Contents

Recommended Starting Material for Isolation of Viral DNA

The size of recommended starting material to use with determined lysis volume are listed here.

Table: Recommended starting material and Lysis Buffer amount

Sample	Size of Starting material	Lysis Buffer Amount
Plasma/serum	Up to 200 µl	200 µl

Protocol 1, Isolation of Viral DNA

Sample type:

- Body fluid
- Serum
- Plasma

Some tips to know

- All centrifugation steps are carried out at room temperature (15–25°C).
- If RNase treatment is desired, Prime-RNase can be ordered separately from ROJETechnologies, Cat NO. EB983013.
- 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.
- If GL Buffer (GLB) forms precipitate, please warm it to 56°C until the precipitate has fully dissolved.

Process

1. Add 25 µl RJ-Protease to a 1.5 ml clean microcentrifuge tube. Add 200 µl sample (plasma, serum, body fluid and etc.) to the tube. Then add 200 µl GLB. Pulse vortex for 15 s and incubate at 56 °C for 12 min.
2. Add 200 µl Ethanol (96-100%) to the lysate, mix by pulse vortexing for 15 s, then centrifuge briefly.

3. Gently, pipette the mixture to a HiPure DR column placed in a 2 ml collection tube (supplied in the kit box). Centrifuge at 8000 rpm for 1 min. Discard flow-through and place back the HiPure DR column in to the collection tube.
4. Add 500 µl BWB1 and centrifuge for 1 min at 8000 rpm, discard both the flow-through and the collection tube. Place back the HiPure DR column in to the collection tube.
5. Add 500 µl BWB2 and centrifuge for 3 min at 14000 rpm. Discard both the flow-through and the collection tube. Place the HiPure DR 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.
6. Pipette 30-50 µl RRB directly onto HiPure DR column. Incubate at room temperature for 1-5 min. Centrifuge it at 12000 rpm for 1 min.

Recommended Starting Material for Isolation of Viral Nucleic Acid

The size of recommended starting material to use with determined lysis volume are listed here.

Table: Recommended starting material and Lysis Buffer amount

Sample	Size of Starting material	Lysis Buffer Amount
Plasma/serum	Up to 200 µl	200 µl

Protocol 2, Isolation of Viral Nucleic Acid

Sample type:

- Body fluid
- Serum
- Plasma

Some tips to know

- All centrifugation steps are carried out at room temperature (15–25°C).
- Equilibrate samples to room temperature.
- Equilibrate ERR to room temperature for elution step.
- Prepare a 56°C thermoblock for use.
- Add carrier RNA reconstituted in ERR to GLB according to following instructions.
- Add ethanol (96–100%) to the bottle containing BWB1[1] concentrate, as described on the bottle. Store reconstituted BWB1 at room temperature (15–25°C).

Note: Always mix reconstituted Buffer BWB1 by shaking before starting the procedure.

- Add ethanol (96–100%) to the bottle containing BWB2 concentrate, as described on the bottle. Store reconstituted BWB2 at room temperature (15–25°C).

[1] Contains chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach

Note: Always mix reconstituted BWB2 by shaking before starting the procedure.

- If GL Buffer (GLB) forms precipitate, please warm it to 56°C until the precipitate has fully dissolved.

Addition of carrier RNA to GLB[2]

Carrier preparation

Add 620 µl ERR to the tube containing 620 µg lyophilized carrier RNA to obtain a solution of 1 µg/µl (for 5 Prep sample add 31 µl ERR to the tube containing 31 µg lyophilized carrier RNA to obtain a solution of 1 µg/µl). Dissolve the carrier RNA thoroughly, divide it into conveniently sized aliquots, and store it at –20°C. Do not freeze–thaw the aliquots of carrier RNA more than 3 times.

Important! Note that carrier RNA does not dissolve in GLB. It must first be dissolved in ERR and then added to GLB.

GLB preparation

Calculate the volume of Buffer GLB–carrier RNA mix needed per batch of samples to be simultaneously processed from Table 1.

Attention! Mix gently by inverting the tube 10 times. To avoid foaming, do not vortex.
Note: The sample-preparation procedure is optimized for 5.6 µg of carrier RNA per sample. If less carrier RNA has been shown to be better for your amplification system, transfer only the required amount of dissolved carrier RNA to the tubes containing GLB.

[2] Contains chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach.

For each microgram of carrier RNA required per preparation, add 5 µl ERR-dissolved carrier RNA per milliliter of GLB. (Using less than 5.6 µg carrier RNA per sample must be validated for each particular sample type and downstream assay.)

Table 1. Volumes of GLB and carrier RNA–ERR mix required for the procedure

Samples Number	GLB amount (ml)	Carrier RNA–ERR amount(µl)	Samples Number	GLB amount (ml)	Carrier RNA–ERR amount (µl)
1	0.22	6.2	13	2.86	80.1
2	0.44	12.3	14	3.08	86.3
3	0.66	18.5	15	3.30	92.4
4	0.88	24.6	16	3.52	98.6
5	1.10	30.8	17	3.74	104.7
6	1.32	37.0	18	3.96	110.9
7	1.54	43.1	19	4.18	117.0
8	1.76	49.3	20	4.40	123.2
9	1.98	55.4	21	4.62	129.4
10	2.20	61.6	22	4.84	135.5
11	2.42	67.8	23	5.06	141.7
12	2.64	73.9	24	5.28	147.8

Process

1. Pipet 25 µl RJ-Protease into a 1.5 ml clean microcentrifuge tube. Add 200 µl of sample (plasma, serum, body fluid etc.) into the tube.

Note: If the sample volume is less than 200 µl, add the appropriate volume of 0.9% sodium chloride solution to bring the total volume up to 225 µl (including RJ-Protease).

2. Add 200 µl GLB (containing 28 µg/ml of carrier RNA). Mix by pulse vortexing for 15 s.

Note: It is crucial that the sample and GLB are mixed thoroughly to yield a homogeneous solution.

Note: Do not add RJ-Protease directly to GLB.

3. Incubate at 56 °C for 15 min in a thermoblock. Then, centrifuge briefly to remove drops from the inside of the lid.
4. Add 250 µl of ethanol (96–100%) to the sample; mix thoroughly by pulse vortexing for 15 s. Incubate the lysate with the ethanol for 5 min at room temperature (15–25°C).

Note: Cool ethanol on ice before adding to the lysate, if ambient temperature exceeds 25 °C.

5. Centrifuge the tube briefly to remove drops from the inside of the lid. Carefully pipette all of the lysate from previous step onto the HiPure VI column without wetting the rim.

6. Centrifuge at 6000 x g (8000 rpm) for 1 min. Discard the flow-through and place back the HiPure DR column in the collection tube.

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

7. Add 500 µl BWB1 and centrifuge at 6000 x g (8000 rpm) for 1 min. Discard the flow-through and place back the HiPure DR column in the collection tube.

Note: This step increases kit performance when processing inhibitory samples.

8. Add 500 µl BWB2 and centrifuge at 6000 x g (8000 rpm) for 1 min. Discard the flow-through and place back the HiPure DR column in the collection tube.
9. Add 500 µl ethanol (96–100%) and centrifuge at 6000 x g (8000 rpm) for 1 min. Discard the flow-through.

Attention! Ethanol carryover into the eluate may cause problems in downstream applications. Removing the HiPure VI column and collection tube from the rotor may also cause flow-through to come into contact with the HiPure VI column.

10. Place back the HiPure VI column in the collection tube. Centrifuge at full speed 20000 x g (14000 rpm) for 3 min to dry the membrane completely. Then discard both the collection tube and flow-through.

Recommended: Place the HiPure VI column into a new 2 ml collection tube (not provided), open the lid, and incubate the assembly at 56 °C for 3 min to dry the membrane completely.

*This step serves to evaporate any remaining liquid.

11. Place the HiPure VI column in a clean 1.5 ml clean microcentrifuge tube (not provided). Apply 20–150 µl ERR to the center of the membrane. Close the lid and incubate at room temperature for 5 min.
12. Centrifuge at full speed, 20000 x g (14000 rpm) for 1 min.

* Ensure that ERR is equilibrated to room temperature.

Important! If elution is done in small volumes (<50 µl), ERR must be dispensed onto the center of the membrane for complete elution of bound RNA and DNA.

Note: Incubation after adding ERR for 5 min at room temperature before centrifugation generally increases DNA and RNA yield.

Genomic DNA Isolation

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
Blood*	Up to 250 µl
Cultured cell	≤5 x 10^6 cells
Buffy Coat	Up to 200 µl
Animal tissue	All tissues except spleen: up to 25 mg (spleen up to 10 mg)
Bacteria cells	2.5 x 10^8 to 2 x 10^9

*White Blood Cell counts are extremely variable from a person to person and therefore, for optimum results its recommended to determine cell counts for choosing the best protocol for purification. For healthy individuals, white blood cell counts normally from 4-10 million per 1 ml blood; however, depending on disease state, cell counts may be higher or lower than the normal range.

Protocol 3, *Isolation of Genomic DNA (Animal blood, Cells, Body fluid, serum, plasma)*

Sample type:

- Animal blood
- Cells
- Body fluid
- Serum
- Plasma

Some tips to know

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

- 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.
- If GL Buffer (GLB) forms precipitate, please warm it to 56°C until the precipitate has fully dissolved.

Process

7. Add 25 µl RJ-Protease to a 1.5 ml clean microcentrifuge tube. Add 250 µl blood (plasma, serum, body fluid and etc.) to the tube. Then add 250 µl GLB. Pulse vortex for 15 s and incubate at 56 °C for 12 min.

   Note: For cell pellets, add 250 µl GLB directly to the pellet or alternatively, for direct lysis of cells grown in a monolayer, add the appropriate amount of GLB to the cell-culture dish (refer to Table). Collect the lysate with a rubber policeman. Transfer the lysate into a microcentrifuge tube. Vortex to mix, and ensure that no cell clumps are visible.

   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 2.

8. Add 250 µl Ethanol (96-100%) to the lysate, mix by pulse vortexing for 15 s, then centrifuge briefly.

9. Gently, pipette the mixture to a HiPure DR column placed in a 2 ml collection tube (supplied in the kit box). Centrifuge at 8000 rpm for 1 min. Discard flow-through and place the HiPure DR column in new collection tube (provided in the kit box).

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

10. Add 600 µl BWB1 and centrifuge for 1 min at 8000 rpm, discard both the flow-through and the collection tube. Place the HiPure DR column in new collection tube (provided in the kit box).
11. Add 600 µl BWB2 and centrifuge for 3 min at 14000 rpm. Discard both the flow-through and the collection tube. Place the HiPure DR 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.

12. Pipette 50-200 µl RRB directly onto HiPure DR column. Incubate at room temperature for 1-5 min. Centrifuge it at 8000 rpm for 1 min.

Note: If the expected DNA yield is more than the yield from pervious step, put the HiPure DR 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 8000 rpm. However, it is possible to pass the follow-through from step 6 once more to obtain DNA with higher concentrations.

Protocol 4, Isolation of Genomic DNA (Buffy coat)

Sample type: Buffy coat

Some tips to know

- All centrifugation steps are carried out at room temperature (15–25°C).
- If RNase treatment is desired, Prime-RNase A can be ordered separately from ROJETechnologies, Cat No. EB983013.
- 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.
- If GL Buffer (GLB) forms precipitate, please warm it to 56°C until the precipitate has fully dissolved.

Process

1. Add 25 µl RJ-Protease to a 1.5 ml clean microcentrifuge tube. Add 200 µl Buffy coat to the tube. Then add 200 µl GLB. Pulse vortex for 15 s and incubate at 56 °C for 12 min.

   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 2.

2. Add 200 µl Ethanol (96-100%) to the lysate, mix by pulse vortexing for 15 s, then centrifuge briefly.

3. Gently, pipette the mixture to a HiPure DR 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 HiPure DR column in new collection tube (provided in the kit box).

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

4. Add 600 µl BWB1 and centrifuge for 1 min at 13000 rpm, discard both the flow-through and the collection tube. Place the HiPure DR column in new collection tube (provided in the kit box).
5. Add 600 µl BWB2 and centrifuge for 3 min at 14000 rpm. Discard both the flow-through and the collection tube. Place the HiPure DR 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.

6. Pipette 50-200 µl RRB directly onto HiPure DR column. Incubate at 56 °C for 3-5 min. Centrifuge it at 13000 rpm for 1 min.

Note: If the expected DNA yield is more than the yield from pervious step, put the HiPure DR column on a new microtube and add another 50-200 µl RRB, incubate for 5 min at 56 °C. Then, centrifuge for 1 min at 13000 rpm. However, it is possible to pass the follow-through from step 6 once more to obtain DNA with higher concentrations.

Protocol 5, Isolation of Genomic DNA (Animal tissues)

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.
- If TL Buffer (TLB) or GL Buffer (GLB) 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. 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 25 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 HiPure DR 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 HiPure DR 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 HiPure DR 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 HiPure DR 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 HiPure DR 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 HiPure DR 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 6, Isolation of Genomic DNA (Gram negative bacteria)

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 (Cat No. EB983013), can be ordered separately from ROJETechnologies, Cat No. EB983013.
- If TL Buffer (TLB) or GL Buffer (GLB) 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 25 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 HiPure DR 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 HiPure DR 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 HiPure DR 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 HiPure DR 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 HiPure DR 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 HiPure DR 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 7, Isolation of Genomic DNA (Gram positive bacteria)

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 GL Buffer (GLB) 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 HiPure DR 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 HiPure DR 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 HiPure DR 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 HiPure DR 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 HiPure DR 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 HiPure DR 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