Genomic DNA QC Using Standard Gel Electrophoresis (For Collaborators)

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Summary

Before shipping your DNA sample(s), please be sure to follow the JGI sample preparation and sample submission guidelines located at http://my.jgi.doe.gov/general/gettingstarted.html.

This protocol describes how to run a standard agarose gel utilizing concentration and size standards as well as Qubit™ fluorometer to evaluate the quality, quantity, and molecular weight of your DNA sample(s). We recommend all DNA samples to be evaluated with this protocol before they are shipped to JGI.

Materials & Reagents

Materials/Reagents/Equipment	Vendor	Stock Number
Disposables		
Microcentrifuge Tubes	VWR	
Pipette Tips		
Qubit™ Assay Tubes	Invitrogen	Q32856
Reagents		
GenePure LE Agarose (Generates)	ISC BioExpress	E-3120-500
50X TAE Buffer	Invitrogen	24710-030
SYBR® Safe DNA gel stain (10,000X concentrate in DMSO)	Invitrogen	S33102
Or Ultra Pure Ethidium Bromide (10mg/ml)	Invitrogen	15585011
5X Loading Dye		
TE Buffer, pH 8.0 500ml	Ambion	9849
DNA Molecular Weight Marker II (0.12–23.1 kbp) (~25ng/ul)	Roche	10 236 250 001
DNA Mass Standards (Lambda DNA) 15, 31, 63, 125, 250, 500ng / 6ul	Life Technologies	14420-012
Quant-iT™ dsDNA BR Assay Kit, 500 assays (2-1000 ng)	Invitrogen	Q32853
Quant-iT™ dsDNA HS Assay Kit, 500 assays (0.2-100 ng)	Invitrogen	Q32854
Equipment		

Pipettes		
12X14 Horizontal Device Comb (25 well 1.5mm)	CLP	75.1214-MT-25D
12X14cm Horizontal Gel Electrophoresis Device	CLP	75.1214
Gel Doc Imager	Bio-Rad	
Qubit™ fluorometer 1.0 or 2.0	Invitrogen	Q32857 or Q32866

EH&S

PPE Requirements:

Safety glasses, lab coat, and nitrile gloves should be worn at all times while performing this protocol. Additional safety equipment is required at designated steps.

Procedure

NOTE: All reagents/stock solutions should be prepared prior to the start of the procedure.

1. **Gel & Sample Preparation**

   1.1 Cast a ~100ml 1% agarose gel with 1X TAE and ethidium bromide (.15ug/ml) or SYBR® Safe DNA gel stain (10,000X concentrate in DMSO). Use a narrow well comb.

   1.2 Transfer 1µl of your genomic DNA sample(s) (concentration between 50ng to 500ng) into clean labeled tube(s) and bring the total volume up to 4µl with 1X TE Buffer, pH 8.0.

   a. If the genomic DNA concentration is thought to be lower than 50ng/µl, then transfer 2-4µl of the sample(s). For example, if the DNA concentration is around 25ng/µl, then transfer 2µl of the DNA sample and add 2µl of TE Buffer to a final volume of 4µl to be loaded on the gel.

   b. If the genomic DNA concentration is thought to be higher than 500ng/µl, then create a dilution of the sample(s) in TE Buffer to reduce the concentration between 50ng/µl and 500ng/µl. For example, if the DNA concentration of the sample is around 1000ng/µl, then create a 1:10 dilution of the sample in TE buffer by adding 1µl of the original sample to 9µl of TE buffer. Then transfer 1µl of 1:10 diluted sample and add 3µl of TE Buffer to a final volume of 4µl to be loaded on the gel.

   1.3 Add 1µl of 5X loading dye. The amount of loading dye should remain consistent among all samples to maintain even DNA migration on the gel. Vortex and spin down sample tube(s).

2. **Gel Electrophoresis**

   Note: Refer to Appendix 1 for the gel loading guide.

2.1 Load the gel according to the format listed below:

a. Well 1 – 6µl of 2.50 ng/µl standard (total mass = 15 ng)

b. Well 2 – 6µl of 5.17 ng/µl standard (total mass = 31 ng)

c. Well 3 – 6µl of 10.50 ng/µl standard (total mass = 63 ng)

d. Well 4 – 5µl of Marker 2 (~25ng/µl)

e. Well 5 – 5µl of DNA sample (from above sample preparation)

Note: If multiple samples are being run, load all samples and complete loading f-i after the last sample.

f. Well 6 – 5µl of Marker 2 (~25ng/µl)

g. Well 7 – 6µl of 20.83 ng/µl standard (total mass = 125ng)

h. Well 8 – 6µl of 41.67 ng/µl standard (total mass = 250ng)

i. Well 9 – 6µl of 83.33 ng/µl standard (total mass = 500ng)

2.2 Run gel for 90 min at ~120V in 1X TAE buffer. If a different electrophoresis set-up is being used, ensure the genomic DNA bands have ran ≥2 cm down from well and separation of marker is apparent.

2.3 Remove gel from gel box and image.

3. **DNA QC Gel Analysis**

3.1 Analyze genomic DNA for molecular weight, quantity, and quality. Refer to the JGI Guidelines document to see the specific guidelines in the following areas for your genome type.

a. **MOLECULAR WEIGHT** (If large long mate-pair analysis is needed, the size of DNA needs to be in the high molecular weight. In this case, DNA band should be above the 23kb band. If possible, pulse field gel should be performed to properly determine the molecular weight)

b. **QUANTITY**

i. Compare genomic DNA band with mass standard bands (15, 31, 63, 125, 250, 500ng) to obtain a concentration estimate. Then use the concentration estimate to calculate the total DNA available for this sample.

ii. If Quantity One Software (Bio-Rad) is available, please refer to Appendix 2 for instructions.

c. **QUALITY**

i. How does the DNA look? Is the DNA a tight band or does it appears to be streaky, displaying signs of degrading and/or shearing? Is RNA present in your sample? A protocol to remove RNA from the sample can be located at http://my.jgi.doe.gov/general/index.html.

Note: Refer to Appendix 3 and 4 for examples of QC gels that have passed and failed JGI DNA QC requirements.

4. **Sample Quantitation with the Qubit™ fluorometer**

4.1 Set up the number of 0.5ml Qubit™ assay tubes that will be needed for the samples and one standard.

4.2 Label the tube lids; these tubes are temperature sensitive so do not hold these too long in your hand as you label them.

4.3 Add 1µl of Quant-iT™ standard (100ng/µl) and exactly 1µl of each of the samples to the appropriate tubes (Careful pipetting is critical to ensure that exactly 1µl was added to each tube to accurately determine the sample concentration).

4.4 Make the Quant-iT™ working solution by diluting the Quant-iT™ dsDNA BR reagent 1:200 in Quant-iT™ dsDNA BR buffer. Use a clean plastic tube each time you make Quant-iT™ working solution. Do not mix the working solution in a glass container.

a. The final volume in each assay tube must be 200µl and each tube will require 199µl. Prepare sufficient Quant-iT™ working solution to accommodate all samples and standard. For example, for 8 samples, prepare enough working solution for the samples and 1 standard: prepare a total volume of 2ml of working solution (10µl of Quant-iT™ reagent plus 1,990µl of Quant-iT™ buffer).

4.5 Load 199µl of Quant-iT™ working solution into each of the tubes used for samples and the standard.

4.6 Mix by vortexing for 2–3 seconds, being careful not to create bubbles. The final volume in each tube should be 200µl.

4.7 Allow all tubes to incubate at room temperature for 2 minutes in dark since the reagent is light sensitive.

4.8 Plug in and press any key to turn on the Qubit™ fluorometer.

4.9 Press **HOME**, use the arrow ↓ and ↑ keys to highlight **Quant-iT™ DNA, BR**, and press **GO** to initiate the assay.

4.10 On the calibration screen, highlight **Use last calibration** and press **GO**.

a. For each assay, there will be an option to run a new calibration or to use the values from the previous calibration. If the values seem off then use the *Calibrating the Qubit™ Fluorometer* from the manufacturer's protocol for calibration guidelines.

4.11 Insert a sample tube into the Qubit™ fluorometer, close the lid and press **GO**.

4.12 When the Qubit™ fluorometer displays the QF value, choose **Calculate sample concentration** and select the volume of sample added to each of the assay tube (which should be 1 µL).

4.13 When the Qubit™ fluorometer displays the concentration of dsDNA in your original sample, record that number (1µg/ml = 1ng/µl).

4.14 Continue reading samples and recording values until all samples are read.

a. If you did not choose **Calculate sample concentration** then you must calculate the concentration of your original sample by using the equation supplied below.

Calculating the Concentration of Your Sample:
The Qubit™ fluorometer gives values for the Quant-iT™ dsDNA BR assay in µg/mL. This value corresponds to the concentration after your sample was diluted into the assay tube. To calculate the concentration of your sample, use the following equation:

$$\text{Concentration of your sample} = \text{QF value} \times (200/x)$$

where:
QF value = the value given by the Qubit™ fluorometer
x = the number of microliters of sample you added to the assay tube
This equation generates a result with the same units as the value given by the Qubit™ fluorometer (i.e., if the Qubit™ fluorometer gave a concentration in µg/mL, the result of the equation will be in µg/mL).

4.15 The following should be done if the concentration reading is out of range:

a. If the concentration reads too low, then use the Quant-iT™ dsDNA HS kit and repeat section 2 and use the ↓ and ↑ keys to highlight **Quant-iT™ DNA, HS** assay.

b. If the concentration reads too high, then dilute the sample to 200-500ng/µl with TE buffer, pH 8.0 and repeat section 2 with **Quant-iT™ DNA, BR** assay.

Reagent/Stock Preparation

1X TAE Buffer
40ml 50X TAE Buffer
1960ml Milli-Q ddH_2O

5X Loading Dye
125ml Nuclease free H_2O
75ml 100% glycerol
0.01g Bromophenol Blue
0.01g Xylene Cyanole FF

APPENDIX 1: GENOMIC DNA QC GEL LOADING GUIDE

Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7	Lane 8	Lane 9
5µl of 3.125ng/µl	**5µl of 6.25ng/µl**	**5µl of 12.5ng/µl**	**5µl of Marker II**	**5µl of sample**	**5µl of Marker II**	**5µl of 25ng/µl**	**5µl of 50ng/µl**	**5µl of 100ng/µl**
Total mass **= 15.625ng**	Total mass **= 31.25ng**	Total mass **= 62.5ng**				Total mass **= 125ng**	Total mass **= 250ng**	Total mass **= 500ng**

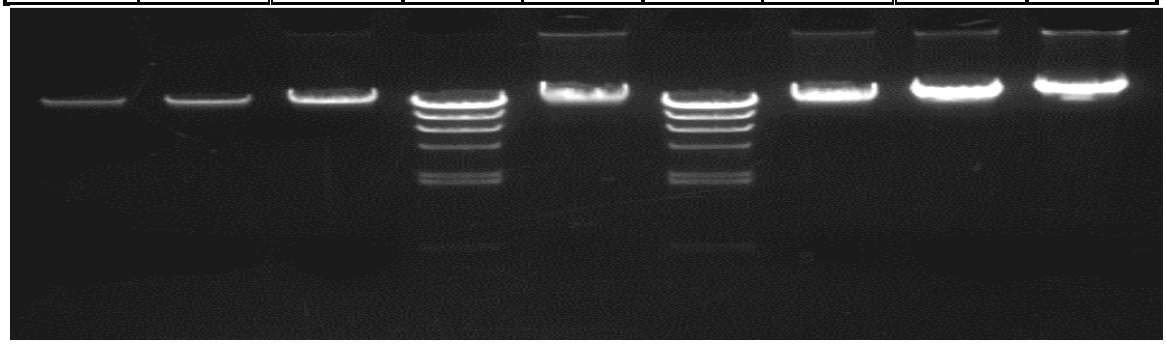


Figure 1: Gel loading Guide.

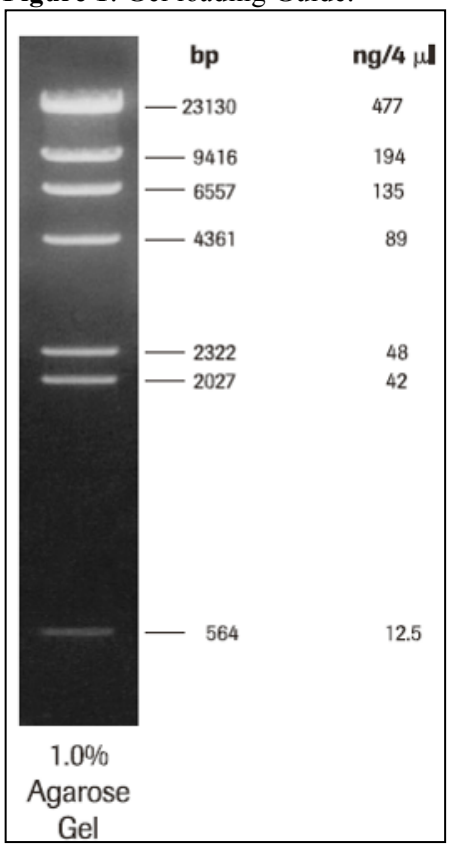
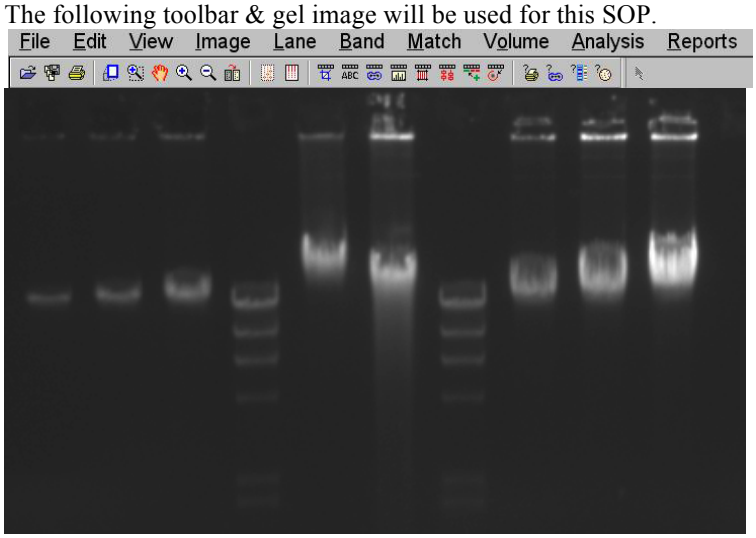


Figure 2: Marker II.

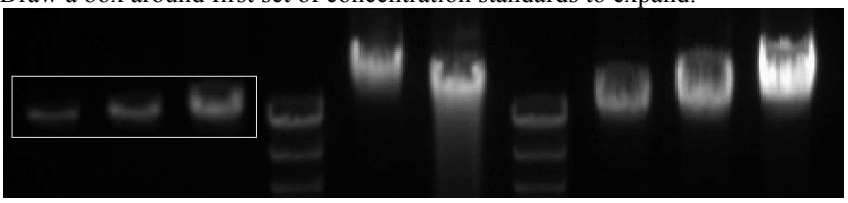
APPENDIX 2: USING QUANTITY ONE SOFTWARE TO ANALYZE DNA QC GELS

1) Click on Quantity One Program Icon 

2) Open gel file image to be analyzed.
The following toolbar & gel image will be used for this SOP.

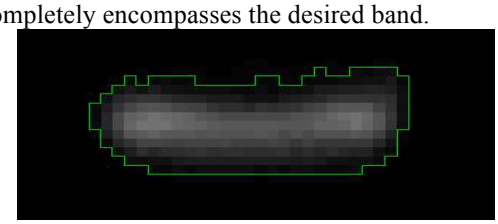


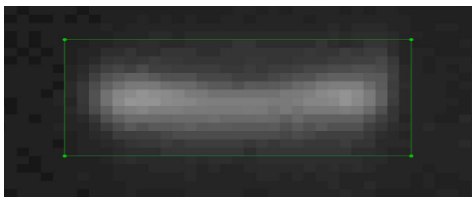
3) Click on the "Draw a box and expand the image inside" Icon 
Draw a box around first set of concentration standards to expand.



4) Click the "Contour" Icon  . The following sub-Icon box appears.

5) Click on the "Volume Contour Tool" Icon  . Place cursor arrow on outer edge of concentration standard, left click & HOLD. Slightly move cursor outward until the contour's bounding outline completely encompasses the desired band.



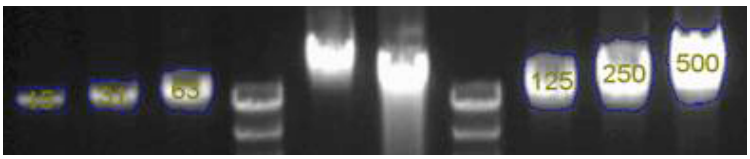


For older versions of the software the contour tool is not available. Use the "Volume Rectangle Tool" Icon  *instead.*

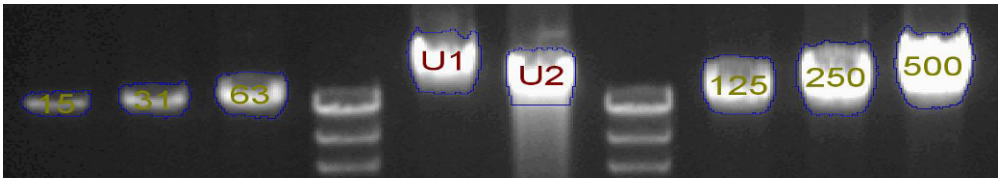
6) Place cursor in center of the bounding area and double click. The "Volume Properties" box will appear.

Make sure "Standard" is selected and input only the numerical value for that particular concentration standard.

Click on the OK button. Repeat for the remaining concentration standards.

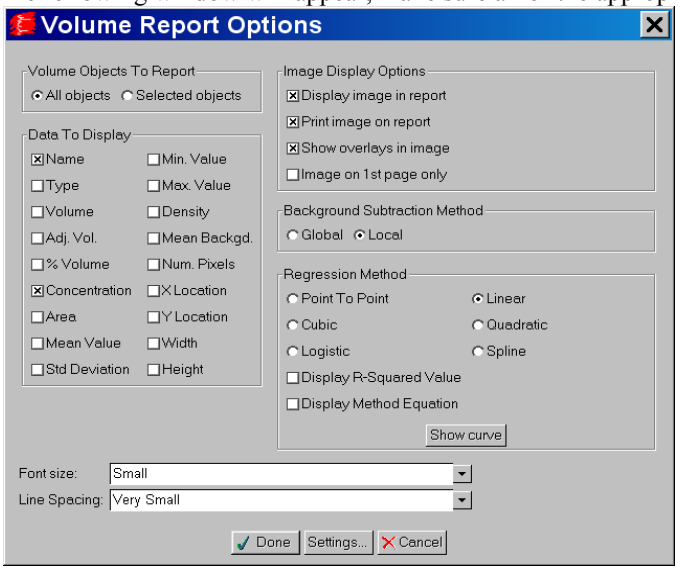


7) After all of the concentration standards have been assigned, repeat Steps #5 & #6 for the unknown samples. Make sure "Unknown" is selected. Remember when outlining the "Unknown samples" ONLY select the high molecular weight section for analysis.

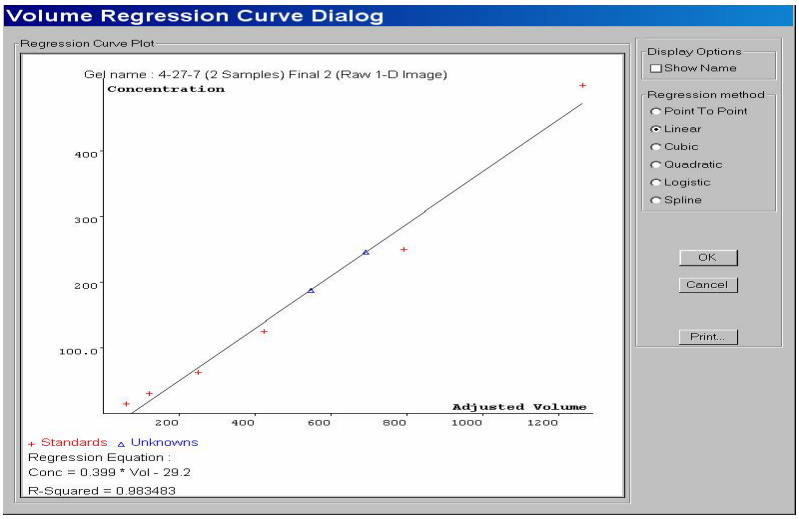


8) Click on the "Display Volume Report" Icon in the sub-Icon toolbar  .

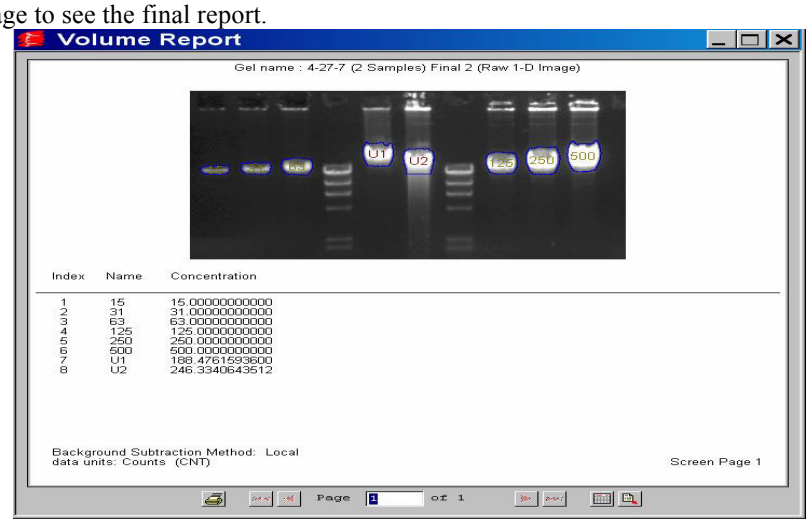
9) The following window will appear, make sure all of the appropriate boxes are selected.



10) Click on the "Show Curve" button to display graphic plot of the standards & samples. Standards are red plus symbols (+) and unknown samples are blue triangles (Δ). All value points should be in close proximity to linear curve plot. The closer the "R-Squared" value *(at the bottom of the page)* is to 1.0 the greater the accuracy. *For this example the R-Squared = 0.983483*



11) Click on the "OK" button. Page returns to "Volume Report Options" displayed in Step #9. Click the "Done" button on that page to see the final report.



12) Determine the "**Concentration**" of each sample in "**ng/ul**".
Sample #1 = **188ng/ul**
Sample #2 = **246ng/ul.**
Note: If the sample volume loaded is greater than 1ul, divide the concentration estimate by the sample volume loaded.

13) Determine the total "**Quantity**" for each sample in "**ug**".
Sample #1) 188ng/ul * 500ul (total volume of sample) = **94,000ng Total (94ug)**
Sample #2) 246ug/ul * 250ul (total volume of sample) = **61,500ng Total (61.5ug)**

APPENDIX 3: EXAMPLE OF QC GELS THAT PASSED JGI DNA QC REQUIREMENTS

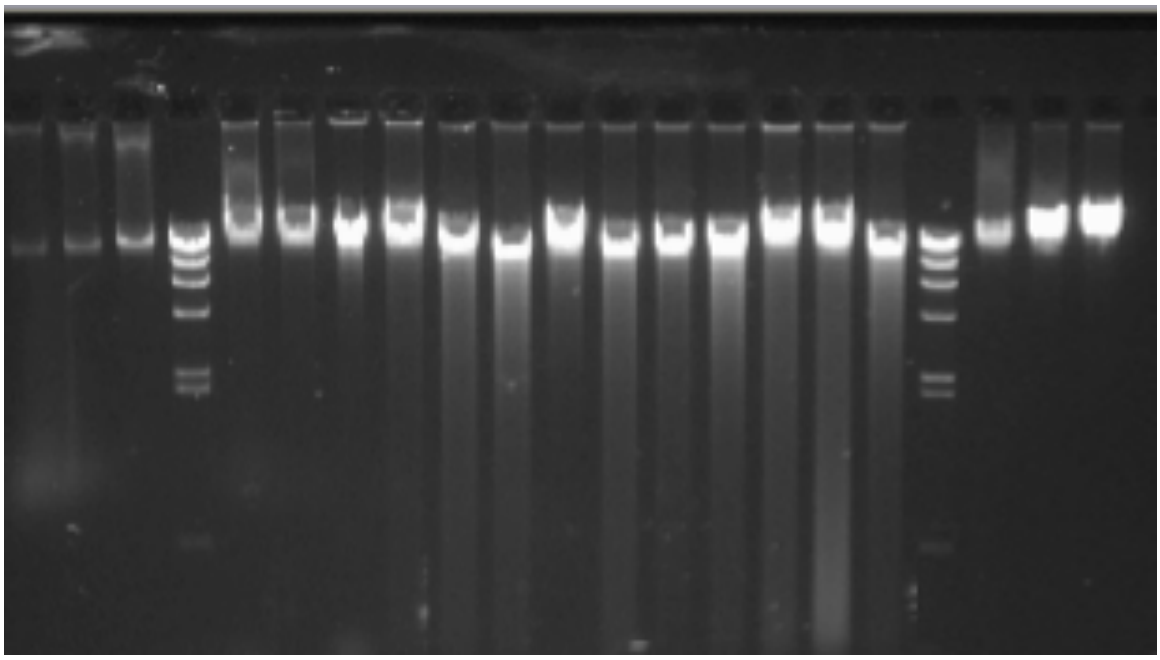


Figure 1: Example of DNA samples that have a tight band with minimal smearing and have molecular weight greater than 23kb.

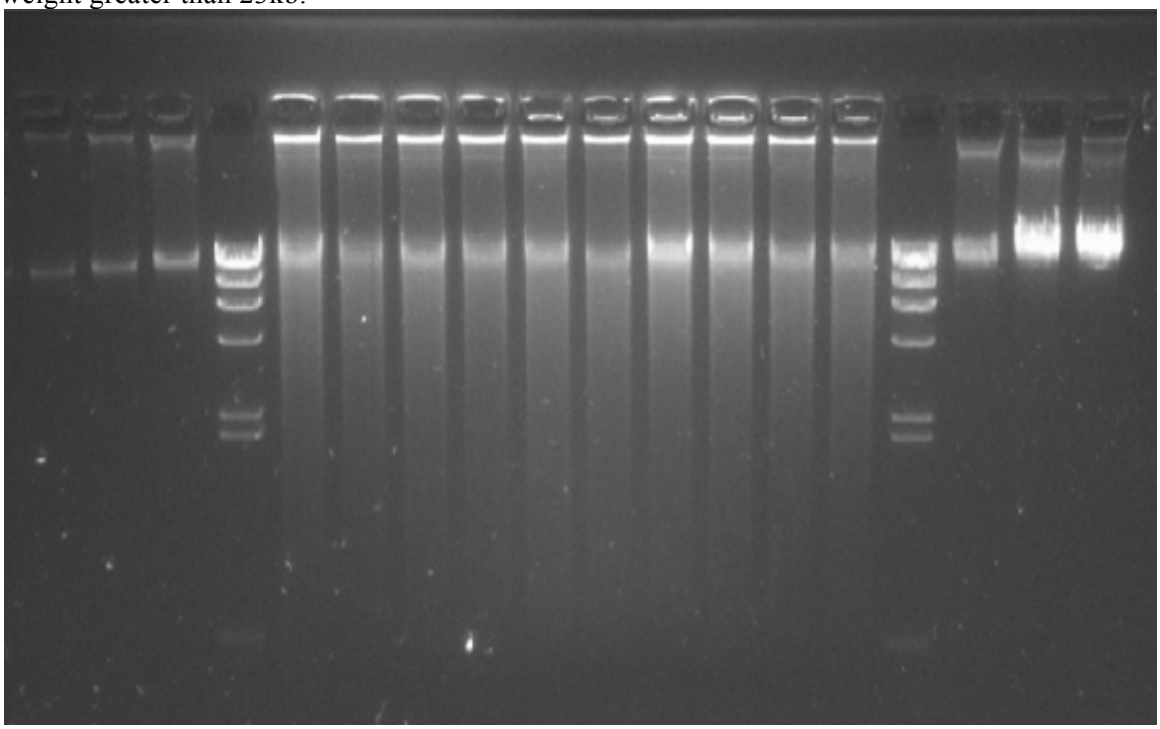


Figure 2: Example of MDA DNA product which has some smearing present but most of the DNA has a molecular weight at 23kb.

APPENDIX 4: EXAMPLE OF QC GELS THAT FAILED JGI DNA QC REQUIREMENTS

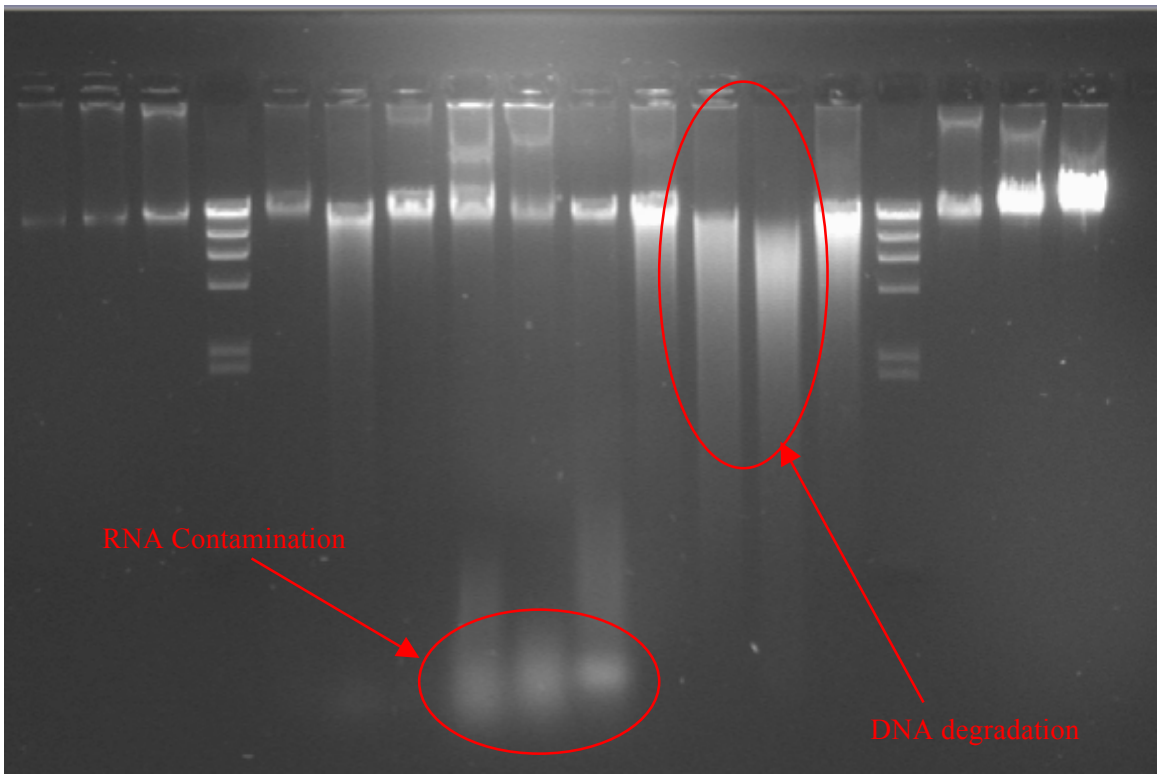


Figure 1: Example of DNA samples with RNA contamination and signs of DNA degradation.

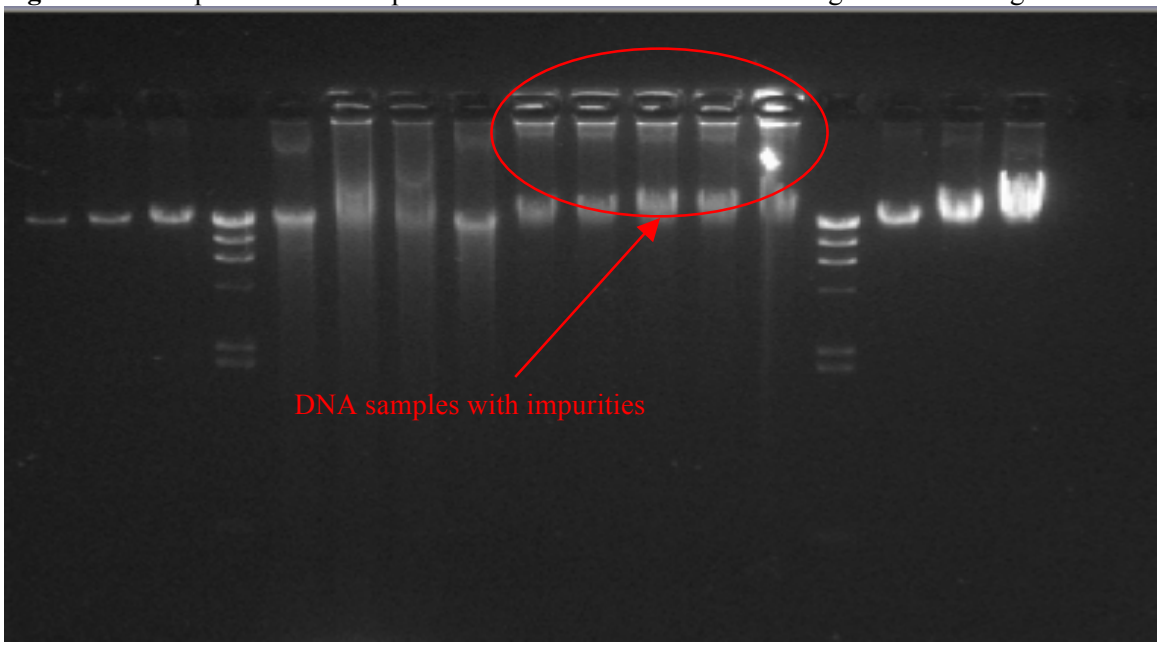


Figure 2: Example of DNA samples with impurities (proteins and polysaccharides that can inhibit chemical reactions during library construction).