

Characterization of a HighThrough-Put, Consistent High Yield, Contaminate Free Genomic DNA Isolation Method

TIM HODGE, NATHAN NOWAK AND JEAN WOLAVER

Many sophisticated molecular biology techniques demand contaminate-free genomic DNA (gDNA). Although there are many methods available to isolate gDNA from tissue, we sought to develop a protocol that produces high yields, is free of contaminants, is consistent from sample-to-sample, is cost effective, and is suitable for a high through-put format. To demonstrate that we have developed such a method we conducted a series of experiments in which we used the proprietary Transnetyx protocol and compared it to standard organic solvent and filter-based isolation techniques using mouse tissue as a source of gDNA. We demonstrate that our method fulfills these requirements and produces high quality gDNA in a high-throughput process.

INTRODUCTION

Genomic DNA is routinely extracted from a wide variety of common biological materials including cells from cultures and tissues, as well as biological liquids such as blood, semen, urine and lymph. This high molecular weight DNA is subsequently used in a large number of molecular biology techniques that include polymerase chain reaction (PCR), southern blotting, northern blotting, restriction analysis, cloning, sequencing, reverse transcription and microarrays. These molecular applications and techniques are often sensitive to trace amounts of contaminants that inhibit, block or compete with the reaction of interest. For example, contaminating proteins, polysaccharides, lipids, oligonucleotides and RNA can interfere with various biological assays. Contamination may also be present from prior molecular techniques or the very techniques used to isolate the gDNA. These may include agarose, polyacrylamide, trace metals such as iron oxide,

dyes, organic solvents, detergents or solution contaminants resulting from amplification techniques such as salts, enzymes or oligonucleotides. Often these contaminants are difficult to detect using the simple spectrophotometry methods generally available in most laboratories.

Historically, two gDNA isolation techniques have been adopted to isolate high molecular weight DNA free of contaminants; organic solvent extraction and filter isolation. The gold standard in gDNA isolation has been to use strong organic solvents, such as phenol and chloroform, to solubilize the protein and lipid constituents of the lysate. This is followed by removal of the organic solvents and precipitation of the DNA with alcohol or ether. Even though this is the most commonly used extraction method, it has many drawbacks. Phenol is a strong organic acid that is highly corrosive and can cause severe burns.

Additionally, it is highly toxic if inhaled, ingested, or absorbed through the skin. Chloroform and ether are also irritants to the skin, eyes, mucosal membranes, and respiratory tract and are carcinogenic, volatile, flammable and toxic. It has been shown that these chemicals can cause liver and kidney damage. Standard safety practices dictate that gDNA isolation by this method be carried out under a fume hood. Safety aside, phenol/chloroform extractions are very labor intensive, not amenable to automation and are centrifuge dependent. If one considers consumable cost, chemical cost, researcher's time, and laboratory overhead it is clear that phenol/chloroform extractions are not cost effective.

To combat the obvious inconvenient and caustic nature of organic solvents, newer, less noxious DNA isolation methods have been developed. Specifically, commercially available filters manufactured with embedded particles of silica or glass mixtures have been designed to selectively bind gDNA in cell lysates exposed to high salt concentration. Unfortunately, filter based isolations are expensive, labor intensive, and rely on centrifugation. To combat the low throughput and centrifugation dependency, some well funded facilities have embraced automated liquid handlers and vacuum manifold systems to carry out gDNA isolations. This approach adds tens-of-thousands to hundreds-of-thousands of dollars in cost making it impractical for the typical laboratory.

METHODS

We have developed an automated extraction protocol that consistently yields high-quality contaminant-free gDNA. To determine how well our protocol works we have compared DNA purified using this method to standard phenol/chloroform extracted gDNA, Qiagen DNeasy extracted gDNA, and two commercially available mouse gDNA preparations.

Transnetyx extraction

One thousand fifty two 0.5cm mouse tail segments of differing mass were cut, lysed, pooled and processed through the automated Transnetyx proprietary gDNA isolation system. The concentration and consistency of sample preparation was measured by A_{260} spectrophotometry. The gDNA was evaluated for protein contamination using a Pierce Biotechnology's microBCA kit (catalog number 23235) and measured with A_{595} spectrophotometry.

Phenol/chloroform extraction

Eight 0.5cm mouse tail segments differing in mass were cut, lysed, pooled and processed at Transnetyx using the method described in Sambrook, J. *et al.* (2001) *Molecular Cloning: A Laboratory Manual*, 3rd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 6.24. The gDNA was quantified using A_{260} spectrophotometry. The gDNA was evaluated for protein contamination using a microBCA kit.

Qiagen DNeasy extraction

Eight mouse tail segments measuring 0.5cm and differing in mass were cut, lysed, pooled and processed at Transnetyx using the method described in Qiagen's DNeasy® Tissue Kit (catalog number 69504). The gDNA was quantitated using A_{260} spectrophotometry. The gDNA was evaluated for protein contamination using a microBCA kit.

Commercial Mouse Genomic DNA preparations

Six replicates of commercial Promega and Clontech gDNA were analyzed. The Promega DNA was isolated using the method described in Sambrook, J. *et al.* (1989) *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 9.14. One hundred microliters were taken from each commercial preparation and evaluated for protein contamination as well as DNA concentration. The gDNA was quantified using A_{260} spectrophotometry and evaluated for protein contamination using a microBCA kit.

RESULTS

The quality of the gDNA produced by each of these isolation methods was quantified in terms of amount recovered and the consistency of the amount recovered. The gDNA concentration, the standard deviation of the concentration and gDNA total yield were measured. Additionally, the quality of the gDNA product was also evaluated in terms of protein contamination. The amount of contaminating protein and the standard deviation of the protein was measured for each isolation method. The greatest concentration of gDNA with the least amount of protein contamination is optimal.

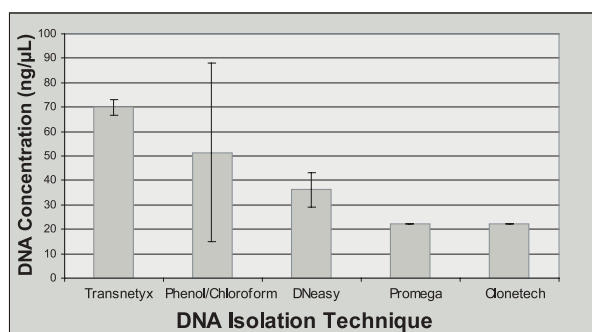


Figure 1. The average Genomic DNA concentrations from different isolation techniques.

All the mouse tail samples that were processed through the various methods yielded high molecular weight gDNA. The Transnetyx method had the highest average gDNA concentration and total yield as determined by A_{260} spectrophotometry. Additionally the Transnetyx isolation method also has the smallest standard deviation with respect to the gDNA concentration. The Phenol/Chloroform isolations also produced high gDNA concentrations and high total yields of gDNA. However, with respect to the gDNA concentration the Phenol/Chloroform extractions produced a very high standard deviation. There is an 11-fold increase in the standard deviation between the Phenol/Chloroform method and the Transnetyx isolation method.

The level of protein contamination from each mouse tail isolation technique was quantified

using Pierce Biotechnology's microBCA kit. The Phenol/Chloroform method had both the highest concentration of contaminating protein and the largest standard deviation of protein. The DNeasy and Transnetyx methodologies had very similar protein concentrations and standard deviations. The Transnetyx method had a slightly lower protein concentration while having a slightly higher standard deviation. The Promega commercial mouse gDNA had the lowest concentration of protein, although no standard deviation could be calculated since the gDNA was purchased in a single tube.

	Lysate Volume (μl)	Sample	Protein Conc (ng/μl)	Std Dev (ng/μl)
Transnetyx	150	288	11.5	8.2
Phenol/Chloroform	150	8	21.8	12.7
Dneasy	150	8	12.8	7.6
Promega	150	1	1.8	**
Clonetech	150	1	8.1	**

Table 1. The average protein contamination concentrations from different isolation techniques.

CONCLUSION

The ability to isolate high quality contaminant-free genomic DNA is critical to biomolecular research. Unfortunately, the widely used methods for gDNA extraction are either hazardous to laboratory personnel (phenol/chloroform extraction) or expensive (silica based filtration methods). We have developed a novel, high-throughput gDNA extraction and purification protocol at Transnetyx to facilitate our genotyping process. We show here that the gDNA produced using this method is high in quality and yield and low in protein contamination. Furthermore, subsequent Transnetyx assays using gDNA produced with our isolation protocol are routinely greater than 99.9% successful. The consistent extraction of high quantity and quality gDNA has enabled the successful downstream processing of thousands of individual samples from different investigators in the United States and abroad. This isolation method has allowed us to develop a robust, high-throughput, genotyping capability.



8110 Cordova Road, Suite 119
Cordova, TN 38016
www.transnetyx.com
901-507-0476 Direct
888-321-2113 Toll Free