

Gentle and Quantitative Recovery of Large Genomic DNA Fragments from Agarose Gels for Genomic Cloning

Gentle, rapid and efficient recovery of even minute amounts of size-separated genomic DNA fragments from low-melting point (LMP) agarose gels can be accomplished using GELase Agarose Gel-Digesting Preparation. GELase Preparation provides for quantitative recovery of large (up to >2 Mb) DNA fragments without the loss or shearing that can occur with electroelution or column purification methods. The DNA recovered from the agarose is often concentrated enough to be used directly for ligation into the BAC, cosmid or fosmid cloning vector. Additional information on using GELase Preparation for genomic cloning is presented in EPICENTRE's product literature for the pWEB™ Cosmid Cloning Kit, the pWEB::TNC™ Cosmid Cloning Kit and the EpiFOS™ Fosmid Library Production Kit.

Here are a few of the many citations describing use of GELase Preparation to recover size-selected genomic DNA fragments from LMP agarose gels for subsequent BAC cloning.

1. Brosch, R. et al. (1998) *Infection and Immunity* **66**(5), 2221.
2. Buchrieser, C. et al. (1999) *Infection and Immunity* **67**(9), 4851.
3. Clemson University Genomics Institute at www.genome.clemson.edu/protocols/
4. Texas A&M University BAC Center at <http://hbz.tamu.edu/bacindex.html>
5. Rondon, M.R. et al. (1999) *Proc. Nat'l Acad. Sci., USA* **96**(11), 6451.
6. Birren, B. et al. (1999) *Bacterial Artificial Chromosomes in Genome Analysis: A Laboratory Manual v.3*, 241, Cold Spring Harbor Press.

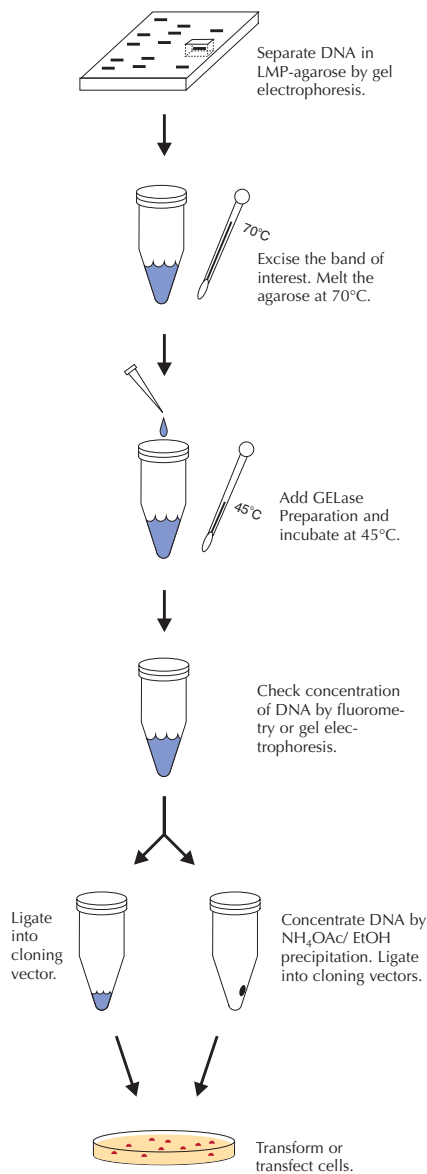


Figure: GELase™ Agarose Gel-Digesting Preparation provides a rapid and gentle method for quantitative recovery of genomic DNA fragments from low melt point (LMP) agarose gels following gel electrophoresis.

The Highest Transformation Efficiency Electrocompetent *E. coli* Cells

With their high efficiency of transformation and lack of size bias against large inserts, TransforMax™ EC100™ Electrocompetent *E. coli* are ideal for producing Bacterial Artificial Chromosome (BAC) libraries as well as for routine cloning and subcloning applications.

Genotype

F⁻ *mcrA* Δ(*mrr-hsdRMS-mcrBC*)
 ϕ80*dlacZ*ΔM15 Δ*lacX74* *recA1* *endA1*
araD139 Δ(*ara, leu*)7697 *galU* *galK* λ⁻
rpsL *nupG*

Important Phenotypes & Applications

- Readily take up large clones for unbiased BAC library production.
- Supports blue/white screening of vectors, including Cloning-Ready pIndigoBAC-5 vectors.
- Restriction minus for efficient cloning of methylated DNA (e.g. mammalian genomic DNA).
- Endonuclease minus (*endA1*) to ensure high yields of clones.
- Recombination minus (*recA1*) to ensure the stability of large cloned inserts.

Transformation Efficiency of TransforMax™ EC100™ Electrocompetent *E. coli* Versus Three Leading Competitors

	Transformation Efficiency (cfu/μg DNA)*
TransforMax™ EC100™ <i>E. coli</i>	9.2 X 10 ⁹
Competitor S	5 X 10 ⁹
Competitor I	4 X 10 ⁹
Competitor L	3 X 10 ⁹

* Average of eight independent transformations using consistent conditions with a pUC vector. Efficiencies vary with electroporator and conditions such as voltage and pulse time. Under optimal conditions, TransforMax™ EC100™ Cells can yield efficiencies >10¹⁰ cfu/μg.

TransforMax™ EC100™ Electrocompetent *E. coli*

EC10005	5 X 100 μl (10 Electroporations)
EC10010	10 X 100 μl (20 Electroporations)

Each includes pUC19 control DNA.

GELase™ Agarose Gel-Digesting Preparation

G09050	50 Units	1 U/μl
G09100	100 Units	1 U/μl
G09200	200 Units	1 U/μl