High-throughput DNA purification with DNeasy 96 — more than just mouse tails

Many laboratories rely on the DNeasy™ 96 Tissue Kit for high-throughput purification of DNA from mouse tails. Now researchers are using DNeasy 96 technology to speed screening of other types of cells and tissues. Here we present the work of two laboratories that use the DNeasy 96 Tissue Kit to purify DNA either from mouse liver, kidney, and salivary gland tissues or from mouse embryonic stem-cell cultures.

DNA purification from various tissues for in vivo antiviral studies

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Mouse-infection models are routinely used for testing antiviral substances in vivo. This usually involves infecting mice with a human virus such as human herpes simplex virus or a mouse-equivalent of a human virus, such as murine cytomegalovirus. To measure the level of infection in different organs, tissue samples are generally titered in plaque assays with cultured cells. Such assays are expensive in addition to being time- and labor-intensive. One alternative is to quantify the amount of viral DNA in the tissues using DNA hybridization techniques. The success of this method depends on a simple and reliable procedure for isolating genomic DNA from different organs. For this purpose, the DNeasy 96 Tissue Kit for mouse tails was tested for high-throughput isolation of DNA from three different types of mouse tissues: salivary gland, liver, and kidney.

Materials and methods

The method was based on the Mouse Tail Protocol in the *DNeasy 96 Tissue Kit Handbook*, June 1997, with modifications as described below. To standardize the results and to minimize the effect of animal-specific differences, 10 similar samples of each type of organ from SCID (severe combined immunodeficiency) mice were pooled and cut into small pieces in a tissue-culture dish using a disposable scalpel. From this homogenate 10 samples of each of 10, 15, 20, 25, and 30 mg were prepared, lysed overnight, and treated with RNase A as described in the protocol. Following addition of Buffer AL/E, the samples were immediately vigorously mixed, loaded into the wells of the DNeasy 96 plate, and centrifuged for 20 min at 6000 rpm (all centrifugation times were doubled relative to those recommended in the standard protocol due to the high viscosity of some samples). The volume of the flow-through was monitored after this step (Table 1). In order to ensure complete removal of Buffer AL/E from the more viscous samples for the purposes of this experiment, a second 20-min spin was performed with all samples. After the DNeasy membrane was washed and dried, the DNA was eluted following incubation for 5 min at 70°C with 200 µl elution buffer. The eluate was reloaded, re-incubated, and re-eluted. DNA yield and purity were then measured spectrophotometrically, and the quality of the DNA was further analyzed on an agarose gel.

Table 1. Percentage of sample volume recovered in flow-through after first centrifugation

	Size of tissue sample				
Tissue type	**10 mg**	**15 mg**	**20 mg**	**25 mg**	**30 mg**
Salivary gland	100	100	100	80–90	30–90
Liver	100	100	100	50	20–30
Kidney	100	100	100	80–90	50

Results

Genomic DNA was successfully isolated from liver, salivary gland, and kidney tissues using the DNeasy 96 Tissue Kit for mouse tails and the modified protocol described above. Previous experiments had shown that yields were greatly reduced when more than 35 mg of tissue was used. Here, sample sizes between 10 and 30 mg were tested. Above 20 mg, reduced flow rates were observed (Table 1), reflecting the higher viscosity of these samples after lysis. This made it necessary to centrifuge twice at each centrifugation step in the Mouse Tail Protocol. ▶

References

1. *Schwarz, H. (1997) Rapid high-throughput purification of genomic DNA from mouse and rat tails for use in transgenic testing. Technical Tips Online (http://www.elsevier.com/locate/tto) T01146.*
2. *Wurst, W. and Joyner, A.L. (1993) Production of targeted embryonic stem cell clones. In: Joyner, A.L., ed.* Gene Targeting: A Practical Approach, *Oxford University Press, Oxford.*
3. *Witzemann, V., Schwarz, H., Koenen, M., Berberich, C., Villarroel, A., Wernig, A., Brenner, H.R., and Sakmann, B. (1996) Acetylcholine receptor ε-subunit deletion causes muscle weakness and atrophy in juvenile and adult mice. Proc. Natl. Acad. Sci. USA* ***93****, 13286.*

Effect of Sample Size on DNA Yield

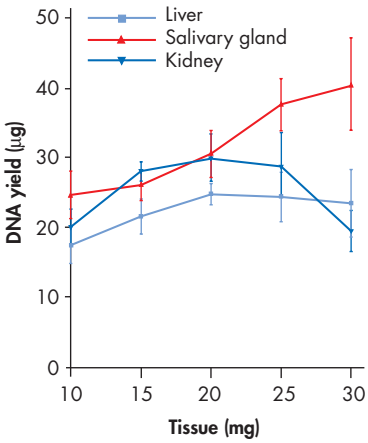

Figure 1 *DNA was purified as described above from tissue samples pooled from 10 mice. DNA yield was determined spectrophotometrically. Each point represents the mean and standard deviation from 10 preparations.*

Effect of Sample Size on DNA Purity

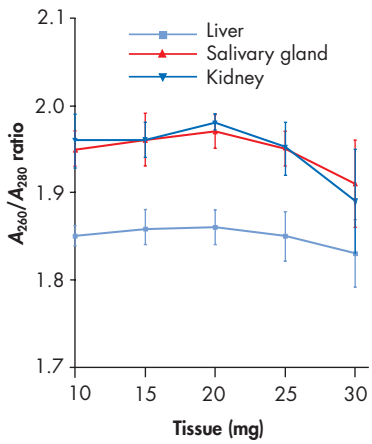

Figure 2 *The DNA purity of the samples described in the legend to Figure 1 was determined spectrophotometrically by measuring the A_{260}/A_{280} ratio.*

Although a slight increase in DNA yield was observed for salivary gland samples up to 30 mg, yields obtained with liver or kidney tissues leveled off or even decreased slightly above sample sizes of 20 mg, respectively (Figure 1). Moreover yield reproducibility decreased in all tissues above 20 mg, as shown by the increasing standard deviations. DNA purity tended to be highest for samples under 20 mg (Figure 2) as well. Figure 3 shows that high-quality DNA was obtained from salivary gland and liver tissues regardless of the amount processed. DNA from kidney tissue exhibited a slight smear. In all cases, the quality of the DNA was satisfactory for hybridization experiments and other downstream applications. The method was also found to yield similarly good results with mouse skeletal muscle and eye tissue (not shown).

In conclusion, maximum DNA purity and yield were obtained using the above protocol with tissue samples of 20 mg or less. Larger samples were more difficult to handle because of their increased viscosity and did not lead to increased yields or purity. We now routinely use 15–20 mg of tissue and have found this sufficient for our needs.

High-Quality DNA from Different Organs

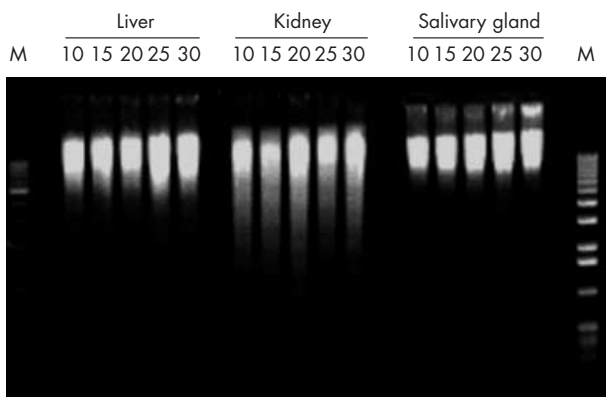

Figure 3 *Agarose gel analyis of 1 µg total DNA isolated from the indicated amounts (mg) of different mouse organs using the DNeasy 96 Tissue Kit for mouse tails.*
M: *1Kb DNA Ladder, Gibco BRL.*

High-throughput screening of embryonic stem-cell clones for a correctly targeted gene

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We use transgenic knock-out and knock-in mice to study the role of different forms of the acetylcholine receptor (AChR) expressed during mammalian muscle development at the neuromuscular junction. Having found the DNeasy 96 Tissue Kit particularly convenient for purification of genomic DNA from rodent-tail biopsies for transgene screening (1), we sought to apply the technique to the screening of the transgenic embryonic stem (ES)-cell lines used to generate the mice as well. Standard methods using crude protease-digested ES-cell extracts (2) in screening procedures are sometimes unreliable, and multi-well cultivation of ES cells easily lends itself to processing in the 96-well format of the DNeasy system.

Materials and methods

ES cells were electroporated with an AChR-ε-subunit–gene-targeting construct containing a neomycin cassette which confers G418 resistance in ES cells. Cells were cultured and selected as described elsewhere (3). Each G418-resistant clone was propagated in three 96-well cell-culture dishes, which were either frozen for later injection into blastocysts, passaged further, or used for DNA preparation using a slight modification of the Mouse Tail Protocol in the *DNeasy 96 Tissue Kit Handbook*, June 1997 as described below.

Cells were lysed directly in the 96-well dish by removing the media and incubating for 1 h at 56°C with 180 µl Buffer ATL and 20 µl Proteinase K. The mixture was resuspended, incubated for a further hour, RNase-digested, and then transferred with a multichannel pipet into a round-well block containing 410 µl Buffer AL/E. The wells were sealed and the block was shaken immediately. The remaining steps were performed as described in the Mouse Tail Protocol, except that the DNA was eluted in a reduced volume of elution buffer (55 µl) to obtain the maximum DNA concentration. A second elution into a fresh tube was carried out with 200 µl elution buffer and kept as a backup.

In this study, PCR was performed with 1µl of each DNA sample. However due to the high variability in ES-clone growth rates, we now routinely use 10–15 µl. One primer used for PCR was complementary to AChR$^{\varepsilon}$ sequences external to the region of homology targeted by the vector and the other was complementary to sequences within the neomycin cassette. Of each 50-µl PCR, 20–30 µl was analyzed on an agarose gel.

For Southern analysis of PCR-positive clones, DNA was extracted from ES cells grown to confluency in 6-well culture plates. Cells were trypsinized and either processed in parallel with tails or 96-well ES-cell samples as described above or processed using individual QIAamp® columns and the Blood & Body Fluid Protocol of the *QIAamp Blood Kit and QIAamp Tissue Kit Handbook* (December 1996). Restriction digestion was performed with 20 µl of the purified DNA.

Results

The modified DNeasy 96 procedure yielded DNA from 192 samples suitable for PCR (Figure 4) in less time than that required by performing time-consuming individual preparations using standard procedures. Of the 192 ES-cell clones screened here, 6 gave positive results after PCR. Southern blot analysis then confirmed that the inserted gene was correctly positioned in 4 clones.

Conclusions

The DNeasy 96 Tissue Kit was successfully used with a variety of sample sources such as stem-cell clones and several types of mouse tissue, yielding DNA that was suitable for downstream PCR and Southern analyses. The convenient and rapid 96-well procedure is ideal for many types of high-throughput screening and monitoring applications. ■

PCR Analysis of ES-Cell Clones

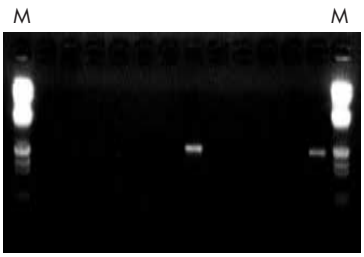

Figure 4 PCR analysis of 1 µl of DNA purified from ES-cell clones using the DNeasy 96 Tissue Kit. A total of 192 samples were processed. 12 are shown here, 2 of which were positive. ***M:*** *lambda–EcoRI-HindIII digest.*

Ordering Information

Product	Contents	Cat. No.
DNeasy 96 Tissue Kits for mouse tails*		
DNeasy 96 Tissue Kit (4)	4 DNeasy 96 plates, Proteinase K and Buffers, Collection Devices	69581
QIAamp Tissue Kits*		
QIAamp Tissue Kit (50)	50 QIAamp Spin Columns, Proteinase K, Reagents and Buffers, Collection Tubes (2 ml)	29304
Accessories		
Plate Rotor 2 x 96	Rotor for 2 DNeasy 96 plates for use with Centrifuge 4-15†	81031
Germany‡: Centrifuge 4-15 (230 V)	Universal laboratory centrifuge with brushless motor (230 V/50 Hz)	81020

** Larger kit sizes available*

† The Plate Rotor 2 x 96 is available exclusively from QIAGEN and its distributors. Under the current liability and warranty conditions, the rotor may only be used in Centrifuge 4-15 or 4K15 from QIAGEN or the freely programmable models of centrifuges 4K15, 6-10, and 6K10 from SIGMA Laborzentrifugen GmbH.

‡ In other countries, please contact your local subsidiary or distributor. Refrigerated version available on request.

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