DNA Loss During Clean-up if Extracts are Not Initially Treated with RNase

Purpose:

RNA obscures both quantitative (when using Nanodrop) and qualitative (using Electrophoresis Gel) tests run on DNA Extracts; therefore, RNA should be removed before quantitative and qualitative tests are performed. RNA can be removed during the initial DNA extraction with the use of RNase or after the initial extraction using either an ethanol or an isopropanol precipitation clean-up (which also uses RNase during the process). There are three goals for this experiment: 1. Determine whether DNA is lost if RNA removal is attempted after initial extraction, 2. Compare the effectiveness of the varying RNA removals (initial extraction RNase treatment, EtOH precipitation, and Isopropanol precipitation), and 3. Compare the reliability of quantitation methods (Qubit and Nanodrop) between samples before and after RNase treatment.

Methods

1. 3 Individuals from ECM collection will be used. Each individual will undergo 3 DNA extractions (1 extraction treated with RNase, 2 extractions **not** treated with RNase).
    a. Individual 1 = 3 equally sized leg extractions
        i. Extraction 1.A = RNase Treatment
        ii. Extraction 1.B = No RNase Treatment
        iii. Extraction 1.C = No RNase Treatment
    b. Individual 2 = 3 equally sized liver extractions
        i. Extraction 2.A = RNase Treatment
        ii. Extraction 2.B = No RNase Treatment
        iii. Extraction 2.C = No RNase Treatment
    c. Individual 3 = 3 equally sized liver extractions
        i. Extractions 3.A = RNase Treatment
        ii. Extraction 3.B = No RNase Treatment
        iii. Extraction 3.C = No RNase Treatment

    *Note: The Liver extracts that weren't treated with RNase should have more RNA than the Leg sample.

2. An aliquot of extracts B and C for all Individuals will be taken in order to run quantitation and a gel later.

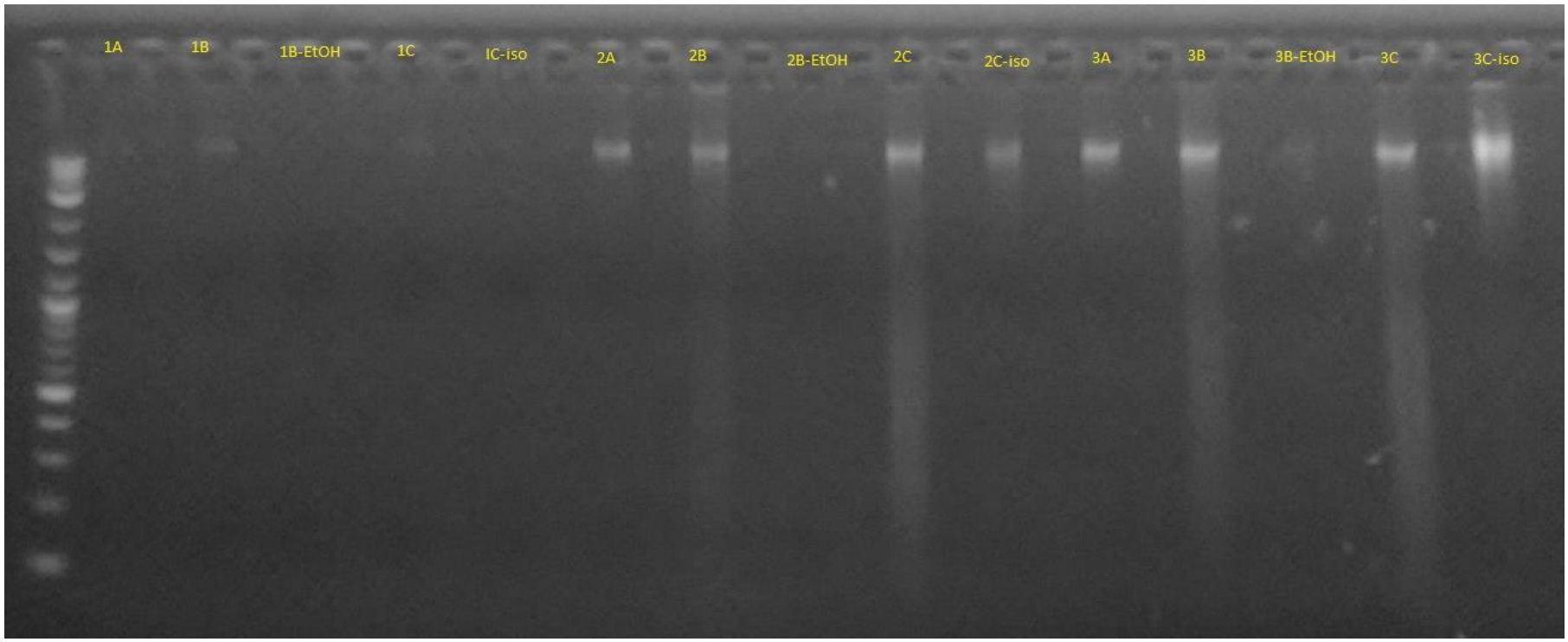
3. Extract B for all individuals will undergo EtOH precipitation; extract C for all individuals Isopropanol precipitation.

4. After Precipitation Clean-ups are complete Nanodrop and Qubit will be performed on all samples:

5. After Precipitation Clean-ups are complete a gel with all samples (pre- and post- cleanup) will be run. Samples will be loaded into every other well to ensure RNA smears don't obscure DNA detection in adjacent wells.

Results

Sample	Before Cleanup					After Cleanup				
	Nanodrop ng/ul	**Nanodrop 260/280**	**Nanodrop 260/230**	**Qubit ng/ul**	**Total ng**	**Nanodrop ng/ul**	**Nanodrop 260/280**	**Nanodrop 260/230**	**Qubit ng/ul**	**Total ng**
1.A (RNase Treated)	**39.2**	**1.93**	**4.60**	**1.91**	**172**	--	--	--	--	--
1.B	**20.7**	**1.70**	**2.04**	**3.05**	**275**	**8.50**	**0.60**	**0.07**	**.097**	**8.73**
1.C	**21.0**	**2.07**	**1.97**	**1.69**	**152**	**-0.30**	**0.20**	**-0.24**	**.116**	**10.4**
2.A (RNase Treated)	**41.1**	**1.94**	**2.54**	**10.3**	**1440**	--	--	--	--	--
2.B	**48.9**	**2.08**	**2.66**	**14.4**	**2020**	**-1.80**	**1.98**	**-0.30**	**.219**	**30.7**
2.C	**142**	**2.04**	**1.61**	**25.5**	**3570**	**30.8**	**1.62**	**1.01**	**16.3**	**2280**
3.A (RNase Treated)	**63.6**	**1.85**	**1.33**	**19.0**	**2660**	--	--	--	--	--
3.B	**139**	**1.98**	**1.58**	**29.9**	**4190**	**12.9**	**1.39**	**0.41**	**2.12**	**297**
3.C	**164**	**1.93**	**1.63**	**31.6**	**4420**	**120**	**1.61**	**0.96**	**43.4**	**6080**



As mentioned above, "A" samples underwent RNase treatment during extractions; "B" and "C" samples underwent RNase treatment after extractions. "B" and "C" samples before cleanup are simply marked **1B**, **1C**, etc. Post-cleanup samples are marked as **1B- EtOH**, **1C- iso**, etc. The smears under the pre-cleanup samples are RNA. After cleanup, the smears are gone, but there is significant sample loss.

Conclusion

In brief: You can treat for RNA after extractions, but you can lose a lot of sample in the process! Sample preparation will be simpler if RNase is applied during extractions.