Contents

Recommended Starting Material

To reach optimized results it is better to follow as listed here. The size of recommended material is written in Table.

Table: Appropriate size of starting material

Sample	Size of Starting Material (min-max)
Liver	1-25 mg
Brain	1-25 mg
Kidney	1-25 mg
Spleen	1-10 mg
Ear	1-25 mg
Muscle	1-25 mg
Skin	1-25 mg
Heart	1-25 mg
Lung	1-25 mg
Mouse tail	1-25 mg
Rat tail	1-25 mg
Bacteria cells	2.5 x 10^{8} to 2 x 10^{9}

Protocol 1

Isolation of Genomic DNA (based on silica technology)

Sample type: Animal tissues (fresh and frozen)

Some tips to know

- All centrifugation steps are carried out at room temperature (15–25°C) in a microcentrifuge.
- If RNase treatment is desired, Prime-RNase A can be ordered separately from ROJETechnologies, Cat NO. EB983013 and EB983014.

- If TL Buffer (TLB) forms precipitate, please warm it to 56°C until the precipitate has fully dissolved. This is due to storage condition and won't influence the efficiency of the buffer.
- If ORB forms precipitate, please warm it to 60°C until the precipitate has fully dissolved. ORB precipitation may occur more often, however this will not influence the efficiency of the buffer.
- Do not forget to add the appropriate amount of Ethanol (96–100%) to BW1 and BW2 buffers (BWB1 and BWB2) as indicated on the bottle, before using for the first time.
- For frozen samples, equilibrate them to room temperature. Avoid repeated freezing and thawing of samples which might lead to a decrease in DNA size.

Process

1. Select one of the sample preparation methods here (a, b or c) then Place the prepared sample in a 1.5 ml microcentrifuge tube.
    a. Cut the tissue into small pieces. Then, Place the sample in to a clean microcentrifuge tube.
    b. Use Micropestle alternatively homogenizer to grind the tissue in TLB Buffer before addition of RJ-Protease.
    c. Ground the samples under liquid nitrogen (recommended for samples which are difficult to lyse).
2. Add 180 µl TLB and then add 20 µl RJ-Protease to the sample. Mix thoroughly by pulse vortexing for 30 s and then incubate at 56°C for 1-2 h until the tissue is completely lysed. Pulse vortex every 10 min during incubation to intersperse the sample, or place it in a thermomixer or shaking water bath.

Optional: This protocol is specialized for DNA isolation. For usual and routine applications RNase treatment isn't needed. If RNA-free genomic DNA is required, add 10 µl Prime-RNase A (Cat No. EB983013), mix by vortexing, and incubate for 5 min at room temperature before going to step 3.

3. Add 200 µl ORB to the mixture, mix by pulse vortexing for 10 s and then incubate at 70°C for 10 min.
4. Add 200 µl absolute Ethanol, then mix thoroughly by pulse vortexing for 15 s.
5. Pipette the mixture from step 4 to a spin column placed in a 2 ml collection tube (supplied in the kit box). Centrifuge at 13000 rpm for 1 min. Discard flow-through and place the spin column in the collection tube again.

   Note: If the lysate did not pass the column, centrifuge at full speed until it passes through the column.
6. Add 700 µl BWB1 and centrifuge for 1 min at 13000 rpm, just discard the flow-through. Place the spin column in the previous collection tube and go to the next step.
7. Add 600 µl BWB2 and centrifuge for 3 min at 14000 rpm. Discard both the flow-through and the collection tube. Place the spin column in a new clean 1.5 ml microcentrifuge tube (not provided).

   Note: To avoid Ethanol carry over, be careful that the column does not come into contact with the flow-through, if it happens discard the flow-through, place the column back in a collection tube and centrifuge for another 1 min at 14000 rpm.
8. Pipette 50-200 µl RRB directly onto spin column. Incubate at room temperature for 1-5 min. Centrifuge it at 12000 rpm for 1 min.

Note: If the expected DNA yield is more than the yield from pervious step, put the spin column on a new microtube and add another 50-200 µl RRB, incubate for 5 min at room temperature. Then, centrifuge for 1 min at 12000 rpm. However, it is possible to pass the follow-through from step 8 once more to obtain DNA with higher concentrations.

Protocol 2

Isolation of Genomic DNA (based on silica technology)

Sample type: Bacteria (gram negative)

Some tips to know

- All centrifugation steps are carried out at room temperature (15–25°C) in a microcentrifuge.
- If RNase treatment is desired, Prime-RNase A can be ordered separately from ROJETechnologies, Cat No. EB983013 and EB983014.
- If TL Buffer (TLB) or OR Buffer (ORB) forms precipitate, please warm it to 56°C until the precipitate has fully dissolved. This is due to storage condition and won't influence the efficiency of the buffer.
- Do not forget to add the appropriate amount of Ethanol (96–100%) to BW1 and BW2 buffers (BWB1 and BWB2) as indicated on the bottle, before using for the first time.
- For frozen samples, equilibrate them to room temperature. Avoid repeated freezing and thawing of samples which might lead to a decrease in DNA size.

Process

1. Calculate the bacteria cell number (refer to appendix 3, in main handbook). Collect the cell by centrifugation at 10000 rpm for 10 min. Discard the supernatant.
2. Add 180 µl TLB and then 20 µl RJ-Protease. Mix thoroughly by pulse vortexing for 30 s, then incubate at 56°C for 1-2 h until the lysate becomes clear. Pulse vortex every 10 min during incubation to intersperse the sample, or place it in a thermomixer or shaking water bath.

   Optional: This protocol is specialized for DNA isolation. For usual and routine applications RNase treatment isn't needed. If RNA-free genomic DNA is required, add 10 µl Prime-RNase A (Cat No. EB983013), mix by vortexing, and incubate for 5 min at room temperature before going to step 3.

3. Add 200 µl ORB, mix by pulse vortexing for 10 s, then incubate at 70°C for 10 min.
4. Add 200 µl absolute Ethanol, then mix thoroughly by pulse vortexing for 15 s.
5. Pipette the mixture from step 4 to a spin column placed in a 2 ml collection tube (supplied in the kit box). Centrifuge at 13000 rpm for 1 min. Discard flow-through and place the spin column in the collection tube again.

   Note: If the lysate did not pass the column, centrifuge at full speed until it passes through the column.

6. Add 700 µl BWB1 and centrifuge for 1 min at 13000 rpm, discard the flow-through. Place the spin column in the previous collection tube and go to the next step.
7. Add 600 µl BWB2 and centrifuge for 3 min at 14000 rpm. Discard both the flow-through and the collection tube. Place the spin column in a new clean 1.5 ml microcentrifuge tube (not provided).

Note: To avoid Ethanol carry over, be careful that the column does not come into contact with the flow-through, if it happens discard the flow-through, place the column back in a collection tube and centrifuge for another 1 min at 14000 rpm.

8. Pipette 50-200 µl RRB directly into spin column. Incubate at room temperature for 1-5 min. Centrifuge it at 12000 rpm for 1 min.

   Note: If the expected DNA yield is more than the yield from pervious step, put the spin column on a new microtube and add another 50-200 µl RRB, incubate for 5 min at room temperature. Then, centrifuge for 1 min at 12000 rpm. However, it is possible to pass the follow-through from step 8 once more to obtain DNA with higher concentrations.

Protocol 3

Isolation of Genomic DNA (based on silica technology)

Sample type: Bacteria (gram positive)

Some tips to know

- All centrifugation steps are carried out at room temperature (15–25°C) in a microcentrifuge.
- For gram positive bacteria like *B. subtilis*, Lysozyme (Cat No. EB983017) should be ordered separately.
- Prepare the lysis buffer as follows:

  20 mM Tris.Cl, pH 8.0

  2 mM sodium EDTA

  1.2% Triton® X-100

  add lysozyme to 20 mg/ml (immediately before use).

- Preheat a heat block or water bath to 37 °C.
- If RNase treatment is desired, Prime-RNase A can be ordered separately from ROJETechnologies, Cat No. EB983013.
- If TL Buffer (TLB) or OR Buffer (ORB) forms precipitate, please warm them to 56°C until the precipitate has fully dissolved. This is due to storage condition and won't influence the efficiency of the buffer.
- Do not forget to add the appropriate amount of Ethanol (96–100%) to BW1 and BW2 buffers (BWB1 and BWB2) as indicated on the bottle, before using for the first time.

- For frozen samples, equilibrate them to room temperature. Avoid repeated freezing and thawing of samples which might lead to a decrease in DNA size.

Process

1. Calculate the bacteria cell number (refer to appendix 3, in main handbook). Collect the cell by centrifugation at 10000 rpm for 10 min. Discard the supernatant.
2. Resuspend the pellet, add 180 µl enzymatic lysis buffer. Incubate 30-60 min at 37 °C.
3. Add 200 µl ORB and then 25 µl RJ-Protease. Mix thoroughly by pulse vortexing for 30 s and then incubate at 56 °C for 30-60 min until the sample is completely lysed. Pulse vortex every 10 min during incubation to intersperse the sample, or place it in a thermomixer or shaking water bath.

   Optional: This protocol is specialized for DNA isolation. For usual and routine applications RNase treatment isn't needed. If RNA-free genomic DNA is required, add 10 µl Prime-RNase A (Cat No. EB983013), mix by vortexing, and incubate for 5 min at room temperature before going to step 4.

4. Centrifuge at 15000 rpm for 2 min. Pour supernatant to clean tube.
5. Add 200 µl absolute Ethanol, then mix thoroughly by pulse vortexing for 15 s.
6. Pipette the mixture from step 5 to a spin column placed in a 2 ml collection tube (supplied in the kit box). Centrifuge at 13000 rpm for 1 min. Discard flow-through and place the spin column in the collection tube again.

   Note: If the lysate did not pass the column, centrifuge at full speed until it passes through the column.

7. Add 700 µl BWB1 and centrifuge for 1 min at 13000 rpm, just discard the flow-through. Place the spin column in the previous collection tube and go to the next step.
8. Add 600 µl BWB2 and centrifuge for 3 min at 14000 rpm. Discard both the flow-through and the collection tube. Place the spin column in a new clean 1.5 ml microcentrifuge tube (not provided).

   Note: To avoid Ethanol carry over, be careful that the column does not come into contact with the flow-through, if it happens discard the flow-through, place the column back in a collection tube and centrifuge for another 1 min at 14000 rpm.
9. Pipette 50-200 µl RRB directly into spin column. Incubate at room temperature for 1-5 min. Centrifuge it at 12000 rpm for 1 min.

   Note: If the expected DNA yield is more than the yield from pervious step, put the spin column on a new microtube and add another 50-200 µl RRB, incubate for 5 min at room temperature. Then, centrifuge for 1 min at 12000 rpm. However, it is possible to pass the follow-through from step 9 once more to obtain DNA with higher concentrations.