

Technically Speaking

## DNA Amplification: Preparation of Template and Recovery of Product

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Many molecular biology applications rely upon the amplification of target sequences from minute quantities of DNA. The isolation of quality template DNA is critical to the success of these techniques. Moreover, the isolation and purification of these valuable amplification sequences is crucial to the success of subsequent applications. Promega's Wizard<sup>®</sup> Genomic DNA Purification Kit and ReadyAmp<sup>™</sup> Genomic DNA Purification System provide rapid and reliable methods of preparing genomic DNA for amplification, while the Wizard<sup>®</sup> PCR<sup>(a)</sup> Preps DNA Purification System and AgarACE<sup>™</sup> Agarose-Digesting Enzyme provide convenient procedures for purifying PCR-amplified DNA fragments.

<sup>(a)</sup>The PCR process is covered by patents issued and applicable in certain countries. Promega does not encourage or support the unauthorized or unlicensed use of the PCR process.

### Q: What systems are available for the isolation of genomic DNA prior to amplification? How do they differ?

Amplification-quality DNA can be isolated using either the Wizard<sup>®</sup> Genomic DNA Purification Kit or the ReadyAmp<sup>™</sup> Genomic DNA Purification System. Both systems provide rapid methods for isolating DNA. One important difference between these two systems is in the characteristics of the purified DNA. The DNA isolated using the Wizard<sup>®</sup> Genomic DNA Purification Kit is double-stranded and of a high molecular weight ( $\geq 50$ kb); DNA isolated using the ReadyAmp<sup>™</sup> Genomic DNA Purification System is single-stranded and of a relatively low molecular weight.

### Q: What is the Wizard<sup>®</sup> Genomic DNA Purification Kit? For what is it used?

The Wizard<sup>®</sup> Genomic DNA Purification Kit uses selective cell lyses and precipitations to rapidly isolate genomic double-stranded DNA (dsDNA). DNA purified with this system is suitable for a variety of molecular biology applications, including amplification, digestion with restriction endonucleases and membrane hybridization (e.g., Southern and dot/slot blots).

### Q: From what sources can DNA be isolated using the Wizard<sup>®</sup> Genomic DNA Purification Kit? What is the expected yield?

The Wizard<sup>®</sup> Genomic DNA Purification Kit is quality control-tested for DNA isolation from 300 $\mu$ l samples of mammalian whole blood. Additionally, Promega scientists have developed protocols for successfully isolating genomic DNA from bacteria, yeast, tissue culture cells and animal tissues. The yield of DNA depends upon a number of factors including type and amount of starting material. Approximate yields from some common sources of starting material are given in [Table 1](#).

**Table 1. Approximate Yield of DNA from Various Sources Using the Wizard<sup>®</sup> Genomic DNA Purification Kit.**

Starting Material	Amount of Starting Material	Approximate Yield
Whole blood	300 $\mu$ l	15 $\mu$ g
Gram(+) bacteria	3.5 x 10 <sup>8</sup> cells	10 $\mu$ g
Yeast	1.8 x 10 <sup>8</sup> cells	5 $\mu$ g
COS cells	1.5 x 10 <sup>6</sup> cells	10 $\mu$ g
Mouse liver	1mg	1.5 $\mu$ g

### Q: What is the ReadyAmp<sup>™</sup> Genomic DNA Purification System? For what is it used?

The ReadyAmp<sup>™</sup> Genomic DNA Purification System uses an ion exchange resin to provide a simple and inexpensive approach to isolate genomic single-stranded DNA (ssDNA) from whole blood or bloodstains for amplification. The process takes less than one hour and requires no organic extractions or ethanol precipitations. The DNA prepared with this system may be used directly in amplification reactions, including *GenePrint*<sup>™(b)</sup> STR Systems' amplifications, without further manipulation

*(b) STR loci are the subject of German Patent No. DE 38 34 636 C2 issued to the Max-Planck-Gesellschaft zur Förderung der Wissenschaften, eV, Germany. Exclusive rights have been assigned to Promega Corporation for uses in human clinical research and diagnostics applications and all forms of human genetic identity. Exclusive rights to human linkage analysis in the research market are assigned to Research Genetics, Inc., Huntsville, Alabama. All other rights are shared by Research Genetics and Promega.*

*The development and use of STR loci is covered by U.S. Patent No. 5,364,759 assigned to Baylor College of Medicine, Houston, Texas. Rights have been licensed to Promega Corporation for all applications. Most applications have been licensed on an exclusive basis. U.S. Patent No. 5,599,666 has been issued to Promega Corporation for allelic ladders for the loci CSF1PO, F13A01, FESFPS, LPL and vWA. PCR primers for the STR loci were developed in several laboratories including that of Dr. C. Thomas Caskey while at Baylor College of Medicine (Houston, Texas), Dr. Peter Gill at the Forensic Science Service (Aldermaston, Reading, Berkshire) and Dr. Jeffrey Murray at the University of Iowa (Iowa City, Iowa).*

*Use of the GenePrint™ STR System requires performance of the polymerase chain reaction (PCR), which is the subject of European Patent Nos. 201,184 and 200,362, and U.S. Patent Nos. 4,683,195, 4,965,188 and 4,683,202 owned by Hoffmann-La Roche. Purchase of the GenePrint™ STR System does not include or provide a license with respect to these patents or any other PCR-related patent owned by Hoffmann-La Roche or others. Users of the GenePrint™ STR System may, therefore, be required to obtain a patent license, depending on the country in which the system is used. For more specific information on obtaining a PCR license, please contact Hoffmann-La Roche.*

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### **Q: What is the expected yield from the ReadyAmp™ Genomic DNA Purification System?**

Typically, 8-10µg of ssDNA can be recovered from 400µl of whole blood or from a 25mm<sup>2</sup> bloodstain sample.

### **Q: What tubes should be used for blood collection prior to DNA isolation?**

DNA may be isolated using the Wizard® Genomic DNA Purification Kit or the ReadyAmp™ Genomic DNA Purification System from whole blood samples collected in EDTA-, heparin- or citrate-coated tubes. No adverse effects upon subsequent manipulation of the DNA, including amplification, have been observed.

### **Q: What are the options for isolating DNA after amplification?**

The AgarACE™ Agarose-Digesting Enzyme was developed by Promega for the simple and quantitative recovery of intact DNA from low melting point agarose gels. AgarACE™ Enzyme completely digests agarose to ethanol-soluble oligosaccharides to release DNA from the gel slice in as little as 15 minutes. The recovered DNA is ready for direct use in a number of procedures such as ligation, random primer labeling and sequencing.

The Wizard® PCR Preps DNA Purification System provides a reliable way to purify dsDNA directly from an amplification reaction or from an agarose gel slice. The PCR product is effectively purified away from contaminants, including salts, primer-dimers and amplification primers.

### **Q: Can PCR products be purified directly from an amplification reaction?**

The Wizard® PCR Preps DNA Purification System can be used to purify PCR products directly from an amplification reaction as well as from gel slices. The Wizard® Resin binds DNA partly based on size: only low-melting point agarose fragments >=200bp are efficiently bound. As a result, PCR products near and above this size are effectively purified away from excess nucleotides, primers and primer-dimers. For direct purification, Wizard® PCR Preps Direct Purification Buffer should be added to the aqueous phase of the reaction. The detergent Triton® X-100 (Union Carbide) in the buffer emulsifies any mineral oil carryover that might interfere with the purification.

### **Q: Why should PCR products be purified before cloning?**

A sample of a PCR amplification should be analyzed by gel electrophoresis with appropriate markers before being used for cloning. If multiple amplification products are observed, excise and purify the appropriate DNA band from the agarose to ensure that only the proper band will be cloned. However, even when the expected amplicon produces a discrete band, primer-dimers can represent a large molar-fraction of amplified product. If the fragment is to be cloned using a T-tailed vector (e.g., the pGEM®-T<sup>(c,d)</sup> and pGEM®-T Easy Vector Systems), primer-dimers in the ligation reaction can lead to an increased number of recombinant clones that lack the insert of interest.

*(c) U.S. Pat. No. 4,766,072 has been issued to Promega Corporation for transcription vectors having two different bacteriophage RNA polymerase promoter sequences separated by a series of unique restriction sites into which foreign DNA can be inserted.*

**Q: Does the choice of agarose affect DNA isolation using the Wizard<sup>®</sup> PCR Preps DNA Purification System?**

The Wizard<sup>®</sup> PCR Preps DNA Purification System Technical Bulletin (#TB118) includes recommendations for isolating PCR products from standard and low-melting point agaroses. More consistent and greater (>60%) yields of DNA are obtained using low melting point agarose.

**Q: Will subsequent reactions be affected by gel purification?**

Gel purification may affect subsequent reactions. The choice of running buffer, which does not affect the recovery of the DNA, may affect subsequent manipulations. For example, carryover of borate ions from TBE buffer can inhibit enzymatic reactions (e.g., sequencing) and elevated pH levels can decrease bacterial transformation efficiencies following ligation. Use TAE gel running buffer to avoid these problems. Furthermore, overexposure of the band of interest to short-wave ultraviolet light during excision can lead to the production of pyrimidine dimers in the DNA that make the fragment unclonable. Use long-wave ultraviolet light or place a glass plate between the gel and the short-wave ultraviolet light while visualizing the bands in a gel to minimize this possibility.

**Q: Can the Wizard<sup>®</sup> PCR Preps DNA Purification System be used to isolate DNA from polyacrylamide gels?**

A DNA fragment can be isolated from a polyacrylamide gel after a passive elution. The gel slice should be soaked in as much as 100µl TE buffer from 30 minutes to overnight at 37°C. The buffer is then mixed with Wizard<sup>®</sup> PCR Preps DNA Purification Resin and processed as described in the Technical Bulletin.

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