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RNJia Bacteria Kit

RNA isolation based on silica technology

- MiniPrep

For RNA Isolation from

Bacteria Cells

By ROJE

Edition, 12/2022

ROJETechnologies has been founded since 2014, and manufactures a wide range of molecular biology kits. We research, develop and create our products in order to make easier and more comfortable approaches to do research in molecular biology. Our target is offering high-quality affordable molecular and diagnostic Kits and reagents, comparable of the world leaders, to research centers, laboratories, clinics, hospitals and diagnostic centers all over the world.

Contents

Kit Content

Component	50 preps
RLB	50ml
TWB1 (concentrate)	16ml
TWB2 (concentrate)	15ml
Nuclease-free Water	5ml
RJ-Protease	1ml
Lysozyme	200mg
HiPure DR Column	50
Collection Tube	50

Storage

Shipment condition is checked by ROJETechnologies. After arrival, all reagents should be kept dry, at room temperature. We suggest storing RJ-Protease at 2-8°C, and for routine use, it is recommended that you aliquot it to 100µl volumes and storage at 2-8°C. When storage condition is as directed, all reagents are stable until expiration date, as indicated on the kit box.

Intended Use

RNJia Bacteria Kit provides the components and procedures necessary for purifying RNA from bacteria. Notice that, RNJia Bacteria Kit is intended for molecular biology applications not for diagnostic use. We recommend all users to study RNA experiments guideline, before starting their work.

Guarantee & Warranty

ROJETechnologies guarantees the efficiency of all manufactured kits and reagents. For more information on choosing proper kits based on your needs, please contact our technical support team. If any product does not satisfy you, due to reasons other than misuse, please contact our technical support team. If problem is due to manufacturing process, ROJE team will replace the kit for you.

Notice to Purchaser

This product is only for experiments and not for commercial use in any kind. No right to resell this kit or any components. For information about out licensing or distributors contact ROJE business team.

Warning and Precautions

Due to chemical material usage that may be hazardous, always make sure to wear suitable lab coat, disposable gloves, and protective eyewear. Material Safety Data Sheet (MSDS) for all products and reagents are provided. They are accessible online at www.rojetechnologies.com.

Quality Control

RNJia Bacteria Kit is tested against predetermined experiments on a lot-to-lot basis according to ROJETechnologies ISO-certified quality management system, to ensure consistent product quality. For your information, the results of all experiments are accessible by addressing REF and Lot number on web at www.rojetechnoloes.com.

Description

RNJia Bacteria Kit Provides a time-saving, reliable and meticulous method for RNA isolation from Bacteria types. RNJia Bacteria Kit is based on spin column technology for isolation of concentrated, highly purified and intact RNA, without use of phenol and chloroform, which is suitable to use for a variety of downstream applications such as Northern blot experiments, Real-time PCR, RNA Sequencing etc.

Procedure

The RNJia Bacteria Kit is designed for isolating RNA molecules from bacteria. Cells should be lysed and homogenized. Then, RNA binding to the silica membrane is achieved selectively, by the addition of ethanol to the lysate. Contaminants are removed by two specific kinds of washing buffers. Pure RNA is finally eluted in nuclease-free water. Isolated RNA is ready to use in downstream applications. It has A260/A280 ratios of 1.9–2.3 by spectrophotometer, confirming high purity.

Equipment & Reagents to Be Supplied by User

- Absolute ethanol
- 1.5ml RNase free microcentrifuge tubes
- RNase free pipets and pipet tips
- Benchtop microcentrifuge
- Vortex

- Thermoblock or water bath
- Syringe and needles Syringe and needles
- Homogenizer

Applications

The isolated RNA and miRNA can be used in many downstream applications:

- RNA Sequencing
- cDNA synthesis
- Microarrays
- RT-PCR and real-time RT-PCR
- Northern, dot blot analysis

Features

Specific features of RNJia Bacteria Kit are listed here in Table 1.

Table 1. RNJia Bacteria Kit features and specifications

Features	Specifications
Elution Volume (µl)	30-50µl
Technology	Silica technology
Main Sample Type	Gram negative & positive bacteria cells
Processing	Manual
Sample Amount	2 x 10^8 to 2 x 10^9 cells
Operation Time Per Reaction (Min)	Less than 50min
Typical Yield for E. Coli (µg)	75µg
Average Purity (A260/A280)	A260/A280= 1.95-2.3
Size of RNA Purified	≥200 nucleotides
Enzyme	RJ-Protease, Lysozyme

Recommended Starting Material

To reach optimized results it is better to follow as listed below. The size of the recommended starting material to use with determined RLB are written in Table 2.

Table 2. Appropriate sample size and amount of RLB

Sample Type	Sample Size	Amount of RLB	Absolute Ethanol Amount
Bacteria Cells	≤5 X10^8	500µl	300µl
Bacteria Cells	5 X 10^8 - 1 X 10^9	800µl	480µl
Bacteria Cells	1 X 10^9 – 2 X 10^9	1000 l	600µl

Sample Storage and Preparation

Bacteria

Typical yields of RNA will vary depending on the cell density of the bacterial culture and the bacterial species, hence before starting, it's recommended to determine your bacterial species. As a guide, bacteria culture preparation and storage conditions are written here.

Storage

Fresh or frozen bacteria samples may be used by RNJia Bacteria Kit. Frozen samples can be kept at -80°C for a long time. As a guide, storage, preparation of stock and conditions are written here.

Bacteria culture

The following protocol is for inoculating an overnight culture of liquid LB with bacteria.

- Prepare liquid Luria-Bertani (LB)

To make 400 mL of LB, weigh out the following into a 500 mL glass bottle:

- 4 g NaCl
- 4 g Tryptone
- 2 g Yeast Extract
- and dH2O to 400 mL

  Loosely close the cap on the bottle and then loosely cover the entire top of the bottle with aluminum foil. Autoclave and allow to cool to room temperature. Now screw on the top of the bottle and store the LB at room temperature.

- Use a single, well-isolated colony from a fresh Luria-Bertani (LB) agar plate to inoculate 1–10 ml of LB medium.
- Loosely cover the culture with sterile aluminum foil or a cap that is not airtight.

- Incubate bacterial culture at 37°C for 12-18 hour in a shaking incubator

Storing condition

- Autoclave microcentrifuge tube or 1-3ml screw cap.
- Grow a fresh overnight culture of the strain in broth. Do not grow the cultures too long. Bacteria strains should be grown to late log phase.
- Label the tube with the strain and date.
- Either %5 to %10 DMSO or glycerol can be used as cryopreservation in the culture medium. Glycerol is usually prepared in aqueous solution at double the desired final concentration for freezing. It is then mixed with an equal amount of cell suspension.
- Aliquot 1 to 1.8ml of bacteria to each vial and seal tightly with screw cap.
- Allow the cells to equilibrate in the freeze medium at room temperature for a minimum of 15min but no longer than 40 min. After 40min, the viability may decline if DMSO is used as the cryoprotectant.
- Place the vials into a pre-cooled (4°C), controlled rate freeze chamber and place the chamber in a mechanical freezer at -70 °C for at least 24 hours.
- Quickly transfer the vials to liquid nitrogen or at -130 °C freezer. After 24 hours at -130 °C, remove one vial, restore the bacteria in the culture medium and check viability and sterility.

Recovery of cryopreserved cells

- Prepare a cultured vessel that contains at least 10ml of the appropriate growth medium equilibrate for both temperature and pH.
- Remove the vial containing the strain of interest and thaw by gentle agitation in a 37 °C water bath (or a bath set at the normal growth temperature for that bacterial strain). Thaw the strain rapidly until all ice crystals have been melted (approximately 2min).
- Remove the vial from the bath and decontaminate it by dipping in or spraying with %70 ethanol. Unscrew the top of the vial and transfer the entire content to the prepared growth medium. Examine the cultures after an appropriate length of time. If the broth shows growth in 1-2 days, streak a plate from the broth and verify that is the correct strain.

Preparation

It is crucial to use the correct amount of starting material. RNA content can vary greatly between different bacteria types. So, counting cells is the most important step before

starting the procedure. The input bacterial cell amount should not exceed 2 × 10^9 cells. For example, for E. coli, depending on culture growth, this is equivalent to 0.5 - 1.0mL of an overnight culture. It is not recommended to exceed 1mL of culture for this procedure. It is important to measure bacterial growth by spectrophotometer before starting the protocol. (For cell counting guideline refer to appendix 7). After counting and selecting the intended cell volume, choose the best protocol and start the isolation process.

RNaseLag

For optimum results, it is recommended to store your samples in RNaseLag. RNaseLag is a Reagent, which provides in vivo stabilization of RNA in bacteria to ensure reliable gene expression analysis.

Procedure

- Calculate the required volume of bacterial culture (refer to Appendix 7).
- Add two volumes of RNaseLag into a tube (order by Cat No RN983016 or RN983017)
- Add one volume of bacterial culture to the tube. Mix by vortexing for 5 sec. Incubate at room temperature (15–25°C) for 5min.
- Centrifuge for 10min at 4000 rpm at universal centrifuge.

  Note: Sometimes the pellet is too clear to be recognized, it is due to RNaseLag treatment, and will not affect the ongoing process.

- Decant the supernatant.
- Pellets can be stored at –20 to –30 °C for up to 2 weeks or at –70 °C for up to one month. For RNA isolation, thaw pellets at room temperature (15–25 °C) and proceed the appropriate RNA isolation protocol.
- Washing Buffer Preparation
- Before the first use, add appropriate amount of ethanol (%96-100) to each washing buffer tube, then mix thoroughly to prepare washing buffer, refer to Table 3. Do not forget to tick the check box on the bottle label to indicate that ethanol has been added. Before each use mix reconstituted buffer by shaking. Storing at room temperature.

Table 3: Washing buffer preparation

Buffer Name	Concentrated Volume	Amount of Ethanol	Final Volume

TWB1	16ml	24ml	40ml
TWB2	15ml	45ml	60ml

Protocols

Phenol-Chloroform Free Based Protocols

Protocol 1: Isolation of RNA (Bacteria, gram negative and positive)

Sample Type: Bacteria (gram negative and positive)

Protocol Type: Enzymatic lysis

Some Tips to Know:

- This protocol needs to be improved by the user for intended bacterial species.
- Set Thermoblock or water bath at both temperature, 40℃ and 60℃ before starting the process.
- Before using the protocol for the first time, add 2-Mercaptoethanol to RLB, %2 of RLB volume. 2-Mercaptoethanol is commercially available and due to safety issue, is not provided in the kit. It can be order separately, by ROJETechnologies (Cat No BU983034) or Sigma-Aldrich (Cat No M3148).

  Attention! It is recommended to prepare it freshly. It is not stable more than 2 weeks.

- If RLB forms precipitate, please warm it to 56 °C until the precipitate has fully dissolved. This is due to storage condition and will not influence the efficiency of the buffer.
- Do not forget to add the appropriate amount of ethanol (molecular biology grade (%96–100) to TWB1 and TWB2, as indicated on the bottle, before using for the first time. Refer to washing buffer preparation section.
- For frozen samples in RNaseLag, thaw them to room temperature. Avoid repeated freezing and thawing of samples, since it may cause RNA degradation.
- If working with RNA for the first time, read Appendix 1 carefully.
- In this protocol, the bacteria cell lysis is accomplished by lysozyme and RJ-Protease. Lysozyme should be prepared as a 20 mg/ml solution in nuclease-free TE buffer.

Attention! It is recommended to prepare lysozyme freshly. It is not stable more than 3 weeks at 4 °C.

Process

- Calculate the bacteria cell number (refer to appendix 7). Collect the cell by centrifugation at 10000 rpm for 10min. Discard the supernatant.
- Add 20µl RJ-Protease and 200µl of prepared lysozyme solution to the pellet. Resuspend by pulse vortexing for 15 s to completely solve the pellet. Incubate the lysate at 40°C for 15min. During the incubation, pulse vortex every 2min for 10 s.
- Add appropriate amount of RLB (refer to Table 2), to the pellet. Vortex for 1min and incubate at 40°C for 5min.
- Add an appropriate absolute ethanol, invert several times (refer to Table 2).
- Transfer up to 700µl of the sample to a HiPure DR Column placed in a 2ml collection tube (supplied in the kit box). Centrifuge for 1min at 13000 rpm. Discard the flow-through.

  Note: If the lysate does not pass the column, repeat the centrifugation once more at full speed for 1min.

- Repeat the previous step by the remaining sample from step 4.

  Note: If the lysis does not pass the column, repeat the centrifugation once more at full speed for 1min.

- Add 700µl TWB1 to the HiPure DR Column. Centrifuge for 1min at 13000 rpm. Discard the flow-through.
- Add 500µl TWB2 to the HiPure DR Column. Centrifuge for 1min at 13000 rpm. Discard the flow-through.
- Add 500µl TWB2 to the HiPure DR Column. Centrifuge for 3min at 14000 rpm. Discard the collection tube with the flow-through.
- Place the HiPure DR Column in a new 1.5ml microtube. Add 30-50µl RNase-free water directly to the HiPure DR Column membrane. Incubate at 60°C for 5 min. Centrifuge for 1 min at 12000 rpm to elute the RNA.

Note: If the expected RNA yield is more than the yield from pervious step, put the HiPure DR Column on a new microtube and add another 30-50µl RNase-free water. Incubate at 60°C for 5 min. Centrifuge for 1min at 12000 rpm. The yield will be nearly same as

previous step. However, it is possible to pass the flow-through from step 10 once more to obtain RNA with higher concentration.

Protocol 2: Isolation of RNA (Bacteria, gram negative and positive)

Sample Type: Bacteria (gram negative and positive)

Protocol Type: Simultaneously mechanical and enzymatic lysis

Some tips to know:

- This protocol needs to be improved by the user for intended bacterial species.
- Set Thermoblock or water bath at both temperature, 40℃ and 60℃ before starting the process.
- Before using the protocol for the first time, add 2-Mercaptoethanol to RLB, %2 of RLB volume. 2-Mercaptoethanol is commercially available and due to safety issue, is not provided in the kit. It can be order separately, by ROJETechnologies (Cat No BU983041) or Sigma-Aldrich (Cat No M3148).

  Attention! It is recommended to prepare it freshly. It is not stable more than 2 weeks.
- If RLB 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 (molecular biology grade, %96–100) to TWB1 and TWB2, as indicated on the bottle, before using for the first time. Refer to washing buffer preparation section.
- For frozen samples in RNaseLag, thaw them to room temperature. Avoid repeated freezing and thawing of samples, since it may cause RNA degradation.
- If working with RNA for the first time, read Appendix 1 carefully.
- In this protocol, the bacteria cell lysis is accomplished by lysozyme and RJ-Protease. Lysozyme should be prepared as a 20mg/ml solution in nuclease-free TE buffer.

Attention! It is recommended to prepare lysozyme freshly. It is not stable more than 3 weeks at 4°C.

Process

- For each sample, weigh 25–50 mg acid-washed glass beads (150–600 µm diameter) in a 2ml Safe-Lock tube.
- Calculate the bacteria cell number (refer to appendix 7). Collect the cell by centrifugation at 10000 rpm for 10min. Discard the supernatant.
- Add 20µl RJ-Protease and 200µl of prepared lysozyme solution to the pellet. Resuspend by pulse vortexing for 15 s to completely dissolve the pellet. Incubate the lysate at 40°C for 15min. During the incubation, pulse vortex every 2min for 10 s.
- Add appropriate amount of RLB (refer to Table 2), to the pellet. Vortex for 1min then incubate at 40°C for 5min.
- Transfer the suspension into the 2ml Safe-Lock tube containing the acid washed glass beads prepared in step 1. Disrupt the cells in the Tissuelyser for 5min at maximum speed.
- Centrifuge at 14000 rpm for 1min. Transfer the supernatant to a new microcentrifuge tube. Add appropriate amount of absolute ethanol (refer to Table 2), invert several times.
- Transfer up to 700µl of the sample to a HiPure DR Column placed in a 2ml collection tube (supplied in the kit box). Centrifuge for 1min at 13000 rpm. Discard the flow-through.

  Note: If the lysate does not pass the column, repeat the centrifugation once more at full speed for 1min.

- Repeat the previous step by the remaining sample from step 6.

  Note: If the lysis does not pass the column, repeat the centrifugation, once more at full speed for 1min.

- Add 700µl TWB1 to the HiPure DR Column. Centrifuge for 1min at 13000 rpm. Discard the flow-through.
- Add 500µl TWB2 to the HiPure DR Column. Centrifuge for 1min at 13000 rpm. Discard the flow-through.
- Add 500µl TWB2 to the HiPure DR Column. Centrifuge for 3min at 14000 rpm. Discard the collection tube with the flow-through.
- Place the HiPure DR Column in a new 1.5ml microtube. Add 30-50µl RNase-free water directly to the HiPure DR Column membrane. Incubate at 60°C for 5 min. Centrifuge for 1min at 12000 rpm to elute the RNA.

  Note: If the expected RNA yield is more than the yield from pervious step, put the HiPure DR Column on a new microtube and add another 30-50µl RNase-free water. Incubate at 60°C for 5min. Centrifuge for 1min at 12000 rpm. The yield will be nearly same as previous step. However, it is possible to pass the flow-through from step 12 once more to obtain RNA with higher concentration.

Troubleshooting

Here we try to cover as many problems as you may see in using this product, however scientists in ROJE Technical Support Team are eager to answer all your questions. Do not hesitate to contact us for more information.

Symptoms	Problem	Suggestion
Low yield	Incorrect starting material	• Refer to appendix 7 for information about cell counting and Table 2 for correct amount of starting material.
	Incomplete cell wall distraction	• While using lysozyme, it may be needed to optimize lysozyme concentration and digestion time. • While using mechanical disruption, it may be needed to lengthen the disruption duration. • Freezing and thawing of the cell pellets treated by RNaseLag makes cell walls disruption easier.
	Not harvesting cells during logarithmic phase	• For highest RNA yield, it is recommended to harvest cells at logarithmic growth phase.
	Ethanol from the washing buffer is present at elution	• Preform another centrifugation before rehydration step to ensure no remaining of ethanol on column. • Carefully remove the column from the collection tube so that the column does not contact the flow-through.
	RNA elution is incomplete	• Perform rehydration step once more, by adding another 30µl rehydration buffer to column and before centrifugation, incubate 5min at 60˚C. • Check that all previous steps are done appropriately.
Degradation	RNase contamination	• All buffers have been tested and are guaranteed RNase-free. RNases can be introduced during use. Refer to Appendix 1 for more information.
Low 260/280 ratio	RNA was diluted in low pH water	• Use 10 mM Tris-HCl with pH ≥ 7.5, or nuclease-free water with pH ≥ 7.5.
	DNA contamination	• Follow precisely the respective protocol, if RNA purification is still problematic further do DNase Treatment.

	Protein contamination	• This is often due to exceeding the amount of starting material. Follow precisely the respective protocol; if RNA purification is still problematic further reduce the amount of starting material.
DNA contamination in downstream application	No DNase treatment	• Perform DNase treatment.
	No incubation with TWB1	• Incubate the spin column for 5min at room temperature after addition of TWB1 and before centrifuging
Not performing well in downstream application	Ethanol carryover	• Preform another centrifugation before rehydration step to ensure no remaining of ethanol on column.
	Salt carryover	• Between washing steps, eliminate remaining flow-through from the rim of collection tube, by blotting on clean paper towels.
Clogged Column	Maximum amount of starting material exceeds the kit specifications	• Refer to specifications to determine if amount of starting material falls within the kit specifications.
	The sample is too large	• Use fewer starting material. The problem can be solved by increasing the g-force and/or centrifuging for a longer period until the lysate passes through the column.
	Centrifuge at low temperature	• The centrifugation temperature should be 20–25°C. Make sure that the centrifuge temperature is set at 25°C.

Technical Assistance

ROJETechnologies guarantee your complete satisfaction. ROJE technical support team is composed of highly trained, experienced scientists; who can troubleshoot most problems you face. Our technical support team can offer expert advice which may help you select a suitable product.

- Contact our technical support at any time by selecting one of these ways:
- Through our telephone and fax number; +982191070705.
- You can submit your question directly to ROJE Technical Support Team from our website (www.ROJETechnologies. com)
- Or send your questions to this email address, technicalsupport@rojetechnologies.com.

Ordering Information

Category	Product Name	Cat No.	Size
RNA Technologies	RNJia Bacteria Kit	RN983022	50 preps
	RNJia Kit	RN983006	100 preps
	RNJia Fibrous Kit	RN983025	50 preps
	RNJia Phenol-Free PB Kit	RN983057	100 preps
	MiRJia Lipid Kit	RN003078	100 preps
	MiRJia Kit	RN003079	100 preps
	RNaseLag	RN983016	50ml
	RNZO	RN983048	25ml
	RNA Loading Set	LD983007	100 Preps
	RNSol H Reagent	RN983061	100 ml

Appendix 1: Handling RNA

RNA is highly sensitive to degradation due to the ubiquitous presence of RNases in the environment. unlike DNases, which require metal ions for activity, RNases do not require cofactors for their enzymatic activity and can maintain activity even after prolonged boiling or autoclaving. Since RNases are active enzymes, and are too hard to be deactivated, do not use any plasticware or glassware without first removing possible RNase pollution. Special precautions should be taken when working with RNA. All reagents and equipment must be specially treated to inactivate RNases prior to use.

The following are some tips and techniques to remember when working with RNA:

General Tips

- Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory mobilizations. These materials are generally RNase-free and do not require pretreatment to inactivate RNase.
- Treat surfaces of benches with commercially available RNase inactivating agents such as RNZO (Order by cat No RN983018 and RN983019).
- Treat non-disposable glassware and plasticware before use, to ensure that it is RNase-free. Thoroughly rinse plasticware with 0.1N NaOH/1mM EDTA, and RNase-free water.
- Use disposable RNase-free plasticware to decrease the possibility of sample contamination.
- Keep purified RNA on ice when aliquots are pipetted for downstream applications.
- Glassware should be immersed in freshly prepared %0.1 (v/v) DEPC in water or ethanol for several hours, followed by draining and autoclaving.

Appendix 2: RNA Storage Condition

RNA samples are commonly stored at −20°C or −80°C, eluted in RNase-free water. Under these conditions, RNA is not degraded after 1 year.

RNA Quality

The ratio of absorbance at 260 nm and 280 nm is used to assess the purity of RNA using UV absorption, by a spectrophotometer or Nanodrop. For pure RNA, A260/A280 ratios should be around 2.1. If the ratio is noticeably lower in either case, it may indicate the presence of protein or other contaminants that absorb strongly at or near 280 nm. High A260/A280 purity ratios are not indicative of an issue.

Appendix 3: RNA Integrity

The integrity of RNA molecules is important for experiments that try to reflect the snapshot of gene expression of RNA isolation. The least expensive method for checking RNA integrity is to run the RNA on a %1 DGE agarose gel and examine the ribosomal RNA (rRNA) bands. The upper ribosomal band (28S in eukaryotic cells and 23S in bacterial cells) should be about twice the intensity of the lower band (18S in eukaryotic cells and 16S in bacterial cells) and should be sharp and tight.

Denaturing agarose gel electrophoresis

Formaldehyde is used to keep the RNA from denaturation, and when RNA is heated in the presence of formaldehyde all secondary structures are eliminated. Therefore, gel electrophoresis of RNA in agarose gels containing formaldehyde provides a good denaturing gel system. Formaldehyde is a potential carcinogen. Take appropriate safety measures and wear gloves when handling.

Gel preparation

- Prepare %1.2-2 gel with TAE 1x buffer (%5 formaldehyde).
- Pour the gel using a comb that will form wells large enough to accommodate at least 20µl.
- Assemble the gel in the tank.
- Use TAE 1x buffer (%5 formaldehyde) as the tank buffer.

RNA sample preparation

- Add 10µg of purified RNA to 6µl of ROJE sample buffer (in RNA Loading Set, order by cat No LD983007) and incubate at 70 °C for 3 min.
- Add 3µl Hinari to the mixture of RNA and sample buffer and mix well by pipetting.
- Then run the mixture in the prepared gel.

Electrophoresis

Load the gel and electrophorese at 5-6 V/cm until the loading dye at least has migrated 2/3 the length of gel.

Results

Visualize the gel on a UV transilluminator. The 28S and 18S ribosomal bands should be sharp and intense. The intensity of the 28S band should be twice more than the 18S band. The following Figure shows different RNA samples, isolated by RNJia kit and run by DGE.

> **Note:** If DNA contamination is present, the high molecular weight smear will be appeared above the 28S band.

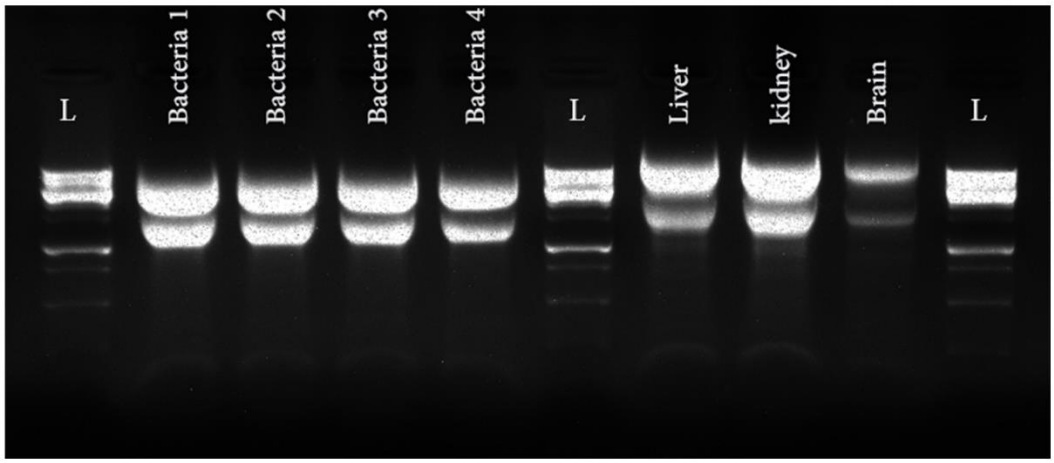

Figure 1. Isolated RNA using RNJia Kit, RNA was isolated from the indicated animal tissues and bacteria cells using the RNJia Kit. For each sample, 10 µg RNA was pretreated by ROJE Sample Buffer and analyzed on Denaturing Gel Electrophoresis (DGE).

Appendix 4: DNA Contamination

Different methods exist for monitoring genomic DNA contamination, for example, using OD measurement or via agarose gel electrophoresis. Nevertheless, genomic DNA traces which are not detectable by the above methods, can produce nonspecific results in sensitive downstream applications. The co-amplification of cDNA and contaminating genomic DNA produce one or more additional PCR fragments that can be traced via agarose gel electrophoresis.

strategies to solve the problem:

- Design PCR primers for span exon boundaries, which includes one or more intronic sequence motifs in the genomic DNA, results in amplification of cDNA template.
- Design a minus control reaction with reverse transcriptase. In that specific reaction, instead of the reverse transcriptase enzyme, water is added to the reaction mixture. PCR product in this control reaction indicate genomic DNA contamination.

Appendix 5: Protein Isolation by RNJia Kit

RNJia Kit is specified for RNA isolation. However, protein can be extracted simultaneously. The denatured protein can be used for SDS-PAGE, western blotting, 2D gel electrophoresis and etc.

Equipment to be supplied by user

- Centrifuge
- Acetone
- Ethanol, molecular biology grade

Process

- Prepare cell lysate (appropriate to sample type) and centrifuge it through a HiPure DR Column, as described in all protocols.
- Do not discard the flow-through. Transfer it to a new clean microtube and Add 4 volumes of ice-cold acetone to it.
- Incubate at -20˚C for 30 min.
- Centrifuge for 10 min at 14000 rpm in a benchtop centrifuge. Discard the supernatant.
- Wash the pellet with 100µl ice-cold ethanol and air-dry.

  Note: For easier resuspension, do not over dry the pellet.
- Resuspend the pellet in the appropriate buffer according to downstream reaction.

Appendix 6: Convert RPM to RCF (centrifuge)

All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})(r)}}$$

Where **RCF** = required gravitational acceleration (relative centrifugal force in units of g); **r** = radius of the rotor in cm; and **RPM** = the number of revolutions per minute required to achieve the necessary g-force.

Appendix 7: Cell Count by a Hemocytometer

Sample preparation

First, resuspend the cell in flask, and then quickly remove your aliquot of cells. For a cell culture growing in a Petri dish, you need to resuspend the cells growing on the bottom of the dish by gently using a pipette to remove cells and media from a dish and then gently expelling them back into the dish. This aspiration of the contents of the Petri dish should be completed several times, each time expelling the cells and media while moving the pipette across the bottom of the dish to gently discharge cells growing on the entire surface. After resuspension, the aliquot is quickly removed from the vessel before the cells settle to the bottom again. For cell culture applications cells are often stained with Trypan Blue so that dead cells can be distinguished from live cells. For example, a 0.5ml suspension of cells would be removed from

the Petri dish and mixed with 0.5ml Trypan Blue solution in an Eppendorf or small test tube. Trypan Blue is a stain that selectively stains dead cells.

Loading Sample

Load your sample quickly and smoothly. It is important to handle the hemocytometer and the cover slip carefully. Never place your fingers on the reflective surface of the slide. Always clean the slide before you load the sample by rinsing the slide and cover slip with %70-95 ethanol. Air-dry or gently wipe the slide and cover slip with lens paper. Place the clean and dry slide on your work surface and place the coverslip on top to cover the reflective surfaces. If you have diluted your cells in a test tube, invert the tube several times to resuspend the cells. Using a micropipette, quickly and smoothly without interruption, add 10 µl of your cell suspension (or 1 drop from a transfer pipette) to the v-shaped groove on each side of the hemocytometer. If your sample moves into the gutters you may not have loaded the sample in the correct location or you may have used too large of an aliquot.

Estimating cell density

count all of cells within each of the four large quadrants in the four corners of each counting chamber on the hemocytometer (see Figure 2). Count all of the cells within each quadrant except those on the far-right edge and lower bottom edge.

Calculate the cell density by this formula:

Average number of cells × dilution factor× 10^4

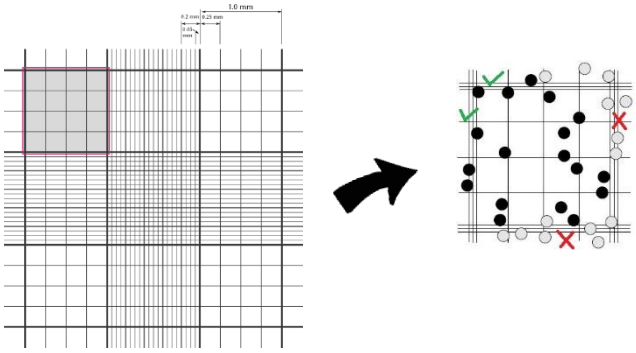

Figure2. Cell counting with hemocytometer

Appendix 8: Preparation of Phosphate Buffered Saline (PBS)

Dissolve the components in about 800mL dH2O. Adjust the pH to 7.4 with HCl. Adjust the volume to one liter. Sterilize by autoclaving. Store at room temperature.

Table 4. PBS preparation

Concentration	Component	G/litter
137 mM	NaCl	8g
2.7 mM	KCl	0.2g
10 mM	Na2HPO4	1.4 g
1.8 mM	KH2PO4	0.25g

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