DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

2800 MITCHELL DRIVE / WALNUT CREEK / CA 94598
www.jgi.doe.gov

Evaluation of Genomic DNA Prepared for Cloning at the DOE Joint Genome Institute

In this package, you have received the following items:

- **DNA Standard Kit (7 tubes):**

  1 Lambda HindIII Size Standard (Marker 2) – 50ul

  6 Lambda DNA Mass Standards – 25ul of each
  (15, 31, 63, 125, 250, 500 ng/ 5ul)

  Store kit at -20 °C upon receipt.

- **Protocol for Genomic DNA QC Using Gel Electrophoresis**

  Following your evaluation of the sample(s) on the QC gel, the Collaborator Sample Information (CSI) form must be completed and submitted on-line (https:// my.jgi.doe.gov/csi/user/login). A well-labeled gel image of your DNA prep in TIFF format is required as part of the submission. Please do not send jpg, pdf, compressed files or files containing more than 3 samples per pixel. The quantification software used at the JGI can't import gel images with the above file types. Also, if gels are overexposed or not run the right distance, as indicated in the protocol, the software will have difficulties quantifying accurately.

The DNA should not be shipped until approval is received from your project manager.

Please contact your project manager with any questions.

Genomic DNA QC Using Gel Electrophoresis

Version Number: 3.0
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Author(s): Eileen Dalin
Reviewed/Revised by: Jan-Fang Cheng, Victor Dorsett

Summary

Utilizing the concentration and size standards provided by the JGI, run an 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		
Reagents		
Marker 2 Lambda HindIII Size Standard (~50ng/ul)	MBI Fermentas	SM0101
DNA MassStandards (Lambda DNA) 15, 31, 63, 125, 250, 500ng / 5ul	JGI (in house)	
GenePure LE Agarose (Generates)	ISC BioExpress	E-3120-500
Ultra Pure Ethidium Bromide (10mg/ml)	Invitrogen	15585011
1X Loading Dye		
50X TAE Buffer	Invitrogen	24710-030
Equipment		
12X14 Horizontal Device Comb (25 well 1.5mm)	CLP	75.1214-MT-25D
12X14cm Horizontal Gel Electrophoresis Device	CLP	75.1214

Procedure

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

1. **Gel & Sample Preparation**

   1.1 Pour one ~100 ml 1% agarose gel containing 1X TAE and ethidium bromide (.15ug/ml). Use a narrow well comb.

   1.2 Transfer 1ul of your genomic DNA sample(s) into clean tube(s).

a. Use more if the genomic DNA concentration is thought to be low <50ng/ul.

b. Dilution of the genomic DNA might be required if the concentration is thought to be >500ng/ul.

1.3 Bring the sample volume up to 5ul with 1X loading dye.

1.4 Mix & spin down sample tube(s).

2. Gel Electrophoresis

Note: Examples of gels displayed in Appendices 1 & 2

2.1 Loading of the gel:

a. Well 1 - 5ul of 15ng standard

b. Well 2 - 5ul of 31ng standard

c. Well 3 - 5ul of 63ng standard

d. Well 4 - 3ul of Marker 2

e. Well 5 – 5ul of DNA sample

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

f. Well 6 - 3ul of Marker 2

g. Well 7 – 5ul of 125ng standard

h. Well 8 – 5ul of 250ng standard

i. Well 9 – 5ul of 500ng standard

2.2 Run gel for ~40 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.

2.4 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 access size)

i. Refer to Marker 2 diagram on Appendix 1. Is the genomic DNA high molecular weight? Fosmid libraries require DNA >40kb in size. Since the regular agarose gel does not have the resolution in the 40kb size range, you should check if DNA band is at least

above the 23kb band. The JGI will run a PFG to better determine the DNA fragment sizes if needed.

b. **QUANTITY**

i. Compare genomic DNA band with 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. Do NOT use nanodrop readings for concentration estimate because they tend to over estimate the DNA concentration if the sample is not super pure.

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 appear 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://www.jgi.doe.gov/sequencing/collaborators/index.html.

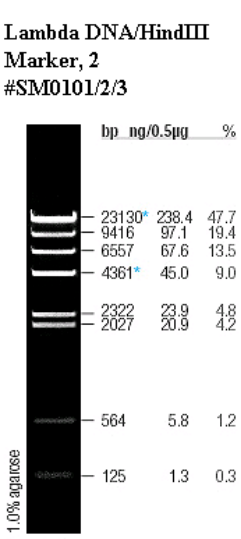
Note: Examples of good and bad DNA samples are displayed in Appendix 3

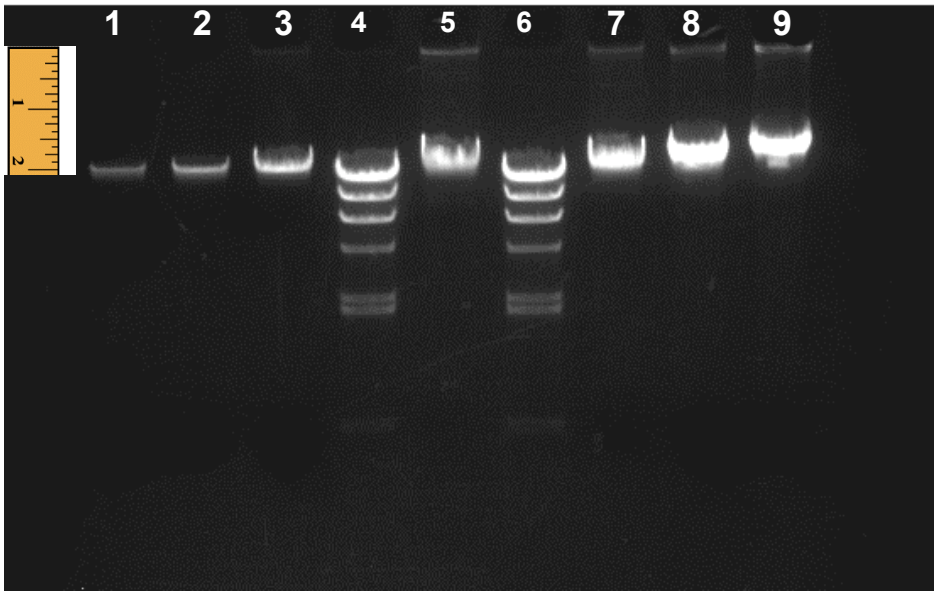
Reagent/Stock Preparation

6X Loading Dye
75ml 100% glycerol
125ml Nuclease free H_20
0.05g Bromphenol Blue
0.05g Xylene Cyanole FF

1X Loading Dye
400ul Nuclease-free H_20
100ul 100% glycerol
100ul 6X loading dye

Appendix 1:



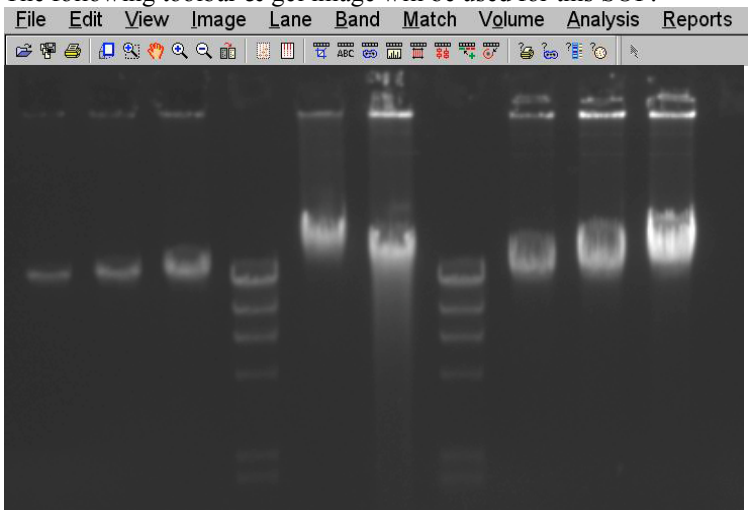


Lane 1: 15ng standard (5ul)
Lane 2: 31ng standard (5ul)
Lane 3: 63ng standard (5ul)
Lane 4: Marker 2 (5ul)
Lane 5: DNA sample (1ul loaded: 300ul total volume)
Lane 6: Marker 2 (5ul)
Lane 7: 125ng standard (5ul)
Lane 8: 250 ng standard (5ul)
Lane 9: 500ng standard (5ul)

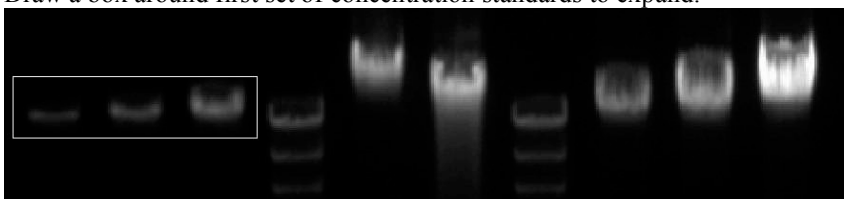
Appendix 2:

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.

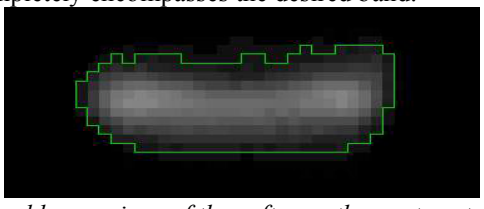


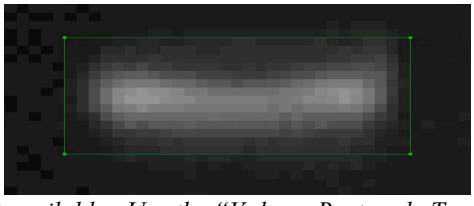
3) Click on the "Draw a box and expand the image inside" Icon 
Draw a box around first set of concentration standards to expand.



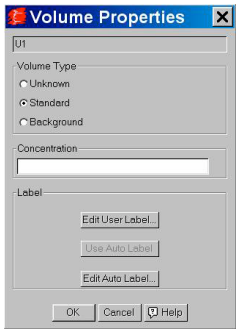
4) Click the "Contour" Icon  . The following sub-Icon box appears.

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.





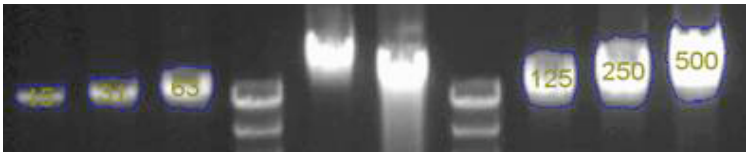
For older versions of the software the contour tool is not available. Use the "Volume Rectangle Tool" Icon  *instead.*



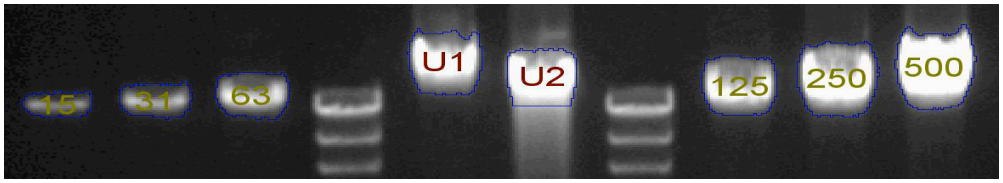
6) Place cursor in center of the bounding area and double click. The "Volume Properties" box will appear.

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.

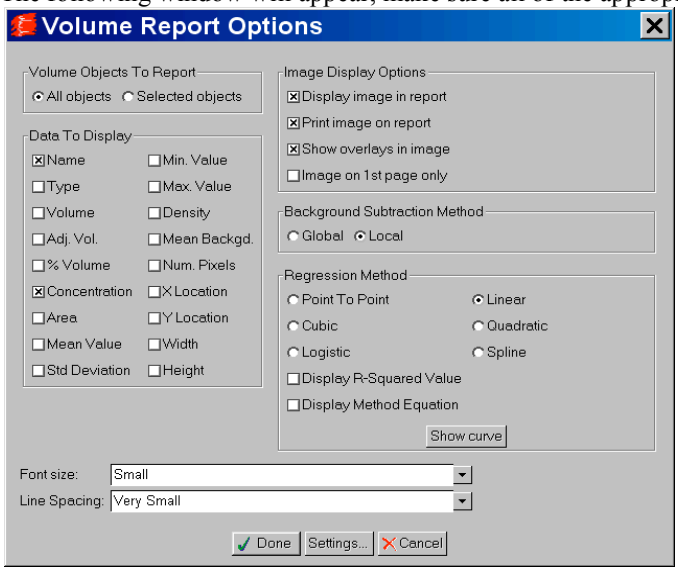


7) After all of the concentration standards have been assigned, repeat Steps #5 & #6 for the unknown samples. Make sure "Unknown" is selected. Remember when outlining the "Unknown samples" ONLY select the high molecular weight section for analysis.

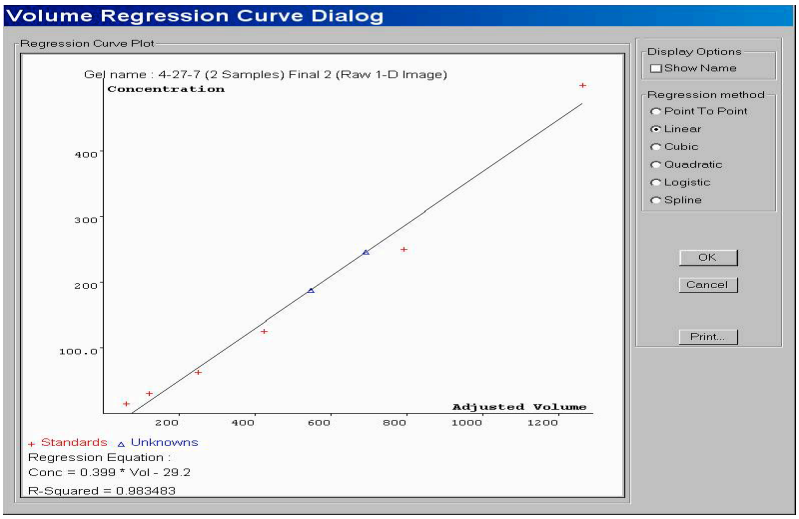


8) Click on the "Display Volume Report" Icon in the sub-Icon toolbar  .

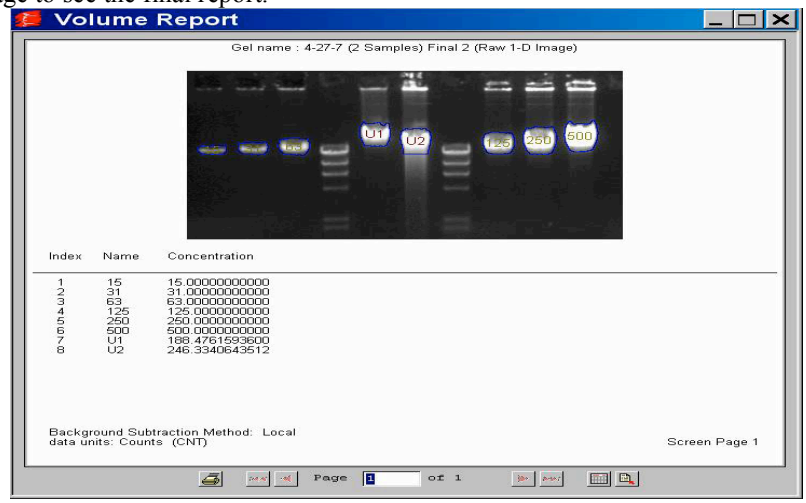
9) The following window will appear, make sure all of the appropriate boxes are selected.



10) Click on the "Show Curve" button to display graphic plot of the standers & samples. Standers 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*



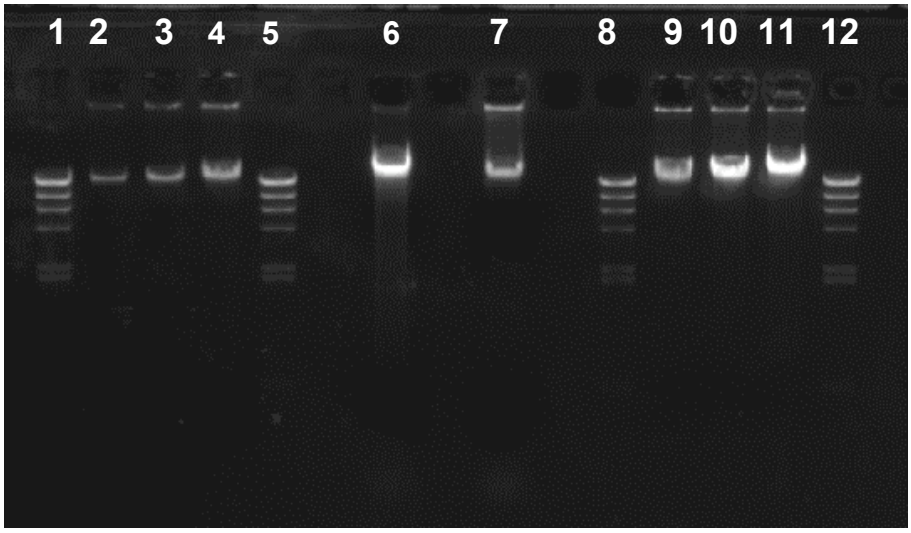
11) Click on the "OK" button. Page returns to "Volume Report Options" displayed in Step #9. Click the "Done" button on that page to see the final report.



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.

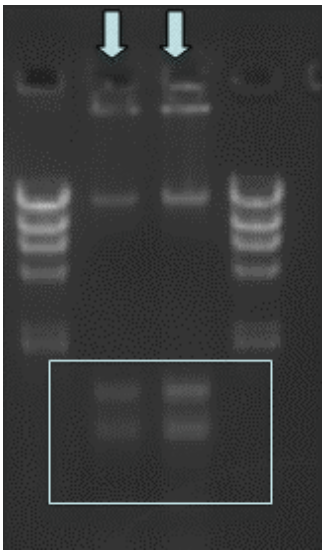
13) Determine the total "**Quantity**" for each sample in "**ug**".
Sample #1) 188ng/ul * 500ul (total volume of sample) = **94,000ng Total (94ug)**
Sample #2) 246ug/ul * 250ul (total volume of sample) = **61,500ng Total (61.5ug)**

Appendix 3:
Example of a good QC gel (both size and mass standards are included)



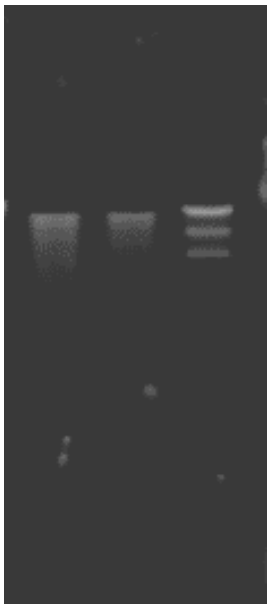
Lane 1: Marker 2 (3ul)
Lane 2: 15ng standard (5ul)
Lane 3: 31ng standard (5ul)
Lane 4: 63ng standard (5ul)
Lane 5: Marker 2 (3ul)
Lane 6: Organism #1 (1ul loaded: 200ul total volume)
Lane 7: Organism #2 (1ul loaded: 350ul total volume)
Lane 8: Marker 2 (3ul)
Lane 9: 125ng standard (5ul)
Lane 10: 250ng standard (5ul)
Lane 11: 500ng standard (5ul)
Lane 12: Marker 2 (3ul)

Appendix 3:
DNA that Failed JGI DNA QC: RNA, Bad Quality, LMW



RNA contamination present, can treat with RNAse I to remove

DNA degraded and/or sheared



MW of DNA must be >40kb if a fosmid library is needed

DNA passed JGI DNA QC: HMW, No RNA, Good Quality, Sufficient Quantity

Some smearing present, but bulk of DNA >23kb band