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Evaluating Alternative RNase-free DNase for RNA Extraction

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Abstract

RNA is used in many laboratories for quantitative experiments. The quality of the RNA is crucial for these experiments and can be affected by DNA contaminants. Such contaminants can be eliminated through the use of RNase-free DNase, but RNase-free DNase produced by companies such as Qiagen can run up to hundreds of dollars. Studies looking at the effectiveness of RNase-free DNase are few even though many labs use it in RNA extraction. The goal of this experiment was to compare Qiagen to RNase-free DNase manufactured by other companies in the hope of finding a DNase that is more cost-efficient but also produces high-quality RNA similar to that of the DNase from Qiagen. The results of this study showed that the RNase-free DNase produced by Thermo Fisher and Promega can purify RNA samples, resulting in a similar quality to the samples treated with the DNase produced by Qiagen and can be used as a more cost-efficient alternative.

Introduction

RNA, a single-stranded nucleotide molecule, plays an important role in the flow of genetic information as the in-between of DNA and protein. The extraction of RNA is used in microbiology laboratories across the world to provide RNA for manipulations such as reverse transcription-polymerase chain reaction (RT-PCR) and cDNA cloning (Shi and Bressan). The purity of RNA is important for these processes. mRNA makes up 1-3% of RNA while ribosomal RNA makes up over 80% of RNA, but DNA and protein can contaminate these RNA samples. To purify the RNA during the process of RNA extraction, RNase-free DNase is used to degrade any DNA within the samples. The purchase of RNA extraction kits for classroom and lab settings can be difficult as kits, such as the RNeasy Mini kit by Qiagen, can run for hundreds of dollars for a mere 50 reactions and the purchase of DNase to purify the RNA for 50 reactions can

cost additional dollars as well. The RNase-free DNase produced by Qiagen has been used regularly in our lab and others as it produces high-quality RNA, but the Qiagen DNase is also extremely expensive at $113 for only 50 reactions. Many studies have used DNase in the past to purify RNA and a few studies have looked at the effects of DNase treatment on different tissues such as a study by Nohazlin et al (2015). Few studies have looked at how the different RNase-free DNase products produced by companies such as Thermo Fisher, Qiagen, and Sigma compare to one another. The goal of this study was to find an alternative RNase-free DNase to the one produced by Qiagen that would produce equal quality RNA and be the most cost-efficient as a way to possibly decrease the overall costs of RNA extraction. For classrooms and laboratories with a limited budget, the cost of RNase-free DNase could negatively influence how RNA extraction is used, and finding a low cost yet efficient DNase could greatly benefit these institutions. In this experiment, we isolated RNA from 293T cells and purified the RNA using five different samples of DNase 1. DNase 1 developed by Qiagen was compared to DNase 1 purchased from Thermo Scientific, Sigma, Promega, and Zymo and the quality and quantity of the RNA purified with each DNase were compared to find the best alternative. To assess the quality and quantity of the RNA samples we extracted, the samples were analyzed using a NanoDrop 1000 spectrophotometer and an Agilent 2100 Bioanalyzer Automated Electrophoresis system. The samples were also run on 1% agarose/formaldehyde gels containing 0.5ug/mL ethidium bromide to visualize the banding patterns and look for possible DNA contaminants.

Materials and Methods

RNA Extraction 293T cells were cultured in media for seven days in a six-well plate until 100% confluency. Four plates were seeded to allow the experiment to be repeated four times. After culturing the cells, the RNA was extracted using the Qiagen RNeasy mini kit and following

the procedure outlined in the kit (Part 1: https://www.qiagen.com/us/resources/resourcedetail?id=0e32fbb1-c307-4603-ac81-a5e98490ed23&lang=en Part 2: https://www.qiagen.com/us/resources/resourcedetail?id=f9b2e5ef-9456-431a-85ed-2a2b9fbd503d&lang=en). 350 uL of the Buffer RLT was added to each plate to lysis the cells and the cells were agitated using a cell scraper for 15 minutes on ice. After the 15 minutes 350 uL of 70% ethanol was added to the wells and mixed by pipetting. From there, we followed the procedure outlined. After step 3 detailed in the protocol, the steps for DNase digestion were followed, detailed in the Quick-Start Protocol RNeasy Mini Kit, Part 2. During these steps the RNase-free DNase was added to the spin column. Sample one was designated as the control and had no DNase added during RNA extraction. Sample two was treated with Qiagen DNase 1. Samples three to six were treated with Thermo Scientific DNase, Promega DNase, Sigma DNase, and Zymo DNase, respectively. The DNase from each manufacturer was at a quantity of 30 units for each reaction diluted with the buffer associated with each DNase. The eluted RNA samples were stored in 1.5 uL collection tubes at -80C. After extracting the RNA, the samples needed to be assessed for quality and quantity. Three techniques were deployed. A NanoDrop 1000 spectrophotometer and an Agilent 2100 Bioanalyzer Automated Electrophoresis system were used and Gel Electrophoresis was performed.

RNA Evaluation by Nanodrop To assess the quality and quantity of the RNA after extracting the samples from the cells, a NanoDrop 1000 spectrophotometer was used and absorbance was measured at 260nm, 230nm, and 280nm. To ensure the quality of the RNA, the samples were kept on ice while in the lab for testing with the Nanodrop. After assessing the RNA samples, the samples were stored in a -70˚C freezer.

RNA Agarose Gel Analysis Two gels were run using a procedure outlined by Promega (https://www.promega.com/resources/pubhub/enotes/what-type-of-analytical-agarose-gel-do-i-need-for-my-rna-samples/). Two 1% agarose/formaldehyde gels containing 0.5ug/mL ethidium bromide were created and loaded with the RNA samples. Formaldehyde was needed in the gel to act as a denaturing agent and thus keep the RNA molecules single-stranded. Ethidium bromide was used in the gel and the loading buffer as a means to improve the visualization of the strands.

RNA Evaluation by Bioanalyzer To confirm and assess the quality and quantity of the RNA, the Agilent 2100 Bioanalyzer Automated Electrophoresis system was used with the assistance of the Uconn Center for Genomic Innovation Lab. Each sample was diluted to 20ng/uL.

Results and Discussion

After the RNA was isolated from the 293T cells, the NanoDrop 1000 spectrophotometer was used to assess the quality and quantity of the RNA samples (Figure 1). The control samples had the lowest average concentration at 574.5 ng/uL and the samples treated with the Zymo DNase had the second-lowest average concentration at 683.7 ng/uL. The RNA samples treated with the Thermo Scientific DNase have the highest average concentration of 1,188.7 ng/µL. The standard deviation of the samples treated with the Thermo Scientific DNase was 256.4 while the control samples, the Sigma treated samples, and the Zymo treated samples had standard deviations of 302.1, 403.9, and 361.4, respectively. The RNA samples treated with Qiagen and Sigma DNase had the next highest average concentrations of 1181.5 ng/µL and 1057.6 ng/µL respectively. The average concentration of RNA extracted using the Promega DNase was 1053.0 ng/uL, putting it just behind the RNA samples treated with DNase from Sigma. Sigma however had a higher standard deviation at 403.9 than Promega and Thermo Scientific having standard deviations of only 241.5 and 256.4 respectively. This suggests that while Sigma DNase provides

a higher average concentration to Promega, Promega DNase produced more consistent results between the two DNase samples (Figure 1A). As seen on the bar chart, the average concentrations of the samples were very similar, but the average concentration of the control is shown to be almost half of the average concentration of the Qiagen samples. Overall the average concentrations of the Thermo-Fisher samples and the Promega samples are close to that of the average concentration of the Qiagen samples. The standard deviations of the Thermo-Fisher samples and the Promega samples are greater than the standard deviation of the Qiagen samples, but the values are also smaller than those of the control, Sigma, and Zymo samples (Figure 1E).

The 260/280 and 260/230 ratios were assessed to determine the quality of the samples. The 260/280 ratios were used to determine the purity of the RNA samples as RNA typically absorbs at 260nm and protein contaminants absorb at 280nm, with ratios about 2.0 suggesting pure samples and low ratios suggesting the presence of contaminants. The 260/230 ratios were used similarly as contaminants may be present that absorb at 230nm. Expected 260/230 ratios were in the 2.0-2.2 range with low ratios suggesting the presence of contaminants. When the two ratios are compared, the 260/230 ratio is expected to be larger and if not, it again suggests the presence of contaminants that absorb at 230 nm. (https://assets.fishersci.com/TFS-Assets/CAD/Product-Bulletins/TN52646-E-0215M-NucleicAcid.pdf). The bar chart of the mean 260/280 ratios showed that mean values were similar for all conditions, but the standard deviation of the Zymo samples was much larger than the standard deviations of the other conditions, showing less consistency between the results of the Zymo samples (Figure 1F). The mean 260/230 ratios were all very close for each condition, though the control mean was slightly less than the others. The Qiagen, Thermo-Fisher, and Promega all gave very similar means and standard deviations (Figure 1G).

A

Concentration RNA in ng/µL

	Control	Qiagen DNase	Thermo Scientific DNase	Promega DNase	Sigma DNase	Zymo DNase
First Extraction	215.7	1357.9	893.7	1015	632.9	79.2
Second Extraction	982.8	1016.8	1596.1	774	1400.8	915.8
Third Extraction	734.4	1184.3	1089.9	1439.2	1517.3	739.4
Fourth Extraction	364.9	1166.8	1175.2	983.7	679.5	999.8
Average	574.45	1181.45	1188.725	1052.975	1057.625	683.55
Standard Deviation	302.054	120.8997	256.3901	241.4736	403.8688	361.3559
Relative Standard deviation	52.58143	10.23316	21.56849	22.93251	38.18639	52.86459

B

	Control			Qiagen		
	Concentration (ng/µL)	260/280	260/230	Concentration (ng/µL)	260/280	260/230
First Extraction	215.7	2.13	1.5	1357.9	2.05	2.09
Second Extraction	982.8	2.13	2.11	1016.8	2.15	2.06
Third Extraction	734.4	2.07	2.08	1184.3	2.1	2.13
Fourth Extraction	364.9	2.06	1.96	1166.8	2.09	2.23
Average	574.45	2.0975	1.9125	1181.45	2.0975	2.1275
Standard Deviation	302.054	0.032692	0.244681	120.8997	0.03562	0.064177
Relative Standard Deviation	52.58143	1.558605	12.79377	10.23316	1.698189	3.016568

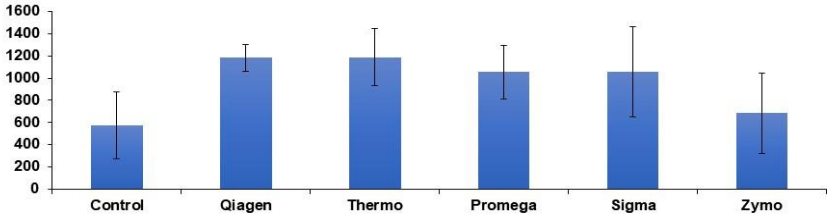
C

	Thermo Scientific			Promega		
	Concentration (ng/µL)	260/280	260/230	Concentration (ng/µL)	260/280	260/230
First Extraction	893.7	2.15	2.14	1015	2.08	2.12
Second Extraction	1596.1	2.11	2.23	774	2.15	2.11
Third Extraction	1089.9	2.08	2.19	1439.2	2.08	2.19
Fourth Extraction	1175.2	2.09	2.21	983.7	2.11	2.19
Average	1188.725	2.1075	2.1925	1052.975	2.105	2.1525
Standard Deviation	256.3901	0.02681	0.033448	241.4736	0.028723	0.037666
Relative Standard Deviation	21.56849	1.2721	1.525552	22.93251	1.364504	1.749886

D

	Sigma			Zymo		
	Concentration (ng/µL)	260/280	260/230	Concentration (ng/µL)	260/280	260/230
First Extraction	632.9	2.06	2.19	79.2	1.99	1.84
Second Extraction	1400.8	2.15	2.25	915.8	2.16	2.27
Third Extraction	1517.3	2.08	2.22	739.4	2.08	2.08
Fourth Extraction	679.5	2.12	2.25	999.8	2.11	2.2
Average	1057.625	2.1025	2.2275	683.55	2.085	2.0975
Standard Deviation	403.8688	0.034911	0.024875	361.3559	0.061847	0.163459
Relative Standard Deviation	38.18639	1.660433	1.116709	52.86459	2.966263	7.793026

E



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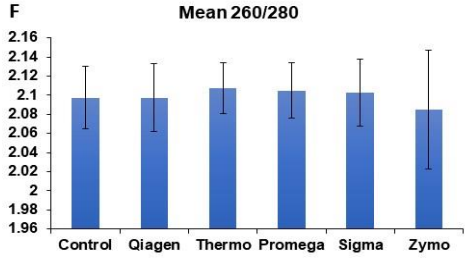

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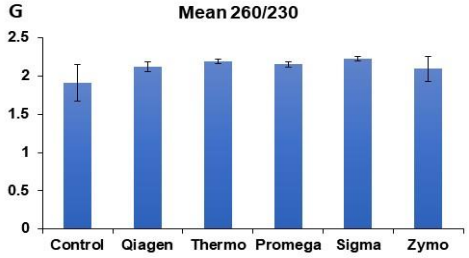
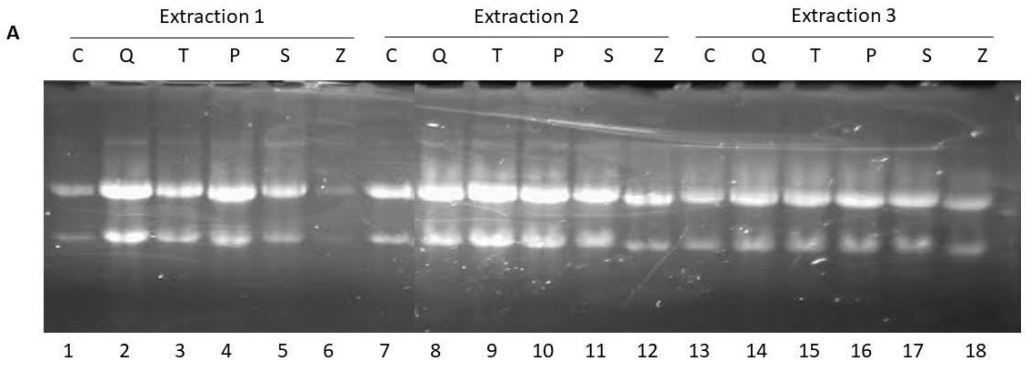


Figure 1: Comparisons of the RNA concentrations obtained under six conditions for four extractions A: Table of the concentration of RNA in samples from extractions 1, 2, 3, and 4 determined by Nanodrop 1000 B, C, and D: Concentrations of RNA, 260/230 and 260/280 ratios of extractions 1, 2, 3. And 4.E: Comparison of mean RNA concentrations F: Comparison of mean 260/280 values G: Comparison of mean 260/230 values

Gel Electrophoresis was performed after the samples were assessed by the NanoDrop. Two 1% agarose/formaldehyde gels containing 0.5ug/mL ethidium bromide were prepared using a procedure from Promega. Quantification of the 28S and 18S RNA was not performed with an RNA ladder. RNA that is not contaminated with DNA will produce two bands on an agarose gel which are made up of ribosomal RNA. The mRNA appears as smears above and below the bands. The ribosomal RNA 28S produces the upper band while the 18S produces the lower band. The upper band is expected to be more intense than the smaller band and if this is not so, the sample is degraded. The weak appearance of both the upper and lower bands would suggest that the concentration of the sample is low. For the first extraction, the only samples that did not produce intense bands were the control and the Zymo DNase treated sample. Both produced weak bands so the samples may have a lower concentration than the other samples. This does fit with the results produced by the Nanodrop. As seen in Figure 1A, the control and Zymo samples for the first extraction both had the lowest concentration of RNA compared to the samples treated with Qiagen, Thermo Fisher, Promega, and Sigma. For the second extraction, each sample produced intense bands with the larger bands being more intense. We see that this matches well with the results of the NanoDrop as the concentration of all of the samples appeared to be high. Here we also see that none of the samples were degraded as none of the larger bands appeared lighter than the lower bands. No other noticeable bands were present so it is unlikely that the RNA was contaminated with DNA. The third extraction produced mostly intense bands, however, the bands produced by the sample treated with the Zymo DNase and the control sample were slightly lighter than the bands produced by the other samples. This compliments the Nanodrop results, in which we see that these two samples did have a lower concentration than the samples treated with Qiagen, Thermo-Fisher, Promega, and Sigma. None

of the samples for the third extraction appeared to be degraded as the smaller bands did not have a greater intensity than the larger bands (Figure 2A). The fourth extraction produced results that were similar to the first extraction as the control sample produced weak bands. The sample treated with Sigma also produced weak bands for this extraction, suggesting that yield of these samples was low. This compliments the Nanodrop results as both the control sample and the Sigma treated sample had a low concentration of RNA compared to the other samples (Figure 2B). By looking at the gel electrophoresis results, we can see that Qiagen, Thermo Scientific and Promega performed better than the control, Zymo, and Sigma as none of the samples from the four extractions were degraded, none appeared to be contaminated by DNA as no DNA bands were present in the samples and each produced bands with high intensity, showing that they had high concentrations which confirmed the NanoDrop results.



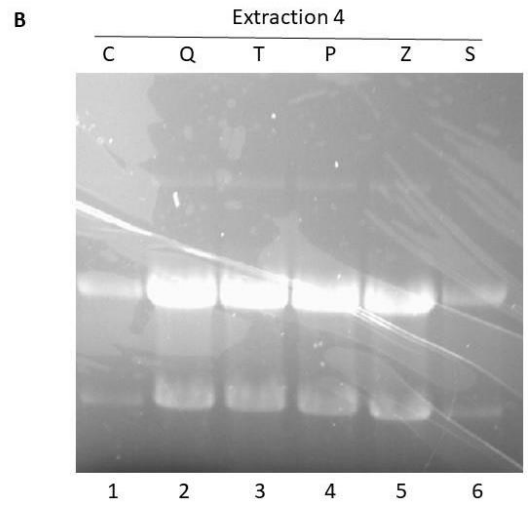


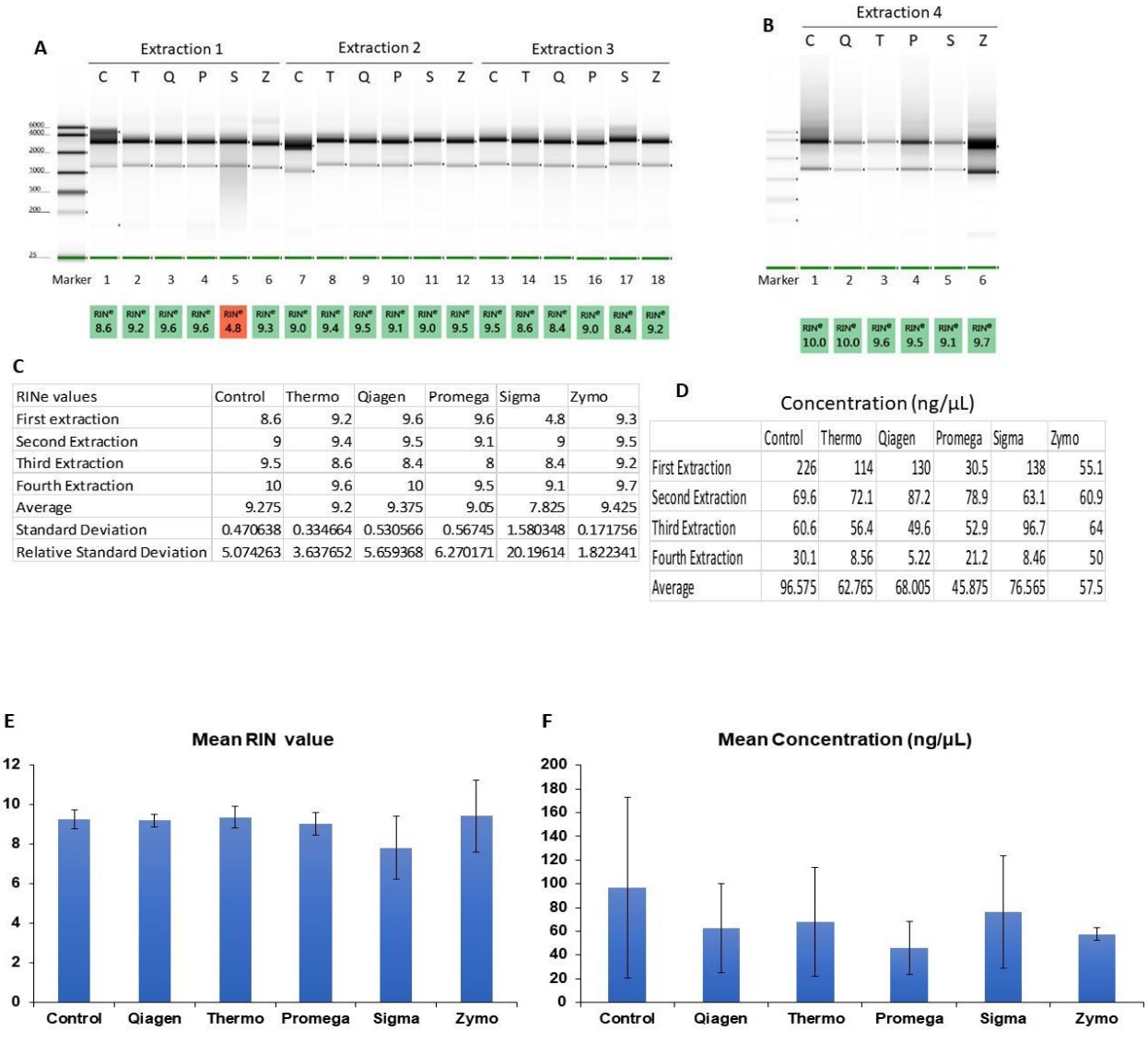
Figure 2: Assessment of quality of RNA obtained from six different treatment conditions A: RNA gel electrophoresis (1% agarose/formaldehyde gel) of RNA from extractions 1, 2, and 3. B: RNA gel electrophoresis (1% agarose/formaldehyde gel) of RNA from extraction 4.

RNA quality assessment was performed using the Agilent 2100 Bioanalyzer Automated Electrophoresis system. RIN values indicate the quality of the RNA with 10 being the highest quality RNA and 1 being the lowest quality. The RNA treated with the DNase from Zymo had the highest average RIN value of 9.425. The RNA sample treated with Qiagen and the control sample had the next highest averages of 9.375 and 9.275 respectively. The samples treated with the Thermo Fisher DNase had an average RIN value of 9.2 and the samples treated with the Promega had an average RIN value of 9.05. The Sigma DNase produced the lowest average RIN value of 7.825 and thus produced the lowest quality of RNA according to the bioanalyzer. Compared to Qiagen, DNase from Thermo Fisher and Promega both produced RNA samples with average RIN values that were close to the average RIN value of the Qiagen samples. The standard deviations of the samples revealed how consistent the results were. The samples treated with the Zymo DNase were the most consistent as the data had a standard deviation of 0.1717. Thermo Fisher had the next best standard deviation of 0.33. The control had a standard deviation of 0.47 while the Qiagen samples and the Promega samples had standard deviations of 0.53 and 0.567 respectively. The Sigma samples had the highest deviation of 1.58, revealing that it is the

least consistent of the 6 conditions (Figure 3C). The bar chart of the mean RIN values showed that each condition produced samples with roughly the same average RIN values with the exception of Sigma. The Zymo samples had the largest standard deviation, showing the least consistency. The Sigma samples also had a large standard deviation. These results suggest that of the five RNase-free DNase, the products produced by Thermo-Fisher and Promega were able to yield RNA samples with a similar quality to that of the Qiagen DNase and were able to give equally consistent results as the standard deviations for both conditions were not much larger than the standard deviation of the Qiagen samples (Figure 3E).

Among the results produced by the bioanalyzer, was the concentration given in pg/μL. Using this information, we calculated the concentration of each sample in ng/uL. Before running the samples on the bioanalyzer, we used the concentration given by the NanoDrop and diluted that to 20 ng/uL with a total volume of 50 uL. To find the total concentration in ng/uL, we first took the concentration in pg/μL that was given by the bioanalyzer and converted this ng/uL. This value however is a diluted value so the undiluted concentration had to be calculated. We calculated the total concentration for each sample and used to calculate the average concentration for each condition. Surprisingly, the control samples produced the highest average concentration of 96.575. The Promega samples had the lowest average concentration of 45.875. The Qiagen samples produced an average concentration of 68.005 and the Thermo Fisher samples did produce an average concentration close to that, 62.765. Both Sigma and Zymo had higher average concentrations than expected which were 76.565 and 57.5 respectively. Promega produced the lowest average concentration of 45.875 (Figure 3D). To better compare the average concentrations of the samples, a table was constructed. The control samples produced the largest mean concentration, but also have the largest standard deviation. The Qiagen and Thermo-Fisher

samples had average concentrations that were close to one another. These results don't agree with the NanoDrop results which show much higher concentrations for each sample. This could be due to errors with the NanoDrop data, but it is also likely that these calculations may not be accurate as there may be dilution errors.



C

RINe values	Control	Thermo	Qiagen	Promega	Sigma	Zymo
First extraction	8.6	9.2	9.6	9.6	4.8	9.3
Second Extraction	9	9.4	9.5	9.1	9	9.5
Third Extraction	9.5	8.6	8.4	8	8.4	9.2
Fourth Extraction	10	9.6	10	9.5	9.1	9.7
Average	9.275	9.2	9.375	9.05	7.825	9.425
Standard Deviation	0.470638	0.334664	0.530566	0.56745	1.580348	0.171756
Relative Standard Deviation	5.074263	3.637652	5.659368	6.270171	20.19614	1.822341

D

	Control	Thermo	Qiagen	Promega	Sigma	Zymo
First Extraction	226	114	130	30.5	138	55.1
Second Extraction	69.6	72.1	87.2	78.9	63.1	60.9
Third Extraction	60.6	56.4	49.6	52.9	96.7	64
Fourth Extraction	30.1	8.56	5.22	21.2	8.46	50
Average	96.575	62.765	68.005	45.875	76.565	57.5

Figure 3: Quality and quantity assessment using an Agilent Bioanalyzer 2100 of samples extracted under six conditions A: Agilent Bioanalyzer 2100 results of extractions 1, 2, and 3. B: Agilent Bioanalyzer 2100 results of extraction 4. C: Table with RIN values of extractions 1, 2, 3,

and 4 along with the average, standard deviation, and relative standard deviation. D: Table of the concentrations of each sample from extractions 1, 2, 3, and 4. E and F: Comparisons of the mean values for the RIN and concentration of the samples.

Conclusion

From the results of NanoDrop and Bioanalyzer, we were able to conclude that the DNase from Thermo-Fisher and Promega obtain a similar quality of RNA compared to the DNase from Qiagen. This is key as the cost of DNase from Qiagen is $113 for 1,500 units while the cost of DNase from Thermo-Fisher is $66.25 for 1,000 units and the cost of DNase from Promega is $53 for 1,000 units. Per unit, the DNase produced by Qiagen is $0.075 while the DNase produced by Thermo-Fisher is $0.066 and the DNase produced by Promega is $0.053. 0.9% per unit of DNase would be saved if Thermo-Fisher was used instead of Qiagen and 2.2% per unit of DNase would be saved if Promega was used instead. The DNase from Thermo-Fisher and Promega offers a cost-efficient DNase that does not sacrifice quantity and quality. The high cost of DNase from companies like Qiagen may negatively impact the use of RNA extraction in labs and in classrooms with a limited budget. For this experiment, we only looked at five different DNase 1 by different companies. Future studies could compare the quality of other DNase produced by other companies so to see if there are alternatives to the DNase produced by Qiagen.

References

Norhazlin, J., et al. “Effect of DNase Treatment on RNA Extraction from Preimplantation Murine Embryos.” *Genetics and Molecular Research*, vol. 14, no. 3, 25 Aug. 2015, pp. 10172–10184., doi:10.4238/2015.august.28.1.

Shi H., Bressan R. (2006) RNA Extraction. In: Salinas J., Sanchez-Serrano J.J. (eds) Arabidopsis Protocols. Methods in Molecular Biology™, vol 323. Humana Press