap RNase Decontamination Solution.
- It is recommended that you use pipette tips with filters to prevent cross-contamination. Use a new tip each time for pipetting different solutions or samples.
- It is recommended that you use the thermocyclers with heated lids for reactions. Preheat the thermocyclers to reaction temperature before use. If the thermocycler does not allow for lid temperature adjustments, the preset lid temperature of 105 ℃ is sufficient.
- Aerosol contamination may cause inaccurate results. It is recommended that you prepare separate working areas in the laboratory for PCR reaction preparation, PCR reaction, and PCR product cleanup. Use designated equipment for each area and clean the area regularly to ensure a sterile working environment (use 0.5% Sodium Hypochlorite or 10% Bleach to clean the working area).
- Avoid skin and eyes contact with samples and reagents. Do not eat or drink the samples and reagents. In case of contact with skin and eyes, rinse immediately with plenty of water and seek medical advice.
- Conform to the law and regulations when disposing of all samples and reagents.
- If you have questions, please contact Technical Support:
  **US-TechSupport@CompleteGenomics.com.**

## 1.7 Workflow

| Section | Workflow | Total time | Hands-on time |
|---|---|---|---|
| 3.1 | RNA/Probe hybridization | 30 min | 5 - 8 min |
| 3.2 | RNase H digestion | 35 - 40 min | 5 min |
| 3.3 | DNase I digestion | 35 - 40 min | 5 min |
| 3.4 | RNA cleanup | 30 - 40 min | 20 - 30 min |
| 3.5 | QC of rRNA depletion product ⏸ | 15 - 60 min | 10 - 20 min |

- Total time: The theoretical use time of 8 reactions. The time will be extended if the number of reactions increases.
- Hands-on time: The total required hands-on time in the process.
- ⏸ Stop point.

# 2 Sample preparation

## 2.1 Sample requirements

### 2.1.1 Compatible sample type and input requirements

This kit depletes rRNA from human, mouse, and rat total RNA. It is compatible with FFPE sample (See Appendix "FFPE sample preparation" on page 17 for instructions).

- For RNA sample with RIN (RNA Integrity Number) ≥7, the recommended amount of RNA input is 10 ng - 1 μg.
- For low-quality RNA sample (RIN< 7), the RNA input should not be less than 200 ng.

### 2.1.2 Total RNA quality requirements

- Use Agilent 2100 Bioanalyzer to perform quality for total RNA sample. RIN value should be ≥7. If RIN<7, increase RNA input (no more than 1 μg) and the PCR cycles appropriately in the library preparation. If RIN is N/A, the sample is unqualified for library preparation.
- RNA purity: $OD_{260/280}$=1.8~2.0, $OD_{260/230}$ ≥ 2.
  The rRNA ratio will increase when $OD_{260/230}$ <2.
  If $OD_{260/230}$<2, total RNA is recommended to be purified with RNA cleanup beads before Chapter 3. The purification method can refer to "Cleanup of RNA sample" on page 16.
- To maximize the effect of rRNA depletion, ensure that there is no DNA contamination. If DNA contamination is found in the RNA sample (use agarose electrophoresis to detect), perform DNase I digestion to remove DNA. Refer to "DNase I digestion of RNA sample" on page 14 for instructions.
- If RNA sample is insufficient, you can try a lower amount of input for depletion. However, a lower amount of input might cause a decreased yield of PCR products and a lower comparison rate in analysis result.

# 3 Library preparation protocol

## 3.1 RNA/probe hybridization

CAUTION Do not shake or vortex the sample in the following procedures. Mix the sample by gently pipetting.

### 3.1.1 Preparation

Mix the reagents before using and store the remaining reagents immediately after use.

**Table 6 Preparing the reagents**

| Reagent | Requirement |
|---|---|
| NF water | User-supplied. Place at room temperature (RT). |
| Hybridization Buffer | Mix thoroughly, centrifuge briefly, and place on ice. |
| rRNA Probe Mix | |

Mix the rRNA Probe Mix by vortexing 5-6 times (3 sec each).

### 3.1.2 RNA/probe hybridization

1. According to the concentration of total RNA, transfer appropriate RNA sample (recommended 200 ng total RNA) to 0.2 mL PCR tube. Add NF water to make a total volume of 18 μL.

2. Individually add Probe Mix and Hybridization Buffer to RNA sample on ice as shown in the table below.

CAUTION Do not prepare an rRNA Probe Mix and Hybridization Buffer mixture.

**Table 7 RNA/Probe hybridization reaction system**

| Reagent | Volume per reaction |
|---|---|
| Total RNA + NF water | 18 μL |
| Hybridization Buffer | 5 μL |
| rRNA Probe Mix | 2 μL |
| Total | 25 μL |

3. Gently **pipette** the solution at least 15-20 times and centrifuge briefly. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

CAUTION After pipetting, make sure that there is no residue on the tip of the pipette; make sure that the number of pipetting times is sufficient to ensure that the hybridization reaction system is evenly mixed, otherwise the final depletion efficiency may be affected.

**Table 8 RNA/Probe hybridization reaction conditions (Volume: 25 μL)**

| Temperature | Time |
|---|---|
| 105 ℃ Heated lid | On |
| 95 ℃ | 2 min |
| 95°C to 22°C | 0.1°C /s |
| 22 ℃ | 5 min |

4. The reaction takes approximately 20 min. After 22 ℃ for 5 min, immediately place the tube(s) on ice for 2 min. Centrifuge briefly and immediately proceed to the next step.

## 3.2 RNase H digestion

CAUTION Do not shake or vortex the sample in the following procedures. Mix the sample by gently pipetting.

### 3.2.1 Preparation

Mix the reagents before using and store the remaining reagents immediately after use.

**Table 9 Preparing the reagents**

| Reagent | Requirement |
|---|---|
| RNase H | Flick and/or invert the tube gently, centrifuge briefly, and place on ice. |
| RNase H Buffer | Thaw on ice, mix by vortexing, centrifuge briefly, and place on ice. |

## 3.2.2 RNase H digestion

1. According to the desired reaction number, prepare the RNase H digestion reaction mixture in a 0.2 mL PCR tube on ice. Mix it well by vortexing, centrifuge briefly, and place on ice.

**Table 10 RNase H digestion reaction mixture**

| Reagent | Volume per reaction |
|---|---|
| RNase H | 2 μL |
| RNase H Buffer | 3 μL |
| Total | 5 μL |

2. Add 5 μL of RNase H digestion reaction mixture to each sample tube (from step 4 in 3.1.2). Gently **pipette** the solution at least 15-20 times, centrifuge briefly, and place on ice.

CAUTION After pipetting, make sure that there is no residue on the tip of the pipette; make sure that the number of pipetting times is sufficient to ensure that the RNase H digestion system is evenly mixed, otherwise the final removal efficiency may be affected.

3. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

**Table 11 RNase H digestion reaction conditions (Volume: 30 μL)**

| Temperature | Time |
|---|---|
| 45 ℃ Heated lid | On |
| 37 ℃ | 30 min |
| 4 ℃ | hold |

If a heated lid cannot be set to 45 ℃, set the temperature to the one closest to 45 ℃.

4. After the reaction, centrifuge the tube(s) briefly and immediately proceed to the next step.

## 3.3 DNase I digestion

CAUTION Do not shake or vortex the sample in the following procedures. Mix the sample by gently pipetting.

### 3.3.1 Preparation

Mix the reagents before using and store the remaining reagents immediately after use.

**Table 12 Preparing the reagents**

| Reagent | Requirement |
|---|---|
| DNase I | Flick and/or invert the tube gently, centrifuge briefly, and place on ice. |
| DNase I Buffer | Thaw at RT, mix by vortexing, centrifuge briefly, and place on ice. |

### 3.3.2 DNase I digestion

1. According to the desired reaction number, prepare the DNase I digestion reaction mixture in a 0.2 mL PCR tube on ice. Mix it well by vortexing, centrifuge briefly, and place on ice.

**Table 13 DNase I digestion reaction mixture**

| Reagent | Volume per reaction |
|---|---|
| DNase I | 2.5 μL |
| DNase I Buffer | 17.5 μL |
| Total | 20 μL |

2. Add 20 μL of DNase I digestion reaction mixture to each sample tube (from step 4 in 3.2.2). Gently **pipette** the mixture at least 15-20 times, centrifuge briefly, and place on ice.

CAUTION After pipetting, make sure that there is no residue on the tip of the pipette; make sure that the number of pipetting times is sufficient to ensure that the DNase I digestion system is evenly mixed, otherwise the final removal efficiency may be affected.

It is normal if bubbles appear during the mixing process. Centrifuge briefly and then flick to remove the bubbles.

3. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

**Table 14 DNase I digestion reaction conditions (Volume: 50 μL)**

| Temperature | Time |
|---|---|
| 45 ℃ Heated lid | On |
| 37 ℃ | 30 min |
| 4°C | hold |

4. After the reaction, centrifuge the tube(s) briefly and place on ice.

## 3.4 RNA cleanup

- Do not shake or vortex the sample in the following procedures. Mix the sample by gently pipetting.
- Use RNase-free non-stick tube in the RNA cleanup.
- Do not disturb or pipette the beads when adding reagents or transferring supernatant. If you accidentally disturb or pipette the beads, pipette the solution and beads back into the tube and restart the separation process.

### 3.4.1 Preparation

Reagent: For use with Agencourt RNAClean XP 40 mL Kit (user-supplied).

**Table 15 Preparing the reagents**

| Reagent | Requirement |
|---|---|
| 80% ethanol | User-supplied. Freshly prepared with **NF water**. |
| NF water | User-supplied. Place at RT. |
| RNA Clean Beads | Allow 30 min to equilibrate to RT before use. Mix thoroughly by vortexing before each use. |

### 3.4.2 RNA cleanup

Transfer all liquid to a new RNase-free 1.5 mL tube (one tube per reaction) in advance if using a 1.5 mL centrifuge tube and a corresponding magnetic rack for purification.

1. Mix the RNA Clean Beads thoroughly. Add 75 μL of RNA Clean Beads to each RNA sample tube (from step 3 in section 3.3.2). Gently **pipette** at least 10 times until all beads are

suspended. Ensure that all of the solution and beads in the tip are transferred into the tube after mixing.

2. Incubate the sample(s) at room temperature for 5 min.
3. Centrifuge the sample tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully remove and discard the supernatant. Do not disturb the beads that contain the RNA.
4. While keeping the tube(s) on the magnetic rack, add 200 µL of 80% ethanol to each tube to wash the beads and tube wall. Wait for 30 sec. Carefully remove and discard the supernatant.
5. Repeat step 4. Try to remove all liquid from the tube. If some liquid remains on the tube wall, centrifuge the tube briefly and place it on the magnetic rack for separation. Remove all liquid by using a low-volume pipette.
6. Keep the tube(s) on the magnetic rack. Open the tube cap and air-dry the beads at room temperature until no wetness or glossiness is visible on the beads' surface. There should be no visible cracking on the surface of the beads.

   *i* Over-drying the beads will result in reduced yield.

7. Remove the tube(s) from the magnetic rack and add an appropriate amount of NF water to elute the RNA. Gently **pipette** the liquid at least 10 times until all beads are suspended.

   *i* In step 7, Elute the RNA sample from the beads, elute the RNA by adding an appropriate volume of NF water according to the following application.

   For example, if you use MGIEasy Fast RNA Library Prep Kit, you need to add 12 µL NF water to elute the RNA, and transfer 10 µL of supernatant at step 9 to proceed to RNA fragmentation reaction.

8. Incubate the sample(s) at room temperature for 5 min.
9. Centrifuge the tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Transfer the supernatant to a new RNase-free PCR tube.

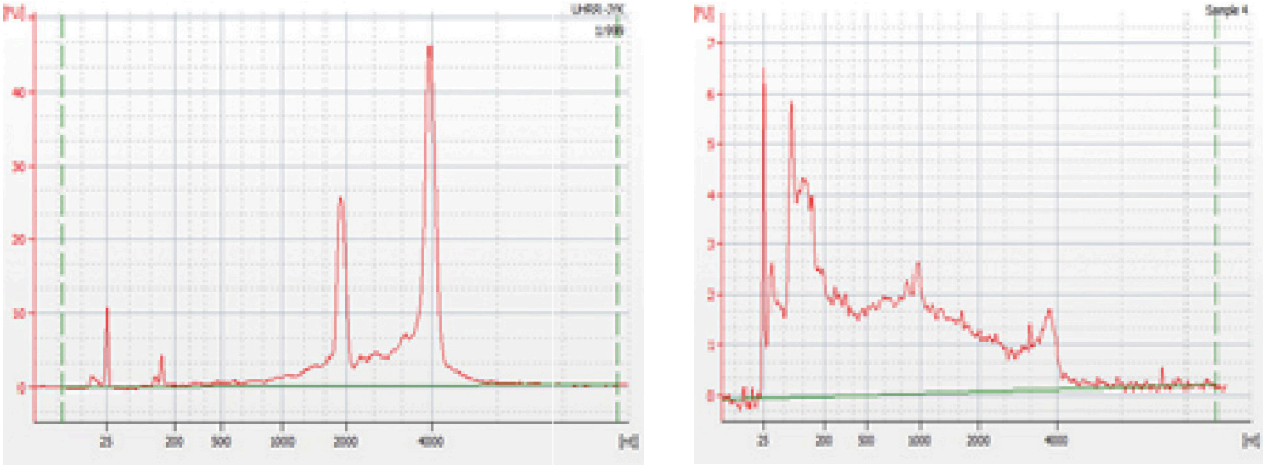
   **Stop point**
   - Cleanup sample can be placed on ice for NGS library preparation or other application.
   - The product can be stored at -20 ℃ overnight or at -80 ℃ for no more than one week (we recommend that you proceed immediately to the next reaction).

## 3.5 QC of rRNA depletion product

After the cleanup of the rRNA depleted RNA sample, perform the quality control following the instructions:

*i* This quality control is optional. If rRNA depleted RNA sample is proceeded by the NGS library construction, it is recommended that you skip this step.

Take 1 μL of the cleanup sample for quality control using the Agilent RNA 6000 Pico chip (Refer to the Agilent RNA 6000 Pico chip manual for the instructions) and Agilent 2100 Bioanalyzer. In Figure 1A, two significant 18 S and 28 S rRNA peaks are present in the Agilent 2100 analysis result of the UHRR sample without rRNA depletion. In Figure 1B, no 18 S and 28 S rRNA peaks are present in the Agilent 2100 analysis result of the rRNA depleted UHRR sample. This result demonstrates the effectiveness of rRNA depletion using DNBSEQ rRNA Depletion Kit.



A B

**Figure 1 Agilent RNA 6000 Pico assay result of purified rRNA depleted RNA**

A. The Agilent 2100 analysis result of the UHRR sample without rRNA depletion;

B. The Agilent 2100 analysis result of the rRNA depleted UHRR sample.

# 4 Appendix

## 4.1 DNase I digestion of RNA sample

### 4.1.1 DNase I digestion of RNA sample

If there is DNA contamination in RNA sample, you need to perform DNase I digestion. DNase I digestion causes a certain loss of RNA sample, the amount of total RNA used in this step needs to be increased by 20% to 30% more than the expected input (the amount of RNA input for rRNA depletion required in this kit). For example, for rRNA depletion, if the required input amount of RNA is 200 ng, the total RNA input should be 250-286 ng for the DNase I digestion.

⚠ CAUTION Do not shake or vortex the sample in the following procedures. Mix the sample by gently pipetting.

#### 4.1.1.1 Preparation

Mix the reagents before using and store the remaining reagents immediately after use.

**Table 16 Preparing the reagents**

| Reagent | Requirement |
|---|---|
| NF water | User-supplied. Place at RT. |
| DNase I | Flick and/or invert the tube gently, centrifuge briefly, and place on ice. |
| DNase I Buffer | Thaw at RT, mix by vortexing, centrifuge briefly, and place on ice. |

#### 4.1.1.2 DNase I digestion

1. Transfer an appropriate amount of RNA sample to a RNase free 0.2 mL PCR tube. Add NF water to make a final volume of 42.5 μL.
2. According to the desired reaction number, dilute the DNase I Buffer in a 1:10 dilution with NF Water (5 μL of 10x DNase I Buffer per reaction).

3. According to the desired reaction number, prepare the DNase I digestion reaction mixture on ice.

**Table 17 DNase I digestion reaction mixture**

| Reagent | Volume per reaction |
|---|---|
| DNase I | 2.5 μL |
| 10x DNase I Buffer | 5 μL |
| Total | 7.5 μL |

4. Add 7.5 μL of DNase I digestion reaction mixture to each sample tube. Gently **pipette** the mixture at least 10 times and centrifuge briefly.
5. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

**Table 18 DNase I digestion reaction conditions (Volume: 50 μL)**

| Temperature | Time |
|---|---|
| 45 ℃ Heated lid | On |
| 37 ℃ | 20 min |
| 4°C | hold |

6. After reaction, centrifuge the tube(s) briefly and place on ice.

## 4.1.2 Cleanup of RNA sample

- Do not shake or vortex the sample in the following procedures. Mix the sample by gently pipetting.
- Use RNase-free non-stick tube in the RNA cleanup.
- Do not touch or pipette the beads when adding reagents or transferring supernatant. If accidentally pipetting the beads, pipette all of the solution and beads back into the tube and restart the separation process.

### 4.1.2.1 Preparation

Reagent: For use with Agencourt RNAClean XP 40 mL Kit (user-supplied).

**Table 19 Preparing the reagents**

| Reagent | Requirement |
|---|---|
| 80% ethanol | User-supplied. Freshly prepared with **NF water**. |
| NF water | User-supplied. Place at RT. |

| Reagent | Requirement |
|---|---|
| RNA Cleanup Beads | User-supplied. Take out 30 min in advance to equilibrate to RT. Mix thoroughly by vortexing before each use. |

### 4.1.2.2 Cleanup of RNA sample

*i* Transfer all liquid to a new RNase-free 1.5 mL tube (one tube per reaction) in advance if using a 1.5 mL centrifuge tube and a corresponding magnetic rack for purification.

1. Mix the RNA Cleanup Beads thoroughly. Add 90 µL of RNA Cleanup Beads to each RNA sample tube (from 4.1.1.2 step 6). Gently **pipette** at least 10 times until all beads are suspended. Ensure all of the solution and beads in the tip are transferred into the tube after mixing.
2. Incubate the sample(s) at room temperature for 5 min.
3. Centrifuge the sample tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid becomes clear. Carefully remove and discard the supernatant. Be careful not to disturb the beads that contain the RNA.
4. While keeping the tube(s) on the magnetic rack, add 200 µL of 80% ethanol to each tube to wash the beads and tube wall. Wait for 30 sec. Carefully remove and discard the supernatant.
5. Repeat step 4. Try to remove all liquid from the tube. If some liquid remains on the tube wall, centrifuge the tube briefly and place it on the magnetic rack for separation. Remove all liquid by using a low-volume pipette.
6. Keep the centrifuge tube(s) on the magnetic rack. Open the tube cap and air-dry the beads at room temperature until no wetness or glossiness is visible on the beads' surface. Cracking on the surface of the beads should not be observed.

   *i* Over-drying the beads will result in reduced yield.

7. Remove the centrifuge tube(s) from the magnetic rack, and add appropriate amount of NF water to elute the RNA. Gently **pipette** the liquid at least 10 times until all beads are suspended.

   *i* If the purified RNA sample needs to be quantitated, elute the RNA with 21 µL of NF water. Transfer 19 µL of supernatant to a new RNase free PCR tube and take 1 µL of product for quantitation using Qubit RNA HS Assay Kit.

   If the quantitation is not performed, elute the RNA with 20 µL of NF water and transfer 18 µL of supernatant to a new RNase free PCR tube for RNA/Probe hybridization.

8. Incubate the sample(s) at room temperature for 5 min.
9. Centrifuge the tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid becomes clear. Transfer the supernatant to a new RNase-free PCR tube and proceed to the RNA/Probe hybridization.

## 4.2 FFPE sample preparation

This kit is also applicable to low-quality total RNA samples such as FFPE. However, as the result of large differences between the quality of different FFPE samples, it is not guaranteed that libraries can be prepared from all FFPE samples. The following instructions take the NGS library construction using the MGIEasy RNA Library Prep kit as an example and list the problems that you need to pay attention to during the NGS library construction from FFPE samples with different qualities.

### 4.2.1 Quality evaluation of FFPE sample

The RIN value is the most common parameter for the evaluation of RNA quality. However, the RIN value cannot accurately assess the quality of the degraded FFPE sample. Particularly, in the NGS library construction, the RIN value of the FFPE sample is not always proportional to the overall success rate of library construction. Therefore, $DV_{200}$ is also used for assessing the success rate of library construction from FFPE samples. The $DV_{200}$ indicates the proportion of RNA fragments larger than 200 nt in the sample. For severely degraded FFPE samples, the $DV_{200}$ value is a reliable indicator for the sample quality.

### 4.2.2 The calculation of $DV_{200}$

Take the analysis result from Agilent 2100 Bioanalyzer as an example for the $DV_{200}$ calculation. The detailed calculation is shown below.

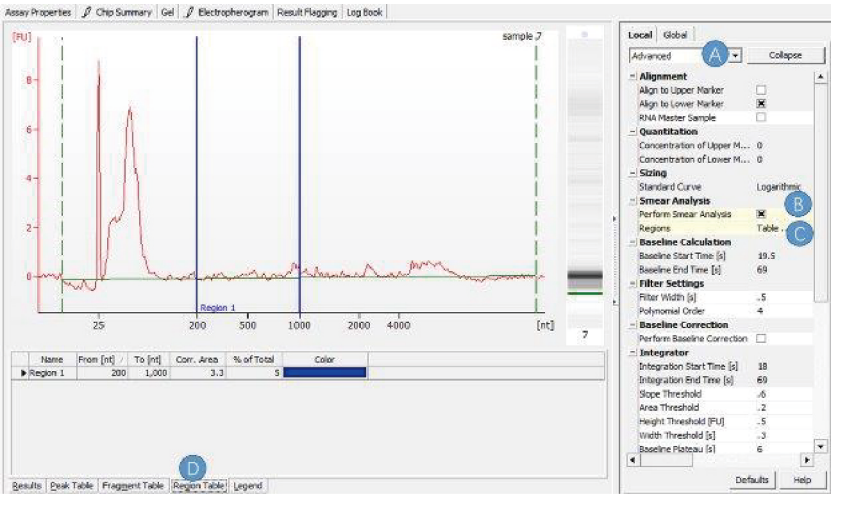



**Figure 2 The calculation of $DV_{200}$**

A. In the Agilent 2100 Bioanalyzer result figure, choose ***Advanced*** under ***Local*** tab.

B. Check the ***Perform Smear Analysis*** option under ***Smear Analysis***.

C. Double-click ***Table*** to enter the range of fragments to be calculated. The figure above shows the example of From 200 bp To 1000 bp.

D. Obtain the proportion of selected fragment shown as a % of Total in the ***Region Table***.

If you need to determine the $DV_{200}$ parameters of the FFPE sample, perform the Agilent 2100 Bioanalyzer analysis (using the RNA analysis chip) on the FFPE sample, and calculate $DV_{200}$ according to the above method. For detailed information, refer to $DV_{200}$ determination for FFPE RNA samples. (https://www.agilent.com/en/promotions/dv200-determination)

## 4.2.3 Recommended input amount of FFPE sample

For FFPE samples, we recommend using different total RNA inputs for samples with a different $DV_{200}$ value in the rRNA depletion. In addition, if the rRNA depleted RNA sample is used for NGS library construction, use different conditions for fragmentation reaction and a different number of PCR cycles in PCR amplification. See the table below for detailed conditions.

**Table 20 The recommended conditions for library construction from FFPE sample**

| $DV_{200}$ Value | Recommended amount of total RNA input | RNAClean XP beads | Fragmentation conditions | PCR cycles |
|---|---|---|---|---|
| ＞70% | 200 ng | 75 μL | 94 ℃，5 min | 15 |
| 50-70% | 200-400 ng | 100 μL | 94 ℃，5 min | 16 |
| 30-50% | 500 ng | 100 μL | No fragmentation | 16 |
| ＜30% | 0.5-1 μg (with a risk of failure of NGS library construction) | 100 μL | No fragmentation | 16 |

**Note**:

a. "Fragmentation conditions" in the above table refers to the conditions of fragmentation in "RNA fragmentation" step in "MGIEasy Fast RNA Library Prep Kit" .

b. "PCR cycles" in the above table refers to the PCR cycles in "PCR amplification" step in "MGIEasy Fast RNA Library Prep Kit" .

FFPE samples from different tissues may have different performances. This table is for reference only. The specific library construction strategy needs to be adjusted according to different RNA samples.

Doc. No.: H-940-001812-00