RIBOSOMAL RNA DEPLETION FOR AVIAN SPECIES

**Novel rRNA-depletion methods for total RNA sequencing and ribosome profiling
developed for avian species**

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ABSTRACT

Deep sequencing of RNAs has greatly aided the study of the transcriptome, enabling comprehensive gene expression profiling and the identification of novel transcripts. While mRNAs are of the greatest interest in gene expression studies as they encode for proteins, mRNAs make up only 3-5% of total RNAs, with the majority comprising ribosomal RNAs (rRNAs). Therefore, applications of deep sequencing to RNA face the challenge of how to efficiently enrich mRNA species prior to library construction. Traditional methods extract mRNAs using oligo-dT primers targeting the poly-A tail on mRNAs; however, this approach is not comprehensive as it does not account for mRNAs lacking the poly-A tail or other lncRNAs that we may be interested in. Alternative methods deplete rRNAs, but such approaches require species-specific probes and the commercially available kits are costly and have only been developed for a limited number of model organisms. Here we describe a quick, cost-effective method for depleting rRNAs using custom-designed oligos. We use chickens as an example species for probe design, and the same approach also apply to mice. Using this protocol, we have not only removed the rRNAs from total RNAs for RNA-seq library construction but also depleted rRNA fragments from ribosome-protected fragments for ribosome profiling. Currently, this is the only rRNA depletion-based method for avian species; this method thus provides a valuable resource for both the scientific community and the poultry industry.

Key words: rRNA, Mitochondrial rRNA, Chickens, RNA-seq, Ribo-seq

INTRODUCTION

The high-throughput sequencing of cellular RNAs (RNA-seq) or ribosome protected fragments (Ribo-seq) has greatly aided in the characterization of the transcriptome, enabling gene expression studies and the identification of novel transcripts and novel open reading frames [Morlan, et al. (2012), Adiconis, et al. (2013)] {Ingolia et al., 2009, #90822}. Protein coding mRNAs are of the greatest interest in gene expression studies. However, efficient RNA deep sequencing faces significant challenges on how to enrich mRNAs in the sample library, as mRNAs only account for 3-5% of total RNA [Alberts, et al. (2002)]. Therefore, applications of high-throughput sequencing approach to RNAs require methods for enriching mRNAs.

One widely-used method for mRNA enrichment uses of oligo dTs to capture the mRNAs with poly-A tails. However, this approach is incapable of capturing mRNAs that are missing poly-A tails due to degradation, or mRNAs such as histones that use alternative mechanisms for 3' end processing [López, et al. (2008)]. An alternative method involves the depletion of ribosomal RNAs (rRNAs). Because ribosomes are required for protein synthesis in the cytoplasm and mitochondria, rRNAs are abundant, accounting for ~80% of total RNA in eukaryotes [Lodish, et al. (2000)]. Cytosolic rRNAs, including 18S, 28S, 5.8S and 5S rRNAs, are processed from 2 rRNA precursors, and the general scheme of their processing is conserved among mammals, invertebrates, yeast, and plants [Tomecki, et al. (2017), Long, et al. (1980)]. One of the rRNA precursors, the 45S rRNA precursor, is transcribed by RNA polymerase I, and then further processed into 18S, 5.8S, and 28S [Winnebeck, et al. (2010)]. The second rRNA precursor, the 5S precursor, is transcribed by RNA polymerase III [Winnebeck, et al. (2010)]. Finally, all mature rRNAs are assembled with ribosomal proteins to form functional small and large

subunits. Distinct from cytosolic rRNAs, mitochondria rRNAs, 12S and 16S rRNAs, are encoded by the mitochondrial genome [Rorbach, et al. (2012)], transcribed and processed within mitochondria, and further assembled with ribosome subunits. These mitochondrial rRNAs are responsible for ribosome functions inside mitochondria [Yang, et al. (2014)].

The depletion of rRNAs is not only a preferred mRNA enrichment method for RNA-seq, but also the only option for Ribo-seq. Ribo-seq is a technique to capture ribosome protected fragments (RPFs) on a genome-wide scale, with a sub-codon resolution [Ingolia, et al. (2009)]. RPFs are acquired by treating tissue/cell lysate with an optimized amount of RNase, which degrades the RNAs that are not protected by ribosomes. After running a sucrose gradient, the monosome fraction will be collected to enrich authentic RPFs. Since rRNA is a significant component of ribosomes, the purified RNAs from sucrose gradient also have an abundance of rRNA. Thus, it is also necessary to deplete rRNA before RPF library construction. rRNA-depletion approaches typically use oligos complementary to rRNAs to either extract them or degrade them from the total RNA sample. A major limitation of such antisense oligo-based approaches, however, is that they require species-specific probes. Thus, the commercially available kits are not only costly and but also have been limited to a small number of model organisms, not including chickens.

The chicken has been widely-used for studying various biological questions. Chickens have a sex-determination system different from that of most mammals, wherein males are the homogametic sex (ZZ) and females are the heterogametic sex (ZW). Chicken primordial germ cells (cPGCs) are currently the only type of PGCs that can be cultured *in vitro* [Park, T. S., & Han, J. Y. (2012)], providing a unique opportunity for extensive biochemical studies, including

genetic manipulation [Naito, et al (2015), Han, J. Y., & Lee, B. R. (2017)]. Studying chickens benefits not only basic research but also the poultry industry. Chicken is one of the most significant food sources in the world, with over 50 billion chickens being consumed every year [Boyd, et al. (2013)]. Furthermore, given the adaptive radiation of birds (over 10,000 species), chickens are valuable models for endangered avian species conservation [Muir, et al. (2003)]. Investigating chicken genomes and transcriptomes may, therefore, shed light on avian evolutionary processes and contribute to conservation biology [Li et al. (2019)].

Here we focus on developing a method for depleting rRNAs from total RNA using oligos specially designed for chickens. These oligos are antisense to cytosolic and mitochondrial rRNAs, and the resulting DNA-RNA hybrids can be removed using RNase H. RNase H is an endoribonuclease that recognizes DNA-RNA hybrid molecules and hydrolyzes the RNA strand [Inoue, et al. (1987)]. DNase is then used to digest the remaining DNA in the mixture. We have succeeded in using this protocol not only to remove rRNAs from total RNAs for RNA-seq library construction, but also to remove rRNA fragments for Ribo-seq.

Every step of the protocol has been optimized. Specifically, we optimized the ratio between RNAs and oligo probe, the ideal brand of RNase H enzyme, the amount of RNase H, as well as the treatment time and temperature. For total RNA, we monitored our depletion efficiency by the ratio of GAPDH to rRNAs and the success of depletion is shown by bioanalyzer profiles. For RPFs, we adopted the optimized condition from total RNA rRNA depletion, but further optimized the temperature of RNase H treatment as the annealing differs by fragment length. We monitored the depletion efficiency by Ribo-seq and the ratio of rRNA reads in the library. Our

strategy has been successfully applied to chickens, and we envision no problems in applying it to other species.

Our protocol is cost-effective and, to the best of our knowledge, is currently the only rRNA depletion-based method for avian species, thus providing a valuable resource for the community. This manuscript outlines the steps for extracting total RNA from animal tissue, depleting rRNAs, and purifying the enriched mRNA sample.

MATERIALS AND METHODS

The RNA depletion protocol outlined here consists of 2 parts: the oligo-based rRNA depletion (fig 1) and the bioinformatic analysis of the result (<https://github.com/LiLabZhaohua/RiboSeqPipeline>).

1. Designing the antisense oligo

To design the antisense oligo, we downloaded the rRNA sequences of both cytosolic ribosomes and mitochondrial ribosomes in chicken species from NCBI. These are mitochondrial rRNAs including the *16S* rRNA (XR_003076910.1), and *12S* rRNA (MH879470), and cytosolic rRNAs which for eukaryotes include *18S* rRNA (AF173612.1), *28S* rRNA (XR_003078040.1), *5.8S* rRNA (XR_003078039.1) and *5S* rRNA (XR_003076910.1). In order to minimize the chances of mismatch, for species not close related to chicken, a specific set of oligos should be designed and synthesized according the following steps.

DNA oligos were designed by converting the original sequences to their reverse complements and splitting the full-length sequences into 50 nt non-overlapping windows using a python script [<https://github.com/LiLabZhaohua/rRNAdepletion>].

1.1. To minimize potential oligo mismatch pairing with other RNAs, the basic local alignment tool (BLAST) was used to find possible pairing with other RNAs in chickens. We confirmed that these oligos do not have significant targets in the chicken transcriptome.

1.2. A pool of DNA probes was made, after receiving the oligos synthesized by Integrated DNA Technologies (Coralville, Iowa 52241), by mixing the 5.8S, 28S, 18S, 16S, 12S and 5S oligos to a final concentration of 0.5 μ M for each oligo.

2. Total RNA isolation using mirVana total miRNA isolation kit

This step is done with the mirVana total miRNA isolation kit (Thermo Fisher cat. AM1560).

Frozen tissue or extremely hard tissues.

2.1. Remove the tissue from the -80 °C refrigerator.

2.2. Cut out 25 mg of frozen tissue and place into a 1.7 uL tube.

2.3. Add 300 uL Lysis/Binding Buffer into a plastic weigh boat or tube on ice.

2.4. Grind frozen tissue into a powder using a homogenizer.

2.5. Add 30 uL miRNA Homogenate Additive to a tube. (Note: It is important to add the Homogenate additive immediately to the tube after the tissue is ground as it prevents the degradation of RNA.)

2.6. Leave mixture on rotator for 10 minutes.

Organic Extraction. Add 300 uL of Acid Chloroform (Thermo Fisher cat. AM9722).

Withdraw the bottom layer, avoiding the upper phase as this is mostly aqueous buffer.

- 160 2.7. Vortex the mixture for 60 seconds to mix well.
- 161 2.8. Centrifuge the mixture for 5 minutes at 16,900 RCF at room temperature. (Note: The
- 162 separation between the top and bottom phase should be clear. If the interphase is not
- 163 compact, repeat the centrifugation process.)
- 164 2.9. Transfer the upper layer to a new 1.7 uL tube. It should be approximately 300 uL. It is very
- 165 important to not disturb the bottom phase since it is mostly tissue and acid phenol.
- 166 ***RNA Isolation Procedure.*** Wash Solution 1 and Solution 2/3 should be pre-mixed with
- 167 ethanol according to the manual. At the end of the final stage, RNA can be eluted with either
- 168 nuclease-free water or in the Elution buffer provided by the kit.
- 169 2.10. Add 375 uL of room temperature 100% ethanol to the aqueous phase.
- 170 2.11. Place column on a collection tube.
- 171 2.12. Pipet the mixture to the column.
- 172 2.13. Centrifuge for 30 seconds at 9300 RCF.
- 173 2.14. Discard flow through.
- 174 2.15. Add 700 uL miRNA Wash Solution 1 (Pre-mixed with ethanol).
- 175 2.16. Centrifuge for 10 seconds.
- 176 2.17. Discard flow through.
- 177 2.18. Add 500 uL miRNA wash Solution 2/3 (Pre-mixed with ethanol).
- 178 2.19. Centrifuge for 10 seconds.
- 179 2.20. Discard flow through.
- 180 2.21. Add 500 uL miRNA wash Solution 2/3 (pre-mixed with ethanol).
- 181 2.22. Centrifuge for 10 seconds.
- 182 2.23. Discard flow through.

2.24. Centrifuge for another 3 minutes to remove the liquid residual.

2.25. Pre-heat the nuclease-free water to 95 °C.

2.26. Transfer the column into a new collection tube.

2.27. Add 100 uL pre-heated Elution Buffer or nuclease-free water.

2.28. Spin for 4 minutes at 11300 RCF at room temperature.

2.29. Collect elutes and store at -80 °C.

3. *rRNA depletion*

For this step, the enrichment for mRNAs and RPFs follows the same steps **except** for the temperature for RNase H treatment and the last step, in which the depletion from total RNAs uses the RNA Clean & Concentrator kit (ZYMO Research cat. R1013) protocol, while the depletion from RPF uses the Acid Phenol protocol.

Depletion Preparation. In this step, we prepare three solutions that will be used for rRNA depletion later.

3.1 For the probe oligo, prepare the 5X rRNA oligo Hybridization Buffer and 10X RNase H and aliquot for use.

3.2 Prepare 5X rRNA oligo Hybridization Buffer: mix 100 mM Tris-HCl with 200 mM NaCl.

3.3 Prepare 10X RNase H Digestion Buffer: mix 500 mM Tris-HCl with 1 M NaCl and 200 mM MgCl₂.

3.1. *rRNA depletion from total RNA*

Probe annealing

3.1.1. Pipette 2 µg of RNA and 15 µL DNA oligo probe into a tube, adjusting the volume of 5X rRNA oligo Hybridization Buffer and water according to Table II.

3.1.2. You may adjust the volume and choose one system in Table II, or use a larger total volume, but a total volume over 45 μ L is not suggested.

3.1.3. You may also dilute the system, using the 1X rRNA oligo Hybridization Buffer, after you mix all the components (not over 45 μ L for 2 μ g RNA).

3.1.4. Use the PCR machine for annealing: Heat to 95 $^{\circ}$ C for 2 minutes and slowly cool down (-0.1 $^{\circ}$ C/sec) to 22 $^{\circ}$ C, then incubate at 22 $^{\circ}$ C for additional 5 minutes.

RNase H treatment

3.1.5. Turn on the PCR machine and set the temperature to 45 $^{\circ}$ C.

3.1.6. Add the solutions and Invitrogen RNase H (Thermo Fisher cat. 18021071) listed in Table III, according to the volumes of annealing.

3.1.7. Incubate at 45 $^{\circ}$ C for 1 hour in the PCR machine.

DNase treatment

3.1.8. Take out samples from the PCR machine and adjust the PCR machine to 37 $^{\circ}$ C.

3.1.9. Immediately add the components listed in Table IV. Note that regardless of which system you chose, the total volume should be 50 μ L.

3.1.10. Incubate at 37 $^{\circ}$ C for 30 minutes in the PCR machine.

RNA purification

This step uses the RNA Clean & Concentrator kit (Thermo Fisher cat. R1013) protocol. Prepare adjusted RNA Binding buffer by mixing equal volumes of buffer and ethanol.

3.1.11. Transfer 50 μ L of RNA to a new 1.7 mL tube.

3.1.12. Add 50 μ L water to the tube.

3.1.13. Add 200 μ L adjusted binding buffer to the tube.

3.1.14. Transfer the mixture into a column.

- 3.1.15. Centrifuge for 30 seconds at 16,900 RCF at room temperature.
- 3.1.16. Discard the flow-through.
- 3.1.17. Add 200 uL RNA Pre-Wash.
- 3.1.18. Centrifuge for 30 seconds at 16,900 RCF at room temperature.
- 3.1.19. Discard the flow-through.
- 3.1.20. Add 200 uL RNA Wash Buffer.
- 3.1.21. Centrifuge for 30 seconds at 16,900 RCF at room temperature.
- 3.1.22. Discard the flow-through.
- 3.1.23. Add 200 uL RNA Wash Buffer.
- 3.1.24. Centrifuge for 2 minutes at 16,900 RCF at room temperature.
- 3.1.25. Discard the flow-through.
- 3.1.26. Transfer the columns into new 1.5 mL tubes (RNase Free tube).
- 3.1.27. Add 15 uL DNase/RNase-Free water directly to the column matrix.
- 3.1.28. Let the columns sit at room temperature for at least 1 minute.
- 3.1.29. Centrifuge for 30 seconds at 16,900 RCF at room temperature.
- 3.1.30. Keep the flow through in the tube and store the tube at -80 °C immediately.

3.2. *rRNA depletion from ribosome protected fragments (RPFs).*

Probe annealing

- 3.2.1. Pipette 2 µg of RNA and 15 µL DNA oligo probe into a 1.7 ml tube.
- 3.2.2. Adjust the volume of 5X rRNA oligo Hybridization Buffer and water according to Table II.
- 3.2.3. You may adjust the volume and choose one system in Table II, or use a larger total volume, but a total volume over 45 µL is not suggested.

3.2.4. You may also dilute the system, using the 1X rRNA oligo Hybridization Buffer, after you mix all the components (not over 45 μ L for 2 μ g RNA).

3.2.5. Use the PCR machine rRNA program for annealing: Heat to 95 $^{\circ}$ C for 2 minutes and slowly cool down (-0.1 $^{\circ}$ C/sec) to 22 $^{\circ}$ C, then incubate at 22 $^{\circ}$ C for additional 5 minutes.

RNase H treatment

3.2.6. Turn on the PCR machine and set the temperature as 37 $^{\circ}$ C for RPF for optimal results. * We have compared the treatment temperature at room temperature, 30 $^{\circ}$ C, 37 $^{\circ}$ C, 42 $^{\circ}$ C, and 45 $^{\circ}$ C, and found that 37 $^{\circ}$ C has the best removal based on our sequencing results (Table I).

3.2.7. Add the solutions and RNase H (Thermo Fisher cat. 18021071) listed in Table III, according to the volumes of annealing.

3.2.8. Incubate at 37 $^{\circ}$ C for 1 hour on PCR machine.

DNase treatment

3.2.9. Remove the samples from PCR machine and adjust the PCR machine to 37 $^{\circ}$ C.

3.2.10. Immediately add the components listed in Table IV. (Note: The total volumes should all be 50 μ L.)

3.2.11. Incubate at 37 $^{\circ}$ C for 30 minutes in the PCR machine.

Purification of RPF

3.2.12. Transfer 50 μ L RNA to a 1.7 mL tube.

3.2.13. Add 200 μ L ddH₂O.

3.2.14. Add 300 μ L Acid Phenol Chloroform (Thermo Fisher cat. AM9722). Note the total volume should be around 550 μ L.

- 3.2.15. Vortex for 1 minute to mix well.
- 3.2.16. Spin at 20,100 RCF for 15 minutes at room temperature.
- 3.2.17. Withdraw the upper phase (around 250 uL) without disturbing the lower phase and transfer the solution to a new 1.7 mL tube.
- 3.2.18. Add 1 µL glycogen (Sigma Aldrich cat. 10901393001).
- 3.2.19. Add 3 volumes (around 750 uL) of 100% ethanol.
- 3.2.20. Leave at 4 °C for an hour, or -20 °C overnight. White precipitate should be visible.
- 3.2.21. Spin down at 16,900 RCF for 30 minutes at 4 °C.
- 3.2.22. Wash with 200 uL of 70% ethanol.
- 3.2.23. Centrifuge at 16,900 RCF for 5 minutes at highest speed at 4 °C.
- 3.2.24. Pour out the liquid.
- 3.2.25. Centrifuge at 16,900 RCF for another 3 minutes at highest speed at 4 °C.
- 3.2.26. Pipette out all the liquid and leave only the pellet in the tube.
- 3.2.27. Dissolve in water at room temperature.
- 3.2.28. Store at -80 °C.

Data Analysis

We analyzed the Ribo-seq as previously described [Sun, Y. H., etc., (2020)]. Ribo-seq pipeline for chicken was developed for this study, which is available in the following link (<https://github.com/LiLabZhaohua/RiboSeqPipeline>).

RESULTS

In this protocol, we describe a simple method for enriching mRNAs from animal tissue using an rRNA depletion-based approach. Each key step of the protocol reported above has been optimized, including the ratio between RNAs and oligo probes, the ideal brand of RNase H enzyme, the amount of RNase H, as well as the treatment time and temperature. This is, to the best of our knowledge, the first rRNA depletion method developed for avian species. Although the probes are designed to target chicken rRNAs, considering the conservation of rRNA sequences, we expect that our probes can be used for diverse avian species.

For total RNAs, we used the ratio of 28S to GAPDH to measure the depletion efficiency. We first compared efficiency of three commercial RNase H enzymes – RNase H (NEB cat. M0297S) and thermostable RNase H (NEB cat. M0523S) from NEB, and RNase (Thermo Fisher cat. 18021071) from Invitrogen with 1 ul probe, 1 ul RNase H treated for 30 mins at 37 °C and 45 °C. The smallest Relative quality value with respect to the control value for RNase H from Invitrogen indicates the best result at 37 °C. Then we compared the Invitrogen RNase H and NEB RNase H with 1 ul and 2 ul at 37 °C and 45 °C with 3 µl probe for 1 hour of RNase treatment and then 30 mins of DNase treatment. The result indicates that Invitrogen has the best efficiency at 45 °C. After the optimal enzyme chosen, the optimal amount of probe was also investigated with 1µl Invitrogen RNase Treatment at 45 °C for 1 h and then DNase treatment for 30 mins. Different ratios of the RNA and probe, including 1:0, 1:1, 1:2, and 1:3 were tested. In the end, the optimal condition for RNA depletion is 1 µg RNA: 3 µl probe, 0.2 µl Invitrogen RNase H treatment at 45°C for 1 h, and DNase treatment at 37°C for 30 min. The result was expressed as the ratio of 28S to GAPDH, decreased > 4,700 fold from 706 to 0.15 after rRNA depletion. The success of rRNA depletion is also demonstrated by the bioanalyzer results, where the 18S and 28S rRNA peaks disappeared after

depletion at the optimal condition which is 1 μ g RNA:3 μ l probe, with 0.2 μ l Invitrogen RNase treatment at 45°C for 1 hour, and then DNase treatment at 37°C for 30 mins. The experiment is considered successful when all ribosomal RNA, including 18S and 28S, are depleted by the end of the procedure. The lack of any rRNA peaks demonstrates the complete removal of rRNA in the rRNA-depletion process (Figure 2B & Figure 2E are two replicates of the samples after depletion). The small peaks that can be seen typically correspond to some small RNA and mRNA.

For Ribo-seq, we used the percentage of rRNA fragment in the sequencing results to measure the depletion efficiency. Because the RNA length differs between full-length mRNAs and RPFs, likely to affect the annealing temperature, we therefore constructed Ribo-seq library with the RNase H treatment temperature at RT, 30°C, 37°C, 42°C, and 45°C respectively. Table I listed the read statistics of the Ribo-seq libraries showing the number of total reads and number of reads mapping to the rRNA sequences. Based on the result in Table I, the optimal result of RPF depletion is seen at 37 °C with the lowest percentage of reads coming from rRNAs. Therefore, 37 °C is used for RPF depletion protocol.

Our results are consistent with what have been reported in mice [Adiconis, et al. (2013)], which indicate the RNase H method is robust and capable of depleting rRNAs from low input samples. As described before [Adiconis, et al. (2013)] that compared to Ribo-Zero, this method has a significantly higher efficiency in rRNA removal and slightly better evenness of coverage, we also found the chicken RNA-seq signals evenly distribute throughout the mRNAs as shown by the metagene analysis of a chicken RNA-seq results (Figure 3).

DISCUSSION

The RNase H method is robust and capable of depleting rRNAs from low input samples [Adiconis, et al. (2013)]. Compared to Ribo-Zero, this method has a significantly higher efficiency in rRNA removal and slightly better evenness of coverage [Adiconis, et al. (2013)] as shown by the metagene analysis of a chicken RNA-seq results (Figure 3).

The whole procedure takes about 2.5 hours starting with purified RNA, and 3.5 hours starting with organic tissue. The procedure time is comparable to those of traditional methods, which take approximately 2 hours. The price of the method of interest would vary from \$20 per reaction starting with total RNA (as seen in Table V) to \$29 starting with organic tissues (seen in Table VI), while the cost of traditional methods varies from \$55 to \$118. By comparing the price and time with other traditional mRNA purification methods, as seen in Table VI, our method is cost-effective and efficient, while maintaining an excellent quality of purification.

Depending on how the oligos are designed and which type of sequence the oligos are specified to target, the same method can potentially be applied for a wide range of organisms and different types of RNA. As long as the oligo is designed to antisense to the specific type of RNA of a specific species, this method should guarantee good quality of purification.

ACKNOWLEDGMENTS

We thank N. Anthony for providing chicken tissues, UR Genomics Research Center for performing bioanalyzer analysis, and members of the Li laboratory for discussion. This work

was supported by Agriculture and Food Research Initiative Competitive Grant no. 2018-67015-27615 from the USDA National Institute of Food and Agriculture to X.Z.L.

DISCLOSURES

The authors have nothing to disclose.

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FIGURE LEGENDS

Figure 1: A flowchart summarizing the protocol

Figure 2: Total RNA bioanalyzer profile, where x axis is RNA size in nucleotides and y axis is the arbitrary fluorescence unit. The profile is typically expressed with 2 major peaks corresponding to the 18S and 28S ribosomal RNA (Figure 2A & Figure 2D). 3 parameters including the RIN number, 260/230 ratio, and 260/280 ratio can be used to measure RNA quality, as well as the effect of DNase treatment.

The RNA integrity number (RIN) is an index for assigning integrity values to RNA measurements. No significant change should be observed for a successful experiment before and after DNase treatment. Figure 2A & 2B and Figure 2D & 2E are two sets of replicates that have a stable RIN before and after DNase treatment indicating the success of the treatment.

The 260/230 values for "pure" nucleic acid are often used to examine whether contamination such as salt or protein exists in the sample or to measure the purity of nucleic acid before and after. Expected 260/230 values are commonly in the range of 2.0-2.2. If the ratio is appreciably lower, it may indicate the presence of contaminants which absorb at 230 nm. The ratio would be decrease largely due loss of rRNA. Sample A & B and D&E are two sets of replicates that have a stable 260/230 ratio before, and after which are considered successfully treated with DNase.

The 260/280 values indicate the purity of RNA. A ratio of ~2.0 is generally accepted as "pure", while pure DNA has a ratio close to 1.8. Sample A & B and D&E are two sets of replicates that

have a stable 260/280 ratio before and after DNase treatment. This result reveals the successful removal of rDNA oligos.

The RNA concentration within the sample is another parameter used to evaluate the success of the RNA depletion. The value should decrease largely after depletion as 90% of the RNA, which is ribosomal RNA, is now depleted. The decrease of the RNA concentration indicates that sample A&C and D&F are two successful treatments.

Figure 3: Metagene plots of RNA-seq at 5' leader, CDS, and 3' trailer of mRNAs. The x-axis shows the median length of all chicken mRNAs, and the y-axis represents the ppm (parts per million).

TABLE LEGENDS

Table I: Read statistics of Ribo-seq libraries constructed with different annealing temperature

Name	Total Reads	rRNA Mapping Reads	% rRNA Mapping Reads
Sample 1 - 30	763,350	372,204	48.8
Sample 2 - 37	165,239	63,454	38.4
Sample 3 - 42	743,014	301,481	40.6
Sample 4 - 45	937,034	388,145	41.4
Sample 5 - RT	973,777	475,268	48.8

This is RPF data, it could be 99%. We chose the optimized temperature.

482 Table II: Probe annealing system

	1	2	3
RNA	2 ug		
5X rRNA oligo Hybridization Buffer	5 uL	6uL	7uL
DNA oligo probe	15 uL		
ddH2O	to 25 uL	to 30 uL	to 35 uL

483

484 Table III: RNase H treatment system

	1	2	3
10X RNase H Digestion Buffer	3 uL	3.5 uL	4 uL
Invitrogen RNase H	1 uL		
ddH2O	1 uL	0.5 uL	0 uL
Total volume	30 uL	35 uL	40 uL

485

486 Table IV: DNase treatment system

	1	2	3
10X Turbo DNase Buffer	5 uL		
Turbo DNase	2 uL		
ddH2O	13 uL	8 uL	3 uL
Total volume	50 uL		

487

Table V: Cost Estimation of the method of interest

Name of Material/ Equipment	Quantity	Company	Catalog Number	Comments/Description
DNA Oligo	32 mL	Integrated DNA Technologies	N/A	\$912, \$5 per reaction
TURBO DNase(2 U/uL)	5000 units	Thermo Fisher	AM2239	\$309, \$0.24 per reaction
RNase H (2 U/μL)	120 units	Thermo Fisher (Invitrogen)	18021071	\$489, \$8.2 per reaction
RNA Clean & Concentrator Kits	50 prep	ZYMO Research	R1013	\$170, \$3.4 per reaction
Acid Phenol Chloroform	400 mL	Thermo Fisher	AM9722	\$197, \$0.14 per reaction
Glycogen	1 mL	Sigma Aldrich	10901393001	\$151, \$3.02 per reaction
Total Cost Estimation				\$2,228 Total \$20 per reaction

Table VI: Other Methods cost Estimation

TruSeq Stranded Total RNA	48 prep	Illumina	20020596	\$5660 Total, \$118 per reaction
SMART Stranded RNA-Seq Kit	48 prep	Takara	634838	\$2920 Total, \$60 per reaction
RiboMinus Human/Mouse Transcriptome Isolation Kit	6 preps	Thermo Fisher	K155001	\$600, \$100 per reaction
NEBNext rRNA Depletion Kit (Human/Mouse/Rat)	6 preps	New England Bio Labs	E6310S	\$330, \$55 per reaction

Supplementary Files:
Supplementary Table I: Sequence used for constructing Probe

5.8S_0	AGCTGCGTTCTTCATCGACGCACGAGCCGAGTGATCCACCGCTAAGAGTT
5.8S_50	GAAGTGTTCGATGATCAATGTGTCTTCAATTACATTAATTCTCGCAGCT
5.8S_100	CGCTCAGGCAGGCGTAGCCCCGGGAGGAACCCGGGGCCGCAAGTGCCTTC
28S_0	CTGACTAATATGCTTAAATTCAGCGGGTCGCCACGTCTGACCTGAGGTCTG
28S_50	CTCTTCACTCGCCGTTACTGAGGGAATCCTCGTTAGTTTCTTTTCTCCTCCG
28S_100	ACACCTCCCGCGCCCCACCGCGGGGCGGGGATTTCGGCGCTGGGCTCTTCC
28S_150	GGACTTGGGCCCCCGAGAGCGGCGCCGGGGATGGGGGCTTCCGTACGCC
28S_200	GGGGGCGCTACCGGCCTCACACCGTCCGCGGGCTGGGCCTCGATCAGAA
28S_250	CTGCATTCCCAAGCAACCCGACTCCGAGAAGCCCCGGGCGCGCGCCG
28S_300	GGTCTCGTGCCGGTATTTAGCCTTAGATGGAGTTTACCACCGCTTTGGG
28S_350	CTCTCTTCAAAGTTCTTTTCAACTTTCCCTTACGGTACTTGTGGCTATC
28S_400	GCGCGGACCCACCCGTTTACCTCTTAACGGTTTTCACGCCCTCTTGAAC
28S_450	GCCCGCGCCGGCCGACCTTGGCCCGCCGGGTGAATCCTCCGGGCGGACT
28S_500	CCCGGCGAAGGGGAGGGACGGAGGGGAGGCGGAGGCGGGGATCCGACGGC
28S_550	CGGCCGGGCGGCGGTCCCCGGCCCGCCCGCCCCCCTGGGCCCCCCCCGC
28S_600	GGTCGCGGCGCACCGCCGCGGAGGAAATGCGCCCGACGGGGGCGGACGC
28S_650	GGCGCCGGGCCACCTGCCCCGCGGCGAGCCCTTCCCAGCCGTCCCGGAGCC
28S_700	TCGGCGACGATCCGGGCCCCGGCGGCTATAACACCCGGCGGCGCTCGCGC
28S_750	CCCCCTCCGGGGGAGGGCGCGGCGGCGGTCTCTCCCTCGGCCCGGGAT
28S_800	CGCGGCCGGCCCGACGCCGGTCCGGCCGCGGGGGGCCCTCCGGGGGGCCG
28S_850	GGAGAGAGAGCGAGCGGCGGCGTACGGCGGCGGCGCGGACGCGCGCGCGG
28S_900	ACCCGACCCCCCGCCCCCGCGCCCGGGACGGAACCGGGGCGGGGAAC
28S_950	CTGGAGGCGCGCGCCACACGCGCGGCGCCGCGCCGAGCCGCGCGCCGGAC
28S_1000	AAGGGGGAAGGTTCCCCCGCGCCCCCGCGGCCTCGCCCGCGCCGGG
28S_1050	CCCGTCCCCCGAACGGGCGCCCCCGCGGGAACGGAGGCGGCCCCGAACAG
28S_1100	CACTGAGCGCAGTCCGCCCCGGTTCGGACAGCGGCGCCGGGGGCGGCGGG
28S_1150	CGCCCTGGCGTGGCCCGAGCCCGGCCCGGCGGCGCCGCGCGGTTCGGGGCG
28S_1200	GTGTTTCAAGACGGGTTCGGGTGGGTAGCCGACGTCGCCGCGGACCCCGGG
28S_1250	GGGCTTTCGCGCGAGCCCGCGACTCGCGCGCGTGCTAGACTCCTTGGTCC

28S_1300	GGGATCCCACCTCAGCCGGCGCGCGCCGGCCCTCACCTTCATTGCGCCGC
28S_1350	CGGGCGAGACGGGCGCGGTGGTGCGCCCGGGCCCTTCCGGCCTGCCGCCCC
28S_1400	CATCTTTCGGGTCCTAGCACGCGCGCTCATGCTCCACCTCCCCGGCGAGG
28S_1450	GACCTCCACCAGAGTTTCCTCTGGCTTCGCCCTGCCCAGGCATAGTTCAC
28S_1500	CGCCCCCTATACCCGGGTTCGGACGACCGATTTGCACGTCAGGACCGCTACG
28S_1550	TGAGGGAAACTTCGGAGGGAACCAGCTACTAGATGGTTCGATTAGTCTTT
28S_1600	ATTCGCTTTACCGGGTAAACTGCACCGCCGCCCGAGCGCCAGCTATCC
28S_1650	TGAAAGTTTGAGAATAGGTTGAGATCGTTTCGGCCCCAAGACCTCTAATC
28S_1700	TCGCATTCCACGGCCCGGCTCCACGCCAGCGAGCCGGGCGTCTTACCCAT
28S_1750	GTTTCATCCCGCAGCGCCAGTTCTGCTTACCAAAGTGGCCCACTGAGCGC
28S_1800	TTTCTGGGCTCTGATGAGCGTCGGCATCGGGCGCCTTAACCCGGCGTTTCG
28S_1850	GGTTCCGACTTCCATGGCCACCGTCCTGCTGTCTAGATCAACCAACACCT
28S_1900	TTTTTCAGGGCTAGTTGATTTCGGCAGGTGAGTTGTTACACACTCCTTAGCG
28S_1950	CGCACCGCCGGCGACGGCCGGGTATGGGCCCCGACGCTCCAGCGCCATCCA
28S_2000	GGCGCACCGCAGCGGCCCTCCTACTCGTCGCGGCGTAGCCCCCGCGGCTC
28S_2050	AAGATCTGCACCTGCGGCGGCTCCACCCGGGCCCCGCGCCCCAGGCTTCCA
28S_2100	GCTCCACTTCGGCCTTCAAAGCTCTCGTTTGAATAGTTGCTACTACCACC
28S_2150	TCGCTTAGGACCGACTGACCCATGTTCAACTGCTGTTACATGGAACCCT
28S_2200	CTGAGGGCAACGGAGGCCATCGCCCGTCCCTTCGGAACGGCGCTCGCCTA
28S_2250	TCTCCGCCGCTCCGGATTTCGGGGATCTGAACCCGACTCCCTTTCGATCGG
28S_2300	GCTTCTCCGGGATCGCTTGCGTTACCGCACTGGGCGCCTCGCGGCGCCCG
28S_2350	GCGCCCGGCCCTTCACAAAGAAAAGAGAACTCTCCCCGGGGCTCCCGCCG
28S_2400	CGACGCTTTCCAAGGCGCGGGCCCCCTCTCTCGGGGCGAACCCGTTCCAGG
28S_2450	CCCCGGATTTTCACGGGGCCAGCGAGAGCTCACCGGACGCCGCCGGAACCG
28S_2500	AGACCTGCTGCGGATATGGGTACGGCCCGGCGCGAGATTTACACCCTCTC
28S_2550	GACTTCCCTTACCTACATTGGTCCAACATGCCAGAGGCTGTTACCTTGG
28S_2600	CCCAGCCCTTAGAGCCAATCCTTATCCCGAAGTTACGGATCCGGCTTGCC
28S_2650	GTCCAGCCGCGGCGCGCGCCAGCCCCGCTTCGCGCCCCAGCCCGACCGA
28S_2700	CCCCGGCGAGCGGGAGCGGGGAAAGGGGGGGCGGGGGCGGGCGGCGCCTC
28S_2750	CGCGTCCAGAGTCGCCGCCGCCGCGCCGCCCGCTGACCCCCCCCCCGGCG
28S_2800	GGCGAGCGGCGCCCGCCGCGAGCTGGGGCGATCCACGGGAAGGGCCCGGCG

28S_2850	GGCCGACGGGAGGGGCGCCGGGAGCGGGGGGCGGAGGGGCAAGGAGGGG
28S_2900	CTCGCGCCCTCGCGGGGAGGGCGCTCGGGACGGGGGCGGCCGGGACGAC
28S_2950	CGGCGGGCCCCCCCCCGCCGCCGCGCCGCGCCGCGGCCGCCGCCGCCGCC
28S_3000	GCGCCGGCCCCGAGACCCCCCGCCCGGGACCGCCCCGCCCGGCGCCGC
28S_3050	CCTGGTCCGCACCAGTTCTAAGCCGGCTGCTAGGCGCCGGCCGAGGCGGG
28S_3100	CCCGCCGCGGGCCTTCGCGATGCTTTGTTTTAATTAAACAGTCGGATTCC
28S_3150	TCTTCACTTTGACATTCAGAGCACTGGGCAGAAATCACATCGCGTCAACA
28S_3200	CTTAAGAGAGTCATAGTTACTCCCGCCGTTTACCCGCGCTTCATTGAATT
28S_3250	GTTTCATCCATTCATGCGCGTCACTAATTAGATGACGAGGCATTTGGCTAC
28S_3300	TCCCTTGGCTGTGGTTTCGCTGGAGAGTAGGTAGGGACAGTGGGAATCTC
28S_3350	GAGTCAAGCTCAACAGGGTCTTCTTTCCCGCTGATTCCGCCAAGCCCGT
28S_3400	GGCCTCCCACTTATTCTACACCTCTCATGTCTCTTCACAGCGCCAGACTA
28S_3450	AGTGGTATTTACCGGCGGCCGGGCGCGGCGCGGGTCGCGCGACCGCGG
28S_3500	TCGGGGCTCGCCCCCGCCTCACCGGGTAAGTGAAAAACGATCAGAGT
28S_3550	GGGTGCGCCCCGGCGCGCGCCGGGCGCTTGGCGCCAGAAGCGAGAGCCCC
28S_3600	ACAGGTGTACCGCCCCAGTCAAACCTCCACCTGACGCTGTCCCCGGAGC
28S_3650	AGGTTTCTGGCCTCCCTGAGCTCGCCTTAGGACACCTGCGTTACGCTTTG
28S_3700	TCGTACTGAAAATCAAGATCAAGCGAGCTTTTGCCCTTCTGCTCCACGGG
28S_3750	AAACCCAAAAAGTCAGAAGGATCGTGAGGCCCCGCTTTCACGGTCTGTAT
28S_3800	CGCCACAAGCCAGTTATCCCTGTGGTAACTTTTCTGACACCTCCTGCTTA
28S_3850	AAGAGCCGACATCGAAGGATCAAAAAGCGACGTCGCTATGAACGCTTGGC
28S_3900	TAGTGGGTGAACAATCCAACGCTTGGTGAATTCTGCTTCACAATGATAGG
28S_3950	GTAAACTAACCTGTCTCACGACGGTCTAAACCCAGCTCACGTTCCCTAT
28S_4000	TCCTCTCGTACTGAGCAGGATTACTAGCGCAACAACACATCATCAGTAGG
28S_4050	TCCAGTGGCTCCTCAGCCAAGCACATACACCAAATGTCTGAACCTGCGGT
28S_4100	GATTCTGACTTAGAGGCGTTCAGTCATAATCCACAGATGGTAGCCTCGC
28S_4150	GCGAGCCCACCGAGGCGCCTCGGCGCTGCGGTATCGCTACGTTTAGGGGG
28S_4200	GCGGCGCTCCGCACCGACCGCCCGCCCGAGGGGGGCGGCGGCCGGCTATC
28S_4250	GCGGGGGAGAGGCGGCGCCACATCTGTCCGCGCTCCGGTCCCGACCACGA
28S_4300	TCGTCTACGAATGATTTAGCACCGGGTTCCCCACGAACATGCGGTACGCG
28S_4350	GCAGCGAGGGAGCTGCTCTGCTACGTACGAAACCCGACCCAGAATCAGG

18S_0	TCGCAGTTTCACTGTACCGCCCGTGTGTACTTAGACATGCATGGCTTAAT
18S_50	GTAACGGGAGGGGAGCGACCAAAGGAACCATAACTGATTTAATGAGCCAT
18S_100	GTCGGCGCTCGTCGGCATGTATTAGCTCTAGAATTACCACAGTTATCCAA
18S_150	CGGGCGAGCCCGGGTTGGTTTTGGTCTGATAAATGCACGCGTCCCCGGAG
18S_200	CCACGGGGGCGTGCGATCGGCTCGAGGTTATCTAGAGTCACCAAAGCCGC
18S_250	ACAGTACCATCGAAAGTTGATAGGGCAGACATTCGAATGGGTCTGTCGCCG
18S_300	GGAATCGAACCCTGATTCCCCGTTACCCGTGGTCACCATGGTAGGCACAG
18S_350	CGCCTGCTGCCTTCCTTGGATGTGGTAGCCGTTTCTCAGGCTCCCTCTCC
18S_400	ATTGTTATTTTTTCGTCACCTACCTCCCCGGGTCGGGAGTGGGTAATTTGCG
18S_450	AGGATTTAAAGTGGACTCATTCCAATTACAGGGCCTCGAAAGAGTCCTGT
18S_500	ATTACCGCGGCTGCTGGCACCAGACTTGCCCTCCAATGGATCCTCGTTAA
18S_550	CTACGAGCTTTTTAACTGCAGCAACTTTAATATACGCTATTGGAGCTGGA
18S_600	AGGCGGTAGCTCGCCTCGCGGCGGACCGCCAGCTCGATCCCAAGATCCAA
18S_650	GACACTCAGTTAAGAGCATCGAGGGGGCGCCGAGAGACAGGGGCTGGGAC
18S_700	CTGCTTTGAACACTCTAATTTTTTCAAAGTAAACGCTTCGGGCCCCGCGG
18S_750	AACCGGAGTCCTATTCCATTATTCTAGCTGGAGTATTCCGGCGGCCAGC
18S_800	CCGGCCGTCCCTCTTAATCATGGCCCCGTTTCCGAAAACCAACAAAATAG
18S_850	TCTTGCGCCGGTCCAAGAATTTACCTCTAGCGGCACAATACGAATGCCC
18S_900	CGTTCTTGATTAATGAAAACATTCTTGGCAAATGCTTTCGCTTTAGTTCG
18S_950	TTATGGTCGGAACCTACGACGGTATCTGATCGTCTTCGAACCTCCGACTTT
18S_1000	TGCCCCGGCGGGTCATGGGAATAACGCCGCCGGATCGCGAGTCGGCATCGT
18S_1050	TTTGCAACCATACTCCCCCGGAACCCAAAGACTTGGGTTTCCCGGGAGC
18S_1100	CAGGCTCCACTCCTGGTGGTGCCCTTCCGTCAATTCCTTTAAGTTTCAGC
18S_1150	GTCCGTGTCCGGGCCGGGTGAGGTTTCCCGTGTTGAGTCAAATTAAGCCG
18S_1200	ATGCACCACCACCCACGGAATCGAGAAAGAGCTCTCAATCTGTCAATCCT
18S_1250	TTCGTTATCGGAATTAACCAGACAAATCGCTCCACCAACTAAGAACGGCC
18S_1300	GACGCCGACCGCTCGGGGGTCGCGTAACTAGTTAGCATGCCAGAGTCTCG
18S_1350	TATTGCTCAATCTCGGGTGGCTGAACGCCACTTGTCCCTCTAAGAAGTTG
18S_1400	CAGTGTAGCGCGCGTGCAGCCCCGGACATCTAAGGGCATCACAGACCTGT
18S_1450	AACGGGTACCCGCGCCTGCCGGCGTAGGGTAGACACAAGCTGAGCCAGT
18S_1500	CGTTCATGGGGAATAATTGCAATCCCCGATCCCCATCACGAATGGGGTTC

18S_1550	CAGGGACTTAATCAACGCGAGCTTATGACCCGCACTTACTGGGAATTCCT
18S_1600	CTCACTAAACCATCCAATCGGTAGTAGCGACGGGCGGTGTGTACAAAGGG
18S_1650	CGACGCTCCGGCAGGGCCGTGGCCGACCCCGCCGGGGCCGATCCGAGGAC
12S_0	GCATGTATATGTCTAGCAAAAACCAATAGAAAGGTTAGGACTAAGTCTTT
12S_50	TTGCTTGGGAAGAAAGGTTTGGGGGCATTTTCACTGGGATGCGGATACTT
12S_100	TAAGCAAGGCGTCTTGGGCTACTGCTGAGTGTGCCTGATACCTGCTCCTT
12S_150	CACTTATTGCTTAAGGTTAATTACTGCTGAGTACCCGTGGGGGTGTGGCT
12S_200	GCTGGCACAAGATTTACCAACCCTGGGTTGCTATGGCTAAGTCAAGTTTA
12S_250	CTCTTTACGCCGGGTAGCTATTGATTTGGGTTTCTTGTATGACCGCGGTG
12S_300	GACAGCTTGGTTGCATTTTAATCTTAGCTGGTGCAGATAACATGTGGCCA
12S_350	GTTTAGGCTAAGATGGATTTGGGTTGGGTTTAGGTGGATCTTAGGCTTAT
12S_400	GGGTATCTAATCCCAGTTTGGGTCTAGCTTTCGTGGGTAAATTAATC
12S_450	GCGGATACATGTGTGATGGGAGGTATCTAGATTTAGGGCTAGGCATAGTG
12S_500	GGCACCGCCAAGTCCTTAGAGTTTAAAGCGTTTGTGCTCGTAGTTCTCAG
12S_550	GTGAATCGTGGATTATCGATTATAGAACAGGCTCCTCTAGGTGGGTTTGG
12S_600	GGTGGGCTGGCGACGGCGGTATGTAGGCTGTGCTGGCAAGGGGTGGTTGG
12S_650	CTGTCTTATTAGCGAGGGGCTATTGAGCTCACTGTTGTTCTTTCATTAGA
12S_700	ATGTTAGAAAATGTAGCCCATTTCTCCCACCCCATAGGCTATACCTTGAC
12S_750	GCTAAATCCTCCTTCTAAGGGCGGGTTTCACGTCCTTTTTTCGTTTGTCT
12S_800	GCCTCAGAGCCGTCTTAAAGTGAGCTTAGGGGGTATGATCTCACTTTACT
12S_850	ATTTATTGATGTTGATGGCTTGTGAAGAGGGTGACGGGCGGTATGTACGT
12S_900	ACTTACCTTGTTACGACTTGCCTCGTCTTTAGCCGGGAGGGGAGGTATAT
16S_0	TAAGGGTACGAGTTTGTCTGGGCTAGAGGGGGGGCAAGTCAAGGCGACCTT
16S_50	GGGACAATTTATAATGTTTTGGTTAAGAGGGGGAGGTAAGTTTTTTATGT
16S_100	GTACAGTTAGCCTCTATTGCGCCGGGGTAGTCTTTTCTATCGCCTATACT
16S_150	TTGCTGTTTTTTGCTTATGGTTTTCATTTGCTATTTTCATCTTCCCTTGCG
16S_200	GCTTGGTTGTTCTTGCTAAATCATGATGCAAAAGGTACAAGGGTTGGTCT
16S_250	CTCGCAAGTAGCTCGCTTGGGTTTCGGGAAGGCAAACTTTAGCTCACTTT
16S_300	AGTCATCCCACTCTTTTGCAACAGAGACGGGTTCGCTCAAATTTTAGCTG
16S_350	CAGGTAACCAGCTATCACCCAGCTCGGTAGGCTTTTCACCTCTACTGGCA
16S_400	AGGTGGGTGTCTTTAGGGGGTGGGTTAAGGGGGAAGTTAGATTCGTTTGA

16S_450	CTGTACCCCCATCGAATTGCTCTTAACCCCAACGTTCTCAAGGTTGACAA
16S_500	GAGGGGTGATTATTATCCGCTGGAGGAGGTTGTGTTCTTTTTTCGATGGAG
16S_550	GCTTTGACGCACTCTTTTGTGTTGGTGGCTGCTTGAAGGCCACAGTGCGGG
16S_600	CTGCTTTGGTTGAGGGAGTCAAATAGGGTTTTAGATTTTTTAATGAGGGA
16S_650	GTTCCAGATTACTCATTTTAGCATTGATTCTTCTATTGTCATAGGTTAAC
16S_700	TGAGTTCTGTTAATAATGTATTAATGTAAGTTTACGCCGTAGGAGGATAG
16S_750	GCTTAACAGATTGAGGAATACGTATTGCTTGTTAGTGTGGGGGTATAAGT
16S_800	GCCGAGTTCCTTCTGTAGGTTTTAATCATCCTGTGGGCGCTCCTGGGTTG
16S_850	TGTTGTTAGCTGAAGGCTATGTTTTTGGGAAACAGTCGGGTCTTTGGTTT
16S_900	CGCGGCCGTTGAACTTTGGGGGTCACTGGGCAGGCATCACCTTCAATACT
16S_950	TCTCAATTTACGGGACAATTGATTGCGCTACCTTCGCACGGTTAGGATAC
16S_1000	TAGATTACCTACAGGAGACAGTTAAGACCTCGTTTAGCCATTCATACAAG
16S_1050	TTCTCGTCTTATGTTACATTCTCGTTTTTGCACGGGAATACTAATTTCA
16S_1100	GGGTTGTGTAAGGTTGTAAGGTGGTCGTGATTTTAAAGTTCCACAGGGTC
16S_1150	CCCCAACCGAAAAATGTCGACCAGGGGTTTATGTGTGGGTGGACCCAGTG
16S_1200	TGAAGAGTTGTGGTCTGTGGGTTTGGAGGATTTTTTTTTCTCCAAGGTCG
16S_1250	CAATTATATTGGGTCTGGTTACTGTTGGTACTTTGAGGAGTTGGTCTTAG
16S_1300	CTCTTGAGGAGATTGCGCTGTTATCCCTGGGGTAGCTTGGTCCATTGCT
16S_1350	AGGTTGTCCTGATCCAACATCGAGGTCGTAAACCTCCTTGTCGATATGGG
16S_1400	AGGACTGTTAATCGTTGAACAAACGAACCCTTAATAGCGGTTGCACCATT
16S_1450	CCATAGATAGAAACCGACCTGGATTGCTCCGGTCTGAACTCAGATCACGT
16S_1500	AGTGGTATTGACCCCACTTCTCCGGTCCTTTCGTACTAGGAGGAGTGTGT
16S_1550	GTTCTTGGCAGTTGAGTTGTATTCATTGCTTAGAAGGTTGGGGTGTGCTC
5S_0	TTCCGAGATCAGACGAGATCGGGCGTTACCAGGGTGGGATGGCCGTAGGC
5S_50	TCCCAGGAGGTCTCCCATCCAAGTACTAACCAGGCCCGACCCTGCTTAGC

Oligo-based rRNA depletion

	steps	cost	time
	Designing antisense DNA oligo		4 hours
	↓		
	Making anti-sense DNA probes	\$3-10	Overnight
	↓		
	Organic Extraction	\$0.14	30 mins
	↓		
	RNA isolation with MirVana total miRNA Isolation Kit	\$9	10 mins
	↓		
rRNA Depletion	Probe Annealing	\$1	20 mins
	↓		
	RNase H treatment	\$8 - 10	1 hour
	↓		
	DNase Treatment	\$1- 5	30 mins
	↓		
	RNA Purification	\$6 -15	1 hour

A **B** **C** **D** **E** **F**