Rapidly recover intact DNA from blood, bone marrow, and cultured cells

New Protocols for Isolating High-Molecular-Weight Genomic DNA

Brenda J. Rogers • Mark J. Dycaico
Stratagene

Stratagene now offers updated protocols to isolate and purify high-molecular-weight genomic DNA using the RecoverEase™ DNA isolation kit. New procedures include DNA isolation from blood, bone marrow, and tissue culture cells. In addition, protocols for liver, lung, kidney, spleen, testes, and brain are also included. Each procedure eliminates the need for toxic phenol or chloroform and requires very little hands-on time to perform. Use the kit to minimize physical manipulation of the DNA —the point in purification methodology where DNA is most susceptible to shearing forces. Fully hydrated high-molecular-weight DNA is ready to use in molecular biological applications in less than 24 hours.

The RecoverEase DNA isolation kit is designed to isolate very high-molecular-weight genomic DNA by reducing the shearing forces that take place in traditional methods using phenol-chloroform extraction and ethanol precipitation. The basic kit procedure requires tissue homogenization, filtration through a filter unit supplied with the kit to remove cellular debris (<3 minutes), isolation of the cell nuclei by centrifugation (<20 minutes), and protease digestion of the nuclei (45 minutes).[1,2] The resulting suspension is transferred to a free-floating RecoverEase kit cup where the digested proteins and low molecular weight contaminants are removed by dialysis (16 to 48 hours), leaving behind fully hydrated DNA, which is ready for immediate use. From previous experiments using field inversion analysis, DNA isolated in this way, produced products that were 500-kb long.[2] Subsequent Southern blot analysis demonstrated that the DNA cut with a variety of restriction enzymes (data not shown). These benefits, along with higher packaging efficiencies, make the RecoverEase DNA isolation kit the recommended method for purifying high-molecular-weight genomic DNA from blood, bone marrow, and cultured cells.

Big Blue® Transgenic Rodent Mutation Assay and Cell Culture System

Genomic DNA isolated with this kit is ideal for recovering lambda shuttle vectors in transgenic in vivo mutation assay systems, such as Stratagene's Big Blue® mutation assay systems.*[3,4,5] An important step in these mutation assays is retrieving large quantities of shuttle vector from the animal's genomic DNA for subsequent analysis of mutations within a target region of the vector. The shuttle vector in Big Blue was derived from bacteriophage lambda and can be retrieved from the animal by subjecting the genomic DNA to the specialized in vitro lambda Transpack® packaging extract.**[,4] Because the shuttle vector is nearly 50-kb long and is present in multiple copies within the genome of the animal, efficient packaging is contingent on genomic DNA preparations of very high-molecular weight.[6] The DNA must also have sufficient purity so as to be free of substances that inhibit the packaging reaction. For these reasons, measuring packaging efficiency in DNA preparations made from shuttle vector transgenics is a good indicator of DNA size and purity. DNA yielded from using the kit averaged a 74% greater recovery of plaque-forming units (pfu) per Transpack packaging reaction over organic extracted DNA overall for liver, kidney, spleen, testes, lung, and brain in Big Blue transgenic mice.[6]

RecoverEase™ DNA Isolation Kit vs. Organic Extraction

The RecoverEase method (see Methods) was compared with the organic extraction technique in relation to DNA yield, quality, and packaging efficiency. The latter technique was taken from Stratagene's Big Blue DNA isolation kit, which uses a modified phenol-chloroform extraction and an ethanol precipitation protocol optimized for isolating high-molecular-weight genomic DNA from transgenic animals containing lambda shuttle vectors. The Big Blue DNA isolation kit was the first kit specifically designed to prepare genomic DNA of size and quality that, when subjected to in vitro packaging extracts, yielded large numbers of rescued shuttle vector.

Blood samples were collected from Big Blue rats, while individual bone marrow samples were taken from both Big Blue mice and Big Blue rats. The Big Blue mouse embryonic fibroblast and Big Blue rat 2 embryonic fibroblast cell lines were also tested. Genomic DNA was isolated from two to five individual samples of each tissue type, using both dialysis and organic extraction methods. The results of the comparison are shown in Table 1. When using 5 to 7 ml of rat blood, the standard organic extraction protocol (Big Blue DNA isolation kit) failed to yield DNA, even after two attempts. However, other organic extraction protocols begin with a blood volume of 20 ml[7] so the lack of DNA recovery may have been due to an insufficient starting blood volume. The dialysis method yielded enough high-molecular-weight DNA from the same blood volume (5 to 7 ml) to package an average of

Table 1

Average Lambda Phage Recovery with RecoverEase™ DNA Isolation Kit and Organic Extraction Methods

Tissue	Tissue source	Method	Average yield[b]	Average pfu/reaction[c]	Average total pfu recoverable[d]	Average pfu/µg DNA[e]
Blood	Big Blue® rats	Dialysis (RecoverEase™ kit)	45 µg	226,000	3,892,000	86,000
		Organic Extraction	NR[f]	NR	NR	NR
Bone marrow	Big Blue mice	Dialysis (RecoverEase kit)	180 µg	898,000	29,126,000	162,000
		Organic Extraction	150 µg	798,000	7,477,000	50,000
	Big Blue rats	Dialysis (RecoverEase kit)	76 µg	200,000	5,884,000	77,000
		Organic Extraction	100 µg	122,000	1, 148,000	11,000
Fibroblast (cultured cells)	Big Blue mice	Dialysis (RecoverEase kit)	138 µg	702,000	28,417,000	206,000
		Organic Extraction	102 µg	636,000	5,959,000	58,000
	Big Blue Rat 2	Dialysis (RecoverEase kit)	96 µg	486,000	15,551,000	162,000
		Organic Extraction	86 µg	419,000	3,929,000	46,000
Overall average[g]		Dialysis (RecoverEase kit)	122 µg	572,000	19,744,000	152,000
		Organic Extraction	110 µg	494,000	4,628,000	41,000

[a] Results are the averages of 2 to 5 samples each.

[b] An aliquot of the recovered DNA was sheared through an 18-gauge needle, and the optical density at 260 nm was used to determine the DNA concentration for each sample. The concentration was multiplied by the volume recovered to give the DNA yield.

[c] DNA (8 µl) was packaged per the Transpack protocol, and an aliquot of the total DNA was plated on the Big Blue® SCS-8 *E. coli* strain to determine the number of pfu per reaction.

[d] The estimated total pfu recoverable was determined using the number of pfu per reaction multiplied by the number of estimated reactions achievable from the total volume of DNA recovered.

[e] The pfu/ug DNA was determined by dividing the total recoverable pfu by the total yield of DNA.

[f] NR: none recovered.

[g] The average of all samples for mice, rats, and tissues combined (for each extraction technique). For comparison purposes, average values are for bone marrow and fibroblast only.

over 3 million pfu of shuttle vector per sample. Among the remaining tissues tested, packaging efficiencies averaged 16% higher with the RecoverEase DNA samples, compared to those prepared using organic extraction. Although this increase is modest compared to previous comparisons in tissues such as liver,[6] note that bone marrow and cultured cells are among the best substrates for the organic extraction method in terms of high rates of packaging success. DNA yields were similar using both techniques. DNA purity was also indistinguishable, with $A_{260/280}$ ratios of 1.8 among all samples (data not shown).

Conclusions

The list of tissues amenable to Stratagene's RecoverEase DNA isolation kit now includes blood, bone marrow, and cultured cell lines. With the kit, reduce the need for hands-on time, eliminate toxic organic solutions, and remove ethanol precipitation and lengthy rehydration steps. Other potential applications for genomic DNA recovery include Southern blot analysis, pulsed-field gel electrophoresis, PCR amplification, genomic library preparation, and shuttle vector recovery in transgenic in vivo mutation assays.

Methods[8]

Blood: Recover rodent whole blood (5 to 7 ml) with the anticoagulant ACD (0.48% citric acid w/v, 1.32% sodium citrate w/v, 1.47% glucose w/v),[7] and add five volumes of Tris-buffered ammonium chloride (140 mM NH4Cl, 17 mM Tris-HCl, pH 7.65). After 5 minutes at 37°C, pellet the lysed blood cells by centrifugation, then wash the pellet with NaCl (0.85%) solution.[7] The pellet may be stored at –80°C. When ready for use, thaw the pellet, and isolate and purify the DNA following the standard kit protocol.

Bone Marrow: Inject lysis buffer through two mouse femurs or one rat femur, and incubate the lysate on ice for 10 minutes; centrifuge and digest/dialyze per the standard kit protocol.

Cultured Cells: Suspend cells (1 to 5 x 10^6) in lysis buffer, eliminate the coarse disruption step, and follow the standard procedure.

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* U.S. Patent Nos. 5,347,075 and 5,824,287 and 5,589,155; European Patent No. 0289121; Japanese Patent No. 2618973

** U.S. Patent No. 5,188,957

RecoverEase™ DNA Isolation Kit		
Coarse filter cups, dialysis cups, cotton applicators, lysis buffer, digestion buffer, RNace-It™ cocktail, proteinase K	15 reactions	#720203
	30 reactions	#720202
Big Blue® DNA Isolation Kit		
All reagents for 30 DNA extractions includes phenol and chloroform in poison-safety pack	with phenol and chloroform	#720200
All reagents for 30 DNA extractions without phenol and chloroform	without phenol and chloroform	#720201
Transpack® Packaging Extract		
50 packaging reactions		#200223
100 packaging reactions		#200221
400 packaging reactions		#200220

Printed in USA, April 2008
5989-8257EN