Effects of Lysis Time and EDTA on Genomic DNA Prepared for Sequencing

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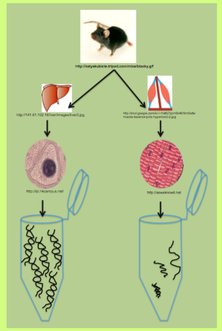
Introduction

Justification



For full genome sequencing, genomic DNA (gDNA) must be of high yield and high molecular weight.

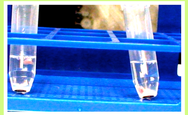
Due to the size preference in fosmid libraries, the ideal yield should be around 0.9-1.0 µg DNA/mg tissue and the ideal length should be ≥38 kilobase-pairs (kbp) long.

- *Yield* is the concentration of DNA (how much double stranded DNA is in the sample)
- *Molecular weight* measures the length of dsDNA in bp.

Background

The current protocol for genomic DNA extraction used in the Genome Sequencing Sample Repository (GSSR) results in very high quality of gDNA. Tissue is lysed overnight (17 hours) using a concentration of 30mM EDTA. There are several factors that effect quality of the resultant DNA:

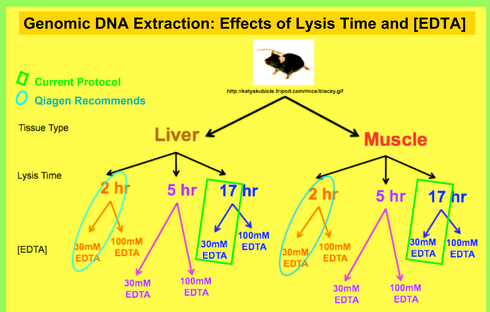
- Length of lysis time
- EDTA concentration
- Tissue type/amount



Muscle tissue samples in lysis

Project Goal:

Determine whether the current GSSR protocol results in the best gDNA quality

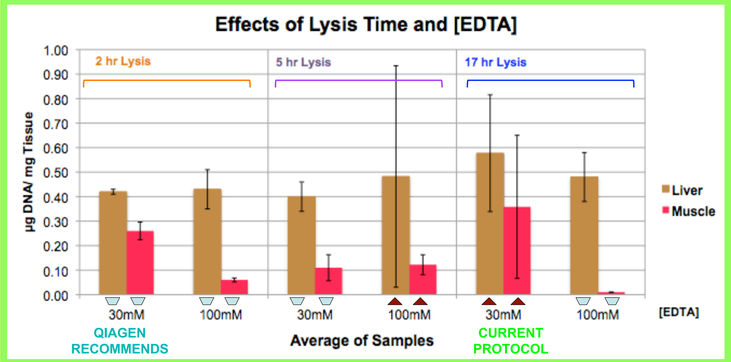


Questions to Answer

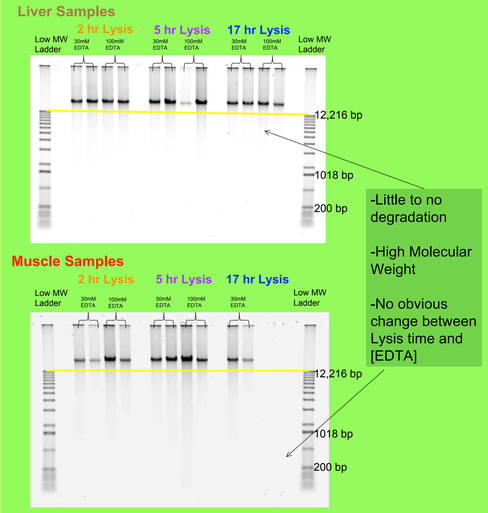
1. Are there effects of different **lysis times** on DNA yield and MW?
2. Are there effects of different **EDTA concentrations** on DNA yield and MW?
3. Is there a best combination of **lysis time** and **EDTA concentration** for all tissues?

Results

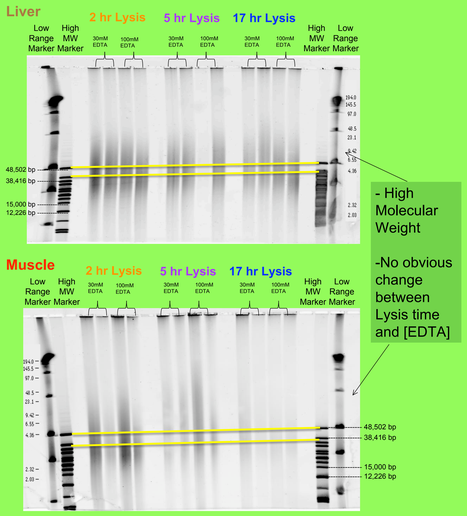
DNA Yields Measured by PicoGreen



2hr Gel Electrophoresis

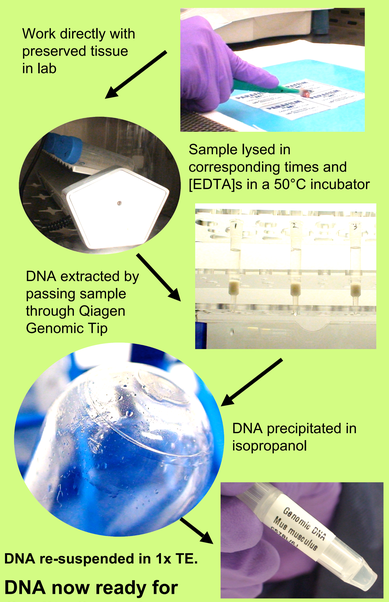


Pulse-Field Gel Electrophoresis



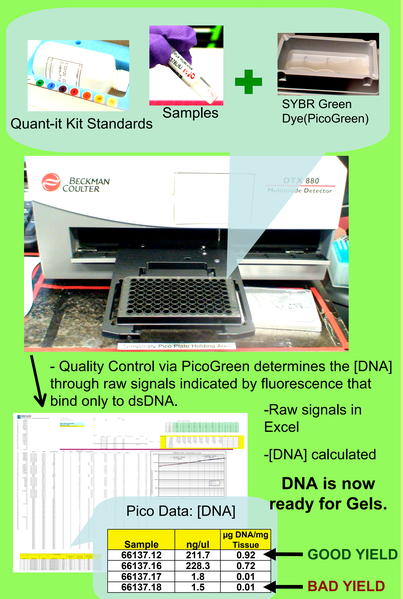
Methods

DNA Extraction

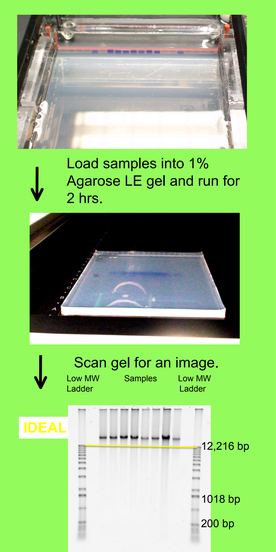


Quality Control

DNA Yields Measured by PicoGreen

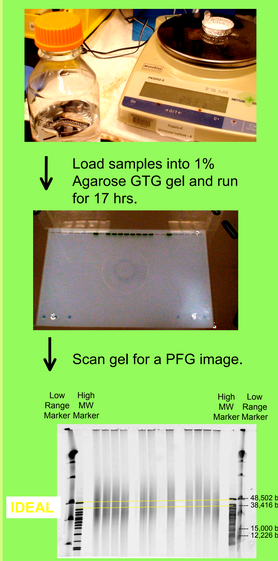


2hr Gel Electrophoresis



* Note: Intensity of the band is not considered when analyzing 2hr gels for size of DNA per base pairs. Position is the important factor since all samples were standardized to have the same amount of DNA loaded in each well.

Pulse-Field Gel Electrophoresis



Conclusions

1. **Lysis time** appears to only affect the yield of gDNA from **muscle tissue.** In **liver tissue**, the **current protocol** (17 hour, 30mM EDTA), shows little difference when compared to other conditions.
2. **EDTA concentration** is not a significant factor for **liver tissue** gDNA quality. However, 30mM EDTA for **muscle tissue** appears to result in better gDNA quality when compared to 100mM EDTA.
3. **There is no best combination of lysis time and [EDTA]** that applies to all tissue types. However, there are trends in the resulting yields from each condition:

- **The current protocol** results in the best yields, although there is a lot of variation between individual samples' yields.
- **The Qiagen recommended protocol** results in high yields as well, although not as high as the **current protocol**. However **the Qiagen recommended protocol** results in the least variable yield between samples.

Other conclusions:

- **Liver tissue** always yields more gDNA compared to **muscle tissue,** as expected.

- **Liver tissue** appears to be less sensitive to changes in lysis time and EDTA concentration than **muscle tissue.**

- The current protocol yields sufficiently high quality gDNA for fosmid library construction.

Acknowledgements

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