

Dominic O’Neil, Stefanie Schröer, Tanya Sperling, Markus Sprenger-Haussels
 QIAGEN Strasse 1, 40724 Hilden, Germany

Introduction

Genetic and genomic analyses from tissue samples require high-quality DNA. Mechanical disruption methods, such as bead milling provide a high yield from tissue samples, but cause damage to nucleic acids. Purely enzymatic methods such as proteinase K digestion can extract nucleic acid without damage, but require long incubation times, and without approaching the yields achieved by mechanical disruption techniques. Therefore, a method is needed which can provide rapid purification of high-yield, high-quality DNA from tissue samples.

Method

The QIAamp Fast DNA Tissue Kit combines mechanical, chemical and enzymatic disruption of tissue samples to provide rapid, high-yield DNA purification – without significant damage to the nucleic acid. Tissue is mechanically disrupted by vortexing with a specially shaped milling bead. The lower power of a vortexer compared to a bead mill prevents DNA shearing. Low chaotropic conditions support mild sample lysis and provides synergy for the simultaneous proteinase K digestion and mechanical shearing.

Results

DNA purified from fresh-frozen and stabilized tissue samples using the QIAamp Fast DNA Tissue Kit showed up to a 10-fold increase in yield compared with standard proteinase K digestion or mechanical milling alone. Disruption was complete in 15 minutes for soft tissues (e.g., liver, kidney), and within 30 minutes for tougher tissue (e.g., stabilized lung, trachea samples). DNA was also efficiently purified from fatty tissues (e.g., brain) and fibrous tissues (e.g., muscle).

Summary

The QIAamp Fast DNA Tissue Kit enables superior DNA purification from tissue compared with existing methods. Technologies including next-generation sequencing make the purification and analysis of DNA from tissue samples increasingly important. Therefore, a method that provides convenient and efficient purification of DNA supports the routine genomic analysis facilitated by these technologies.

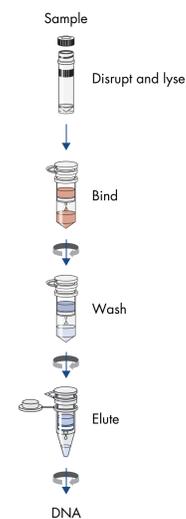
Combined Enzymatic, Chemical and Mechanical Tissue Lysis

Tissue samples are added to tubes containing specially shaped beads that homogenize tissue during mechanical agitation. Mechanical lysis proceeds in a gentle chaotropic solution, in which the components for enzymatic digestion are already present. The chaotropic buffers denature proteins to make them more accessible to proteinase K. Synergistically, proteinase K digestion softens tissue, making it more susceptible to mechanical disruption. The mechanical disruption in turn liberates additional material for the chaotropic salts and proteinase K. The three components together provide rapid tissue homogenization.

The method was developed for various downstream applications, including (q)PCR and next-generation sequencing.



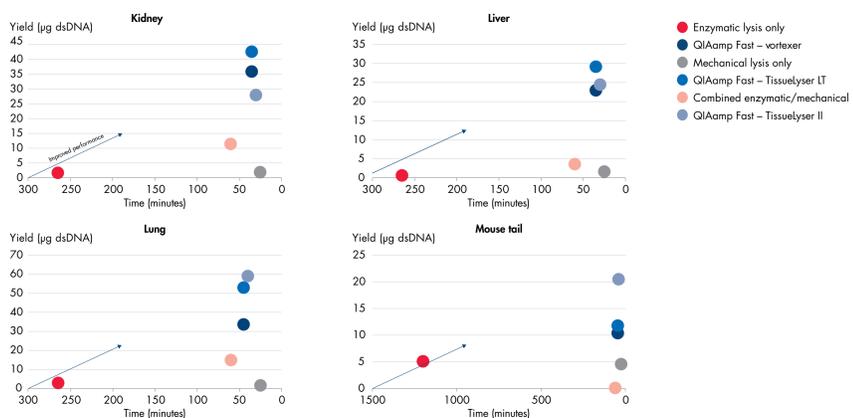
Bealco beads for gentle tissue disruption.



QIAamp Fast DNA Tissue procedure.

Combination Lysis Enables Rapid, High-Yield DNA Purification

DNA was purified from 15 mg of stabilized rat kidney, lung and liver and 1 cm fresh-frozen mouse tail tip. The QIAamp Fast DNA Tissue Kit was compared with spin-column-based purification kits from other suppliers (with solely mechanical or enzymatic lysis or a less optimized combination of mechanical and enzymatic homogenization). In all cases, the QIAamp Fast DNA Tissue Kit gave the best combination of time and yield.

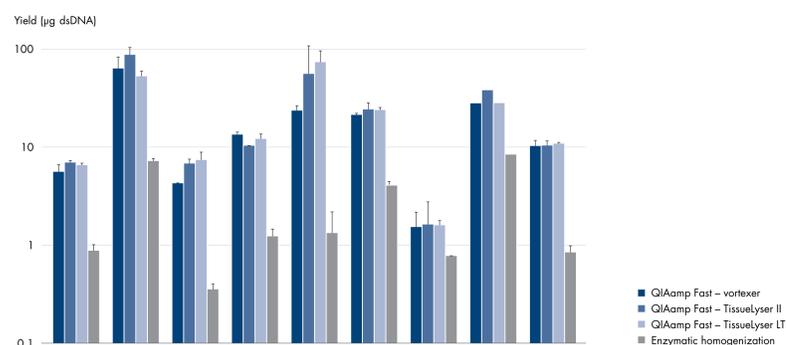


Consistently high yields of DNA and rapid purification times with the QIAamp Fast DNA Tissue Kit.

Efficient Purification from Tough, Fibrous or Fatty Tissues

Rat and mouse tissues – including fatty tissue (e.g., brain), fibrous tissue (e.g., heart) and tough tissue (e.g., trachea) were processed using the QIAamp Fast DNA Tissue Kit in comparison to kits using enzymatic homogenization alone.

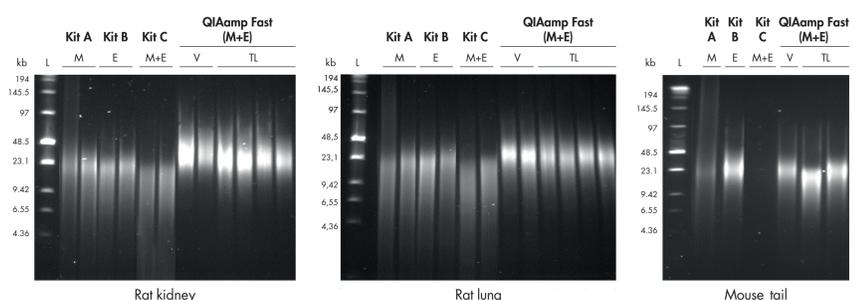
Yields (determined by dsDNA-specific assays) of all tissues were up to 10-fold higher using the QIAamp Fast DNA Tissue Kit. The QIAamp Fast DNA Tissue Kit can be used with vortexers, the Tissuelyser LT or Tissuelyser II with comparable performance.



Up to 10-fold higher yields with the QIAamp Fast DNA Tissue Kit, compared with enzymatic lysis alone.

DNA Quality is Improved With Combination Lysis

DNA was purified using either the QIAamp Fast DNA Tissue Kit or methods from other suppliers. DNA quality was analyzed using pulsed-field gel electrophoresis (PFGE). DNA purified using the QIAamp Fast DNA Tissue Kit showed highest integrity. The speed of purification preserves DNA integrity compared with enzymatic digestion alone, while the comparatively gentle mechanical agitation improves DNA integrity over other mechanical homogenization methods.



High-quality DNA with the QIAamp Fast DNA Tissue Kit. Genomic DNA was purified from 15 mg stabilized rat kidney and lung and 1 cm fresh-frozen mouse tail tip using either the QIAamp Fast DNA Tissue Kit (with vortex, Tissuelyser II or Tissuelyser LT) or a spin-column-based kit (with solely mechanical or enzymatic or a combination of mechanical and enzymatic homogenization). Equal amounts of DNA were analyzed using PFGE, except for the mouse tail sample from Kit C, where due to low yields, the maximum possible amount of eluate was analyzed. E: Enzymatic lysis; M: Mechanical lysis; M+E: Mechanical and enzymatic lysis; TL: Tissuelyser II or LT; V: Vortexer.

Conclusions

The rapid and gentle lysis provided by the the QIAamp Fast DNA Tissue Kit demonstrated superior results compared with existing methods.

- DNA yield was up to 10-fold higher with the the QIAamp Fast DNA Tissue Kit than with other enzymatic and/or mechanical homogenization techniques.
- The QIAamp Fast DNA Tissue Kit has been shown to work with a wide variety of tissue types – including fatty, fibrous and tough tissues.
- DNA integrity (in terms of molecular weight) extracted from tissue is improved with the QIAamp Fast DNA Tissue Kit, compared with methods from other suppliers.
- Since DNA purification is a major step in almost all genetic and genomic applications, the QIAamp Fast DNA Tissue Kit offers a new approach for tissue investigation.

The QIAamp Fast DNA Tissue Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

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