Genomic DNA QC Using Standard Gel Electrophoresis (For Collaborators)

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Author(s): Mansi Chovatia
Reviewed/Revised by: Jan-Fang Cheng, Shweta Deshpande, Hope Tice

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 utilizes the concentration and size standards provided by the JGI, run a standard agarose gel to evaluate the quality, quantity, and molecular weight of your DNA sample(s).

Materials & Reagents

Materials/Reagents/Equipment	Vendor	Stock Number
Disposables		
Microcentrifuge tubes	VWR	
Pipette tips		
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
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 / 5ul	JGI	
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	

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 50ng to 500ng of your genomic DNA sample(s) 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 of the sample 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 – 5µl of 3.125ng/µl standard (total mass = 15.625ng)

   b. Well 2 – 5µl of 6.25ng/µl standard (total mass = 31.25ng)

   c. Well 3 – 5µl of 12.5ng/µl standard (total mass = 62.5ng)

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

e. Well 5 – 5µl of DNA sample (Xµl DNA + Yµl 1X TE Buffer, pH 8.0 + 1µl 5X loading dye = 5µl total volume to be loaded on the gel)

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 – 5µl of 25ng/µl standard (total mass = 125ng)

h. Well 8 – 5µl of 50ng/µl standard (total mass = 250ng)

i. Well 9 – 5µl of 100ng/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. Save photo as a TIFF file and upload the image on the Collaborator Sample Information (CSI) Online Form.

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** (Pulse-field gel recommended to properly assess size)

i. Refer to the Marker 2 image in Appendix 1. Is the genomic DNA high molecular weight? Fosmid libraries and large insert 454 Paired End libraries require DNA ≥40kb, check if DNA band is above the 23kb band.

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. The JGI will run the TIFF file through quantification software to determine the quantity of the DNA sample(s).

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.

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

SOP Approval

DEPARTMENT	APPROVED BY	DATE
Lab Supervisor	Shweta Deshpande	8/5/2010
Process Optimization	Jan-Fang CHeng	8/4/2010

Change History

Updated on 03/16/2010: Version 1.0 Released on 09Aug2010- Per PCN 46, original SOP was divided into 2 and each of them is released as version 1.0

- The Genomic DNA QC Using Standard Gel Electrophoresis SOP version 3.0 has split into two different SOPs:
  - Genomic DNA QC Using Standard Gel Electrophoresis (for Collaborators) version 1.0
  - JGI Genomic DNA QC version 1.0
- Updated both of the SOPs with proper PPE statement.
- Updated both of the SOPs to include the use of SybrSafe Gel stain.
- Updated both of the SOPs with newer and more recent DNA QC gel images and ladder images.
- Added JGI sample submission guidelines website in the summary section for Genomic DNA QC Using Standard Gel Electrophoresis (for Collaborators) version 1.0.
- Updated RNAase treatment protocol location for Genomic DNA QC Using Standard Gel Electrophoresis (for Collaborators) version 1.0.
- Standardized the amount of glycerol to be added in the loading dye for each sample:
  - Removed 1X Loading Dye from both of the SOPs. We will use 5X Loading dye to maintain the 7.5% glycerol as the final concentration when loading the sample. The 5X

Loading Dye has 37% glycerol, adding 1ul of 5X Loading Dye to a 4ul sample will yield a 7.5% glycerol as the final concentration.

- Renamed 6X Loading Dye to 5X Loading Dye since we use 1ul of 5X loading dye with 4ul of DNA sample.

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**

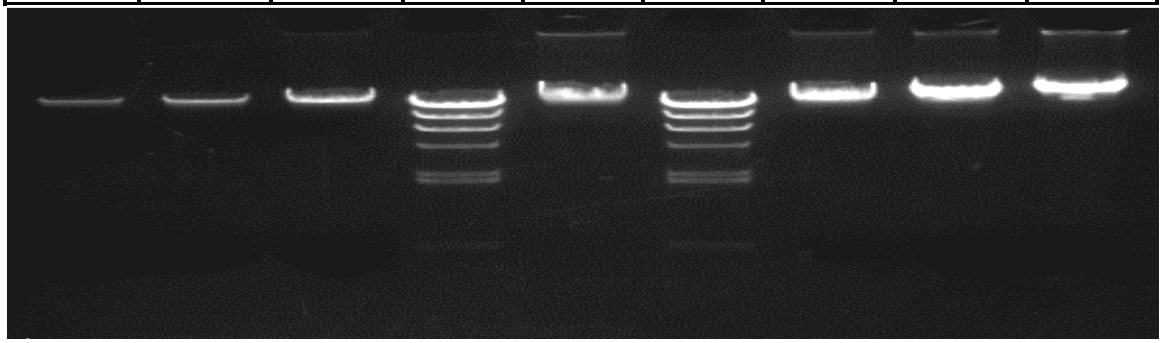


Figure 1: Gel loading Guide.

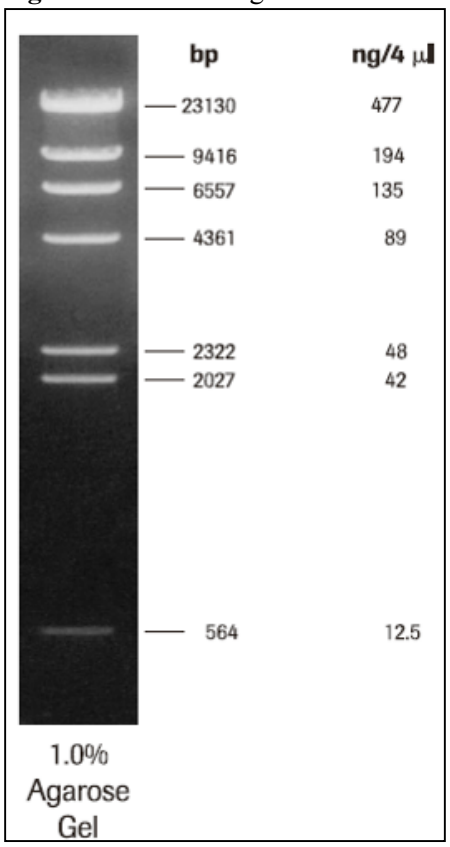
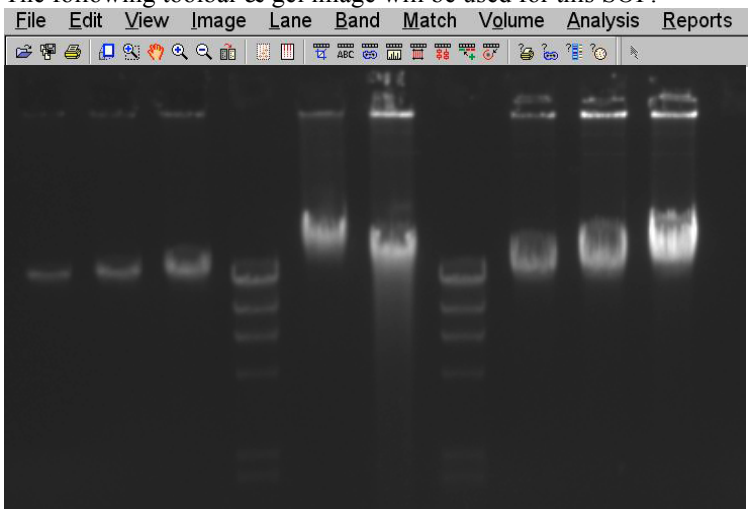


Figure 2: Marker II.

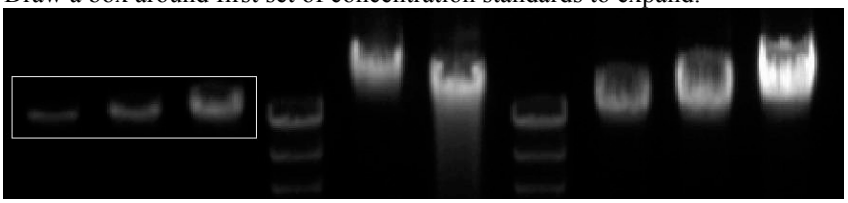
APPENDIX 2: USING QUANTITY ONE SOFTWARE TO ANALYZE DNA QC GELS

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.

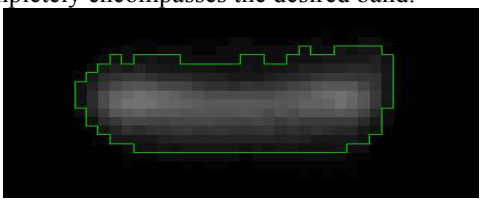


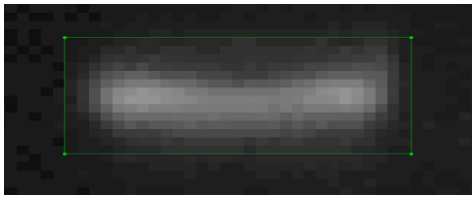
3) Click on the "Draw a box and expand the image inside" Icon 
Draw a box around first set of concentration standards to expand.



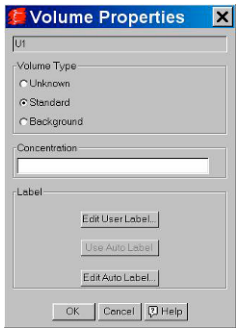
4) Click the "Contour" Icon  . The following sub-Icon box appears.

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.





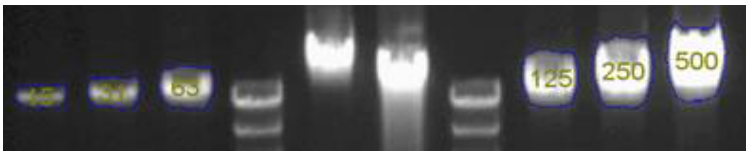
For older versions of the software the contour tool is not available. Use the "Volume Rectangle Tool" Icon  *instead.*



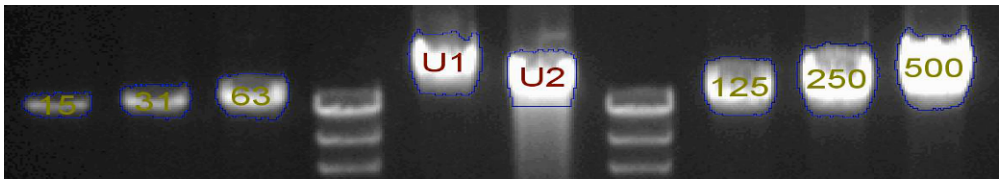
6) Place cursor in center of the bounding area and double click. The "Volume Properties" box will appear.

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.

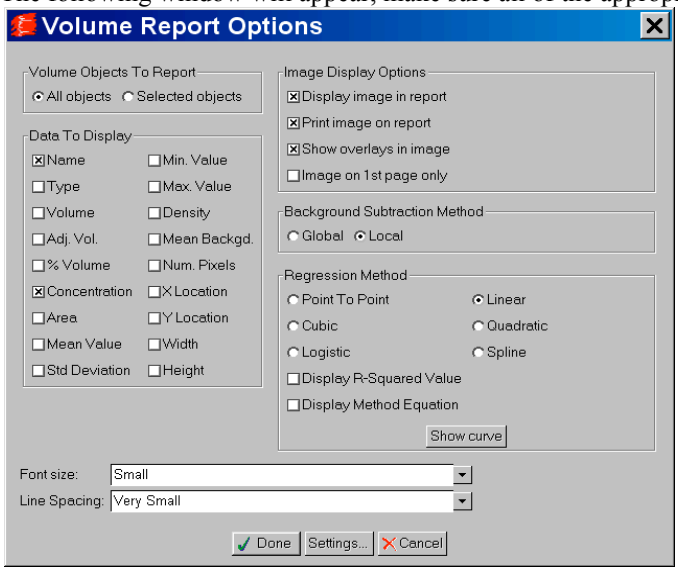


7) After all of the concentration standards have been assigned, repeat Steps #5 & #6 for the unknown samples. Make sure "Unknown" is selected. Remember when outlining the "Unknown samples" ONLY select the high molecular weight section for analysis.

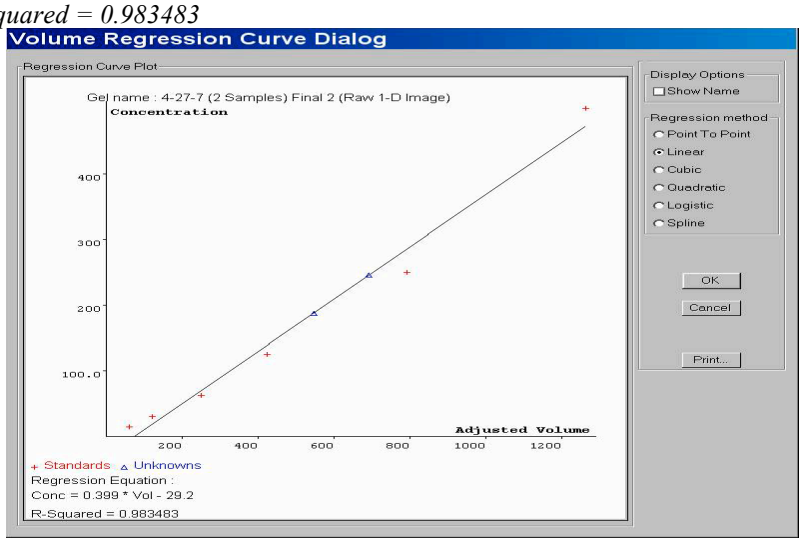


8) Click on the "Display Volume Report" Icon in the sub-Icon toolbar  .

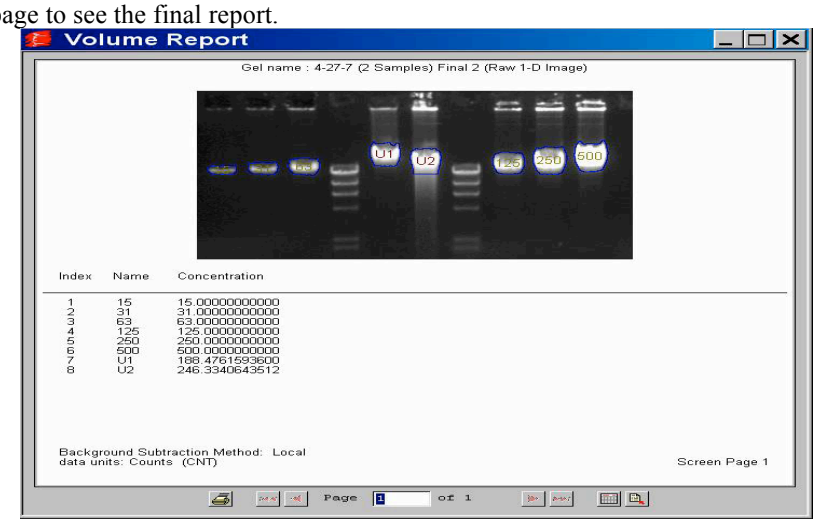
9) The following window will appear, make sure all of the appropriate boxes are selected.



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

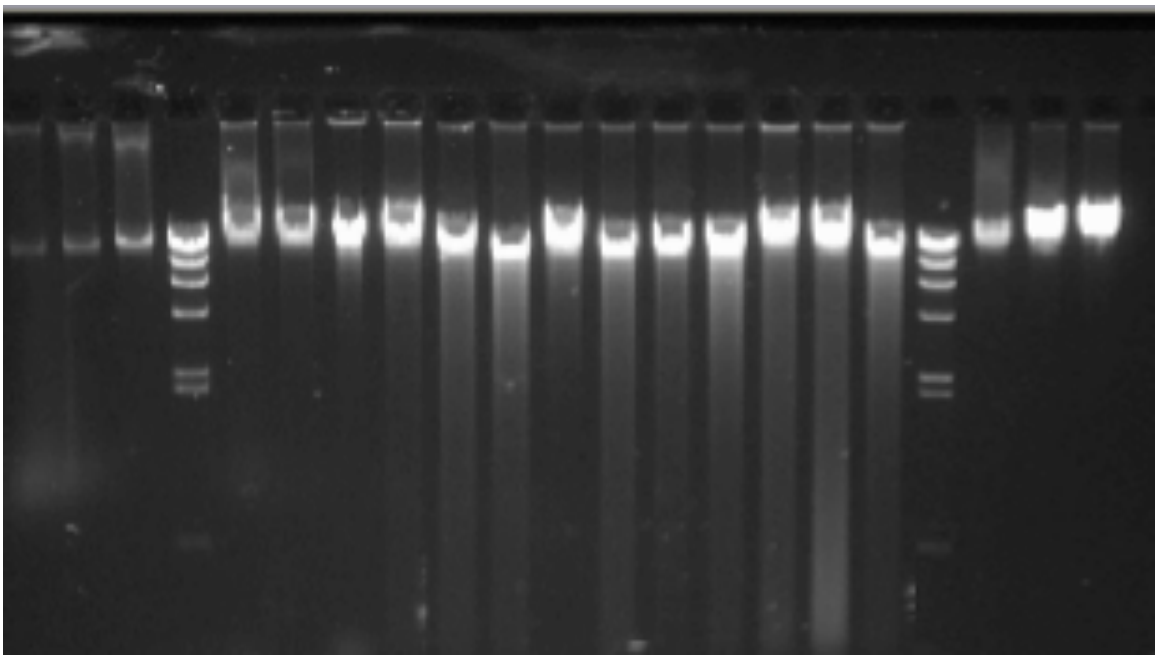


Figure 1: Example of DNA samples that have a tight band with minimal smearing and have molecular weight greater than 23kb.

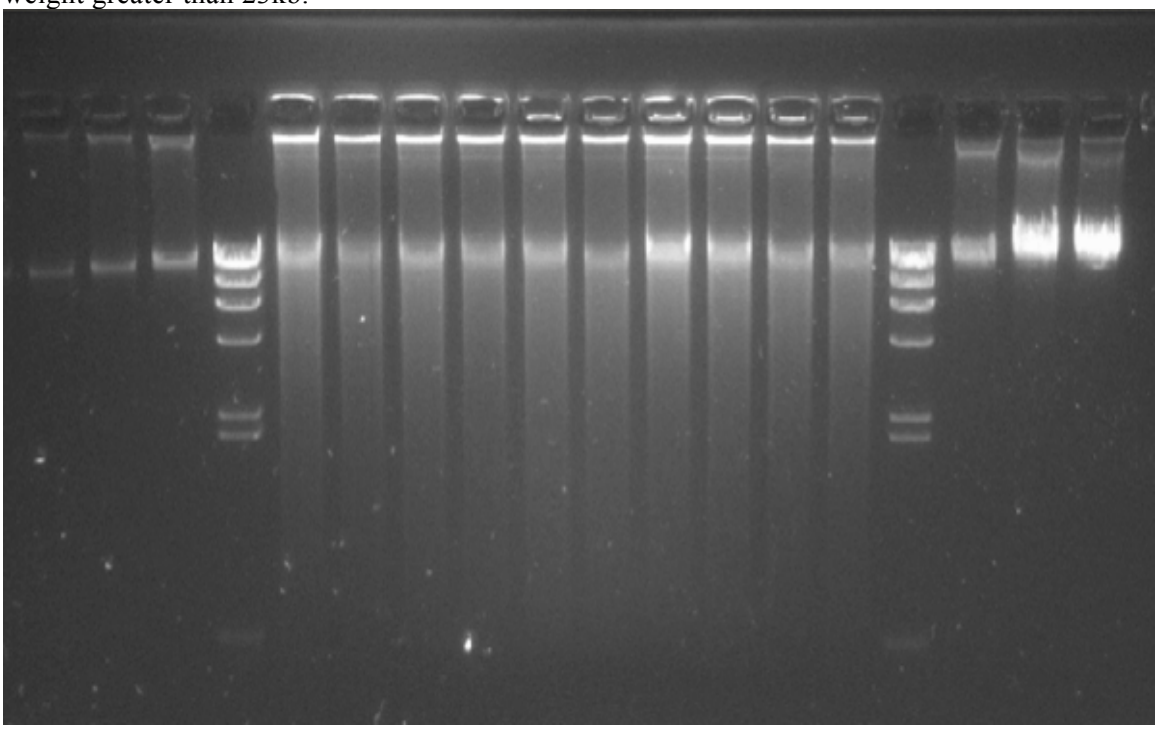


Figure 2: Example of MDA DNA product which has some smearing present but most of the DNA has a molecular weight at 23kb.

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

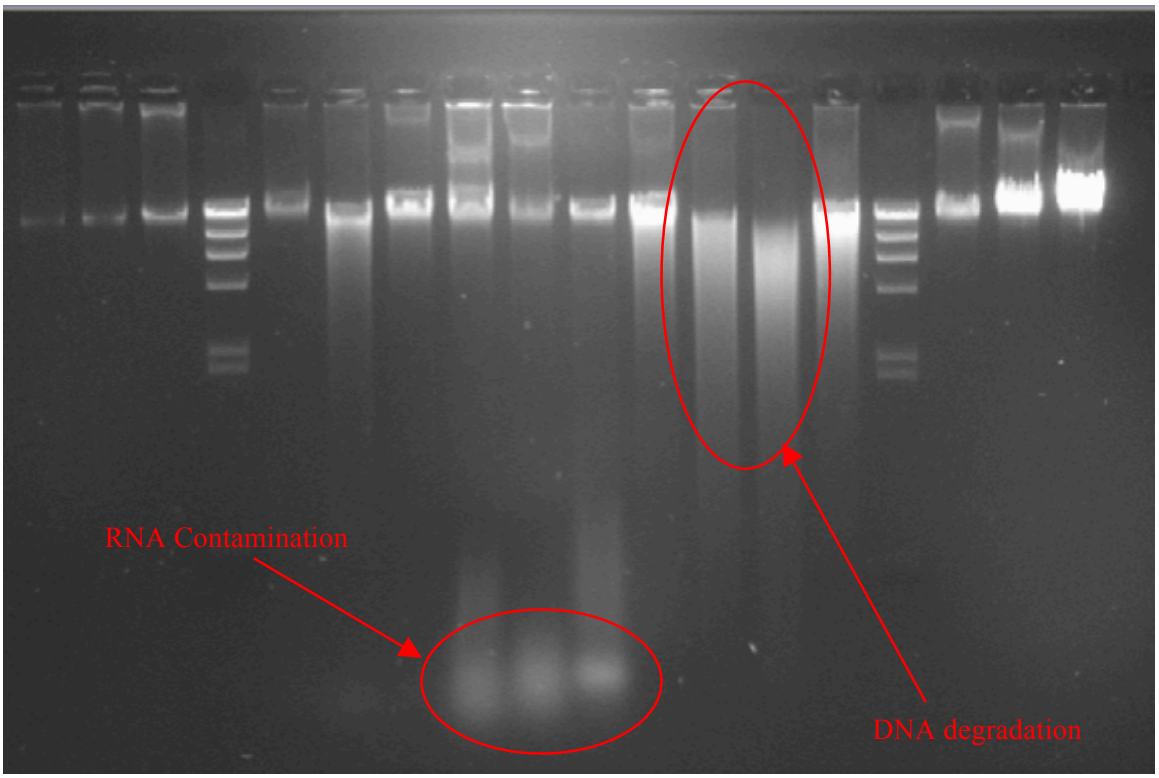


Figure 1: Example of DNA samples with RNA contamination and signs of DNA degradation.

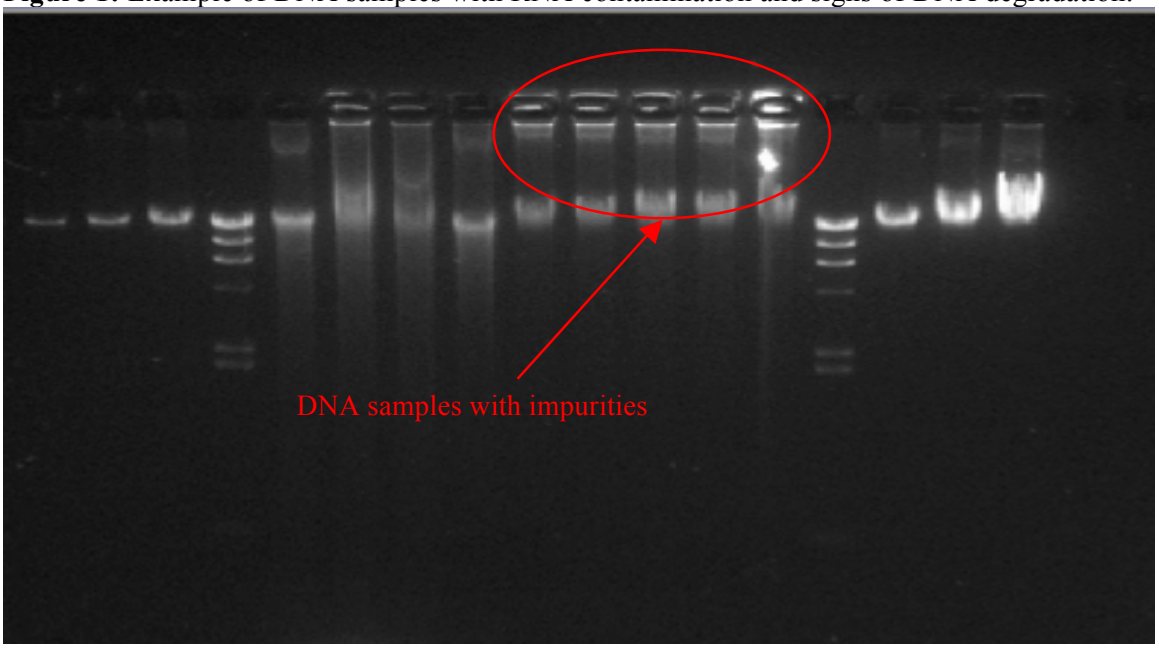


Figure 2: Example of DNA samples with impurities (proteins and polysaccharides that can inhibit chemical reactions during library construction).