



**Promega**

## Technical Bulletin

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# AgarACE™ Agarose-Digesting Enzyme

INSTRUCTIONS FOR USE OF PRODUCTS M1741 AND M1743.



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# AgarACE™ Agarose-Digesting Enzyme

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## I. Description

AgarACE™ Enzyme<sup>(a)</sup> is a unique agarose-digesting enzyme developed by Promega for simple and quantitative recovery of intact DNA or RNA from agarose gels. Isolated from a marine *Flavobacterium*, AgarACE™ Enzyme completely digests agarose to ethanol-soluble oligosaccharides in 15 minutes. AgarACE™ Enzyme is sufficiently thermostable that low melting point (LMP) agarose melted at 65–75°C does not have to be equilibrated to the reaction temperature before hydrolysis. Simply place the tube containing the molten agarose at 42–47°C and add AgarACE™ Enzyme. With slight modifications to the protocol, AgarACE™ Enzyme can also effectively hydrolyze non-LMP agaroses, although the higher temperatures required to melt them may be deleterious to the DNA or RNA. Finally, AgarACE™ Enzyme does not require buffer exchange in order to exhibit high agarose hydrolytic activity; it works equally well in TAE or TBE buffers used in normal electrophoretic procedures.

Promega has tested the performance of DNA isolated from agarose gels with AgarACE™ Enzyme in a number of downstream applications. Nucleic acids recovered using AgarACE™ Enzyme are ready for direct use in a number of procedures such as ligation, nick translation and sequencing. In other applications, an ethanol precipitation of the isolated nucleic acid is recommended before use. A guide to application compatibility is found in Section IV and Section VIII.E. The same information can be applied to other applications that are not specifically mentioned in this Technical Bulletin. Most interferences can be traced to gel running buffer components or hydrolyzed agarose, not to the AgarACE™ Enzyme directly. These interferences usually can be eliminated by the methods described.

### Selected Citations Using AgarACE™ Enzyme:

- Blodgett, J.A.V., Zhang, J.K. and Metcalf, W.W. (2005) Molecular cloning, sequence analysis, and heterologous expression of the phosphinothricin tripeptide biosynthetic gene cluster from *Streptomyces viridochromogenes* DSM 40736. *Antimicrob. Agents Chemother.* **49**, 230–40.

AgarACE™ Enzyme was used to prepare DNA fragments during the creation of an *S. viridochromogenes* genomic library.

- Odersky, A. *et al.* (2002) Repair of sequence-specific <sup>125</sup>I-induced double-strand breaks by nonhomologous DNA end joining in mammalian cell-free extracts. *J. Biol. Chem.* **277**, 11756–64.

AgarACE™ was used to purify linear pUC19-MDR1 after a double-strand breakage assay with a triplex-forming oligonucleotide. The linear pUC19-MDR1 construct was purified from a 1.5% NuSieve® agarose gel.

For additional peer-reviewed articles citing use of this product, please visit:  
[www.promega.com/citations](http://www.promega.com/citations)

## II. Product Components and Storage Conditions

| Product                           | Size | Cat.# |
|-----------------------------------|------|-------|
| AgarACE™ Agarose-Digesting Enzyme | 25u  | M1741 |
|                                   | 500u | M1743 |

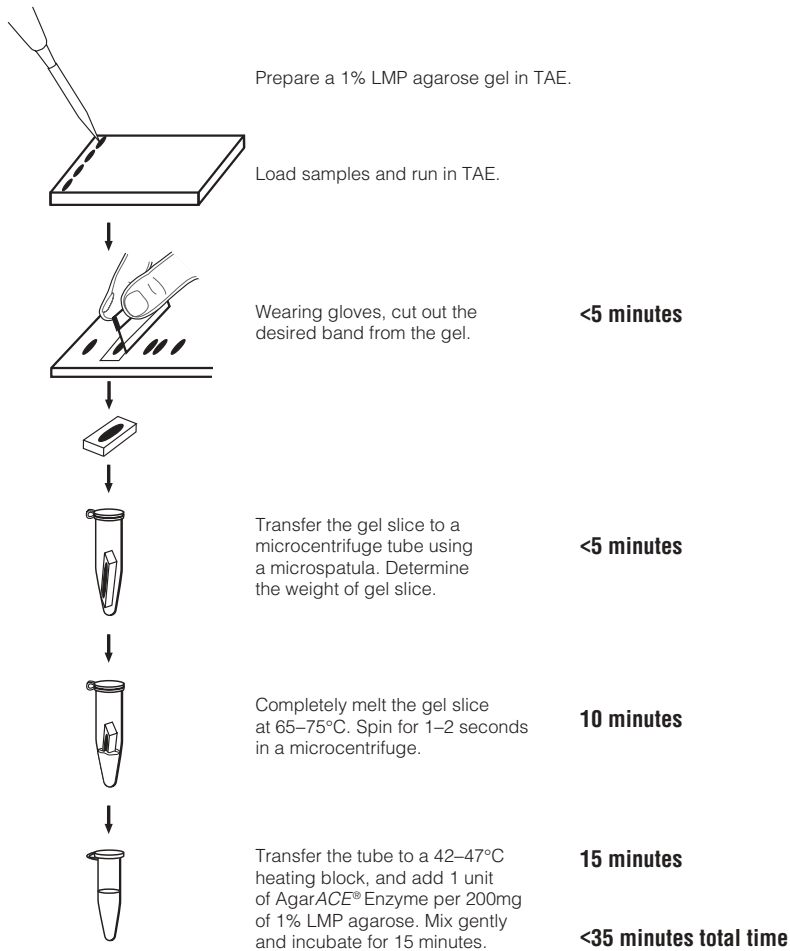
AgarACE™ Enzyme is supplied in a storage buffer containing 20mM Tris-HCl (pH 7.3 at 23°C).

**Unit Definition:** One unit of AgarACE™ Enzyme completely degrades 200μl (200mg) of molten 1% Cambrex SeaPlaque® agarose in 1X TBE (pH 8.3) in 15 minutes at 42–47°C as judged by release of reducing sugars.

**Storage Conditions:** Store AgarACE™ Enzyme at -15 to -25°C. AgarACE™ Enzyme may be stored at 2–8°C for up to 3 weeks. AgarACE™ Enzyme is stable to at least 10 freeze-thaw cycles and is stable at room temperature (15–30°C) for at least 1 day.

## III. General Considerations

Figure 1 depicts a basic protocol for the digestion of a 1% (w/v) Promega LMP, Preparative Grade Agarose gel in 1X TAE buffer using AgarACE™ Enzyme. A more detailed protocol is described in Section V. If you are using TAE and a 1% agarose gel prepared from preparative grade LMP agarose, this basic protocol should be sufficient for your needs. In general, using different buffers or agarose types will have very little effect on the number of units of AgarACE™ Enzyme required. However, the protocol must be modified when non-LMP agaroses are used (these changes are noted in the protocol in Section V). Using a different agarose percentage or digestion time effect how much AgarACE™ Enzyme is needed. The following subsections provide information on the amount of enzyme required under various digestion conditions.



**Figure 1. Schematic diagram of the basic AgarACE™ Enzyme protocol.** This diagram outlines the steps of the basic protocol for isolating nucleic acids from a 1% LMP agarose gel slice and run in 1X TAE (pH 9.3). Depending upon the actual running conditions, and the application in which the isolated nucleic acid will be used, you may need to pretreat the gel before isolation or the nucleic acid after isolation (Section IV). We recommend that you wear gloves and use nuclease-free instruments to minimize nuclease contamination.

### III.A. Extended Digestions

Table 1 indicates the amount of enzyme to use for different digestion times. If enzyme dilution is required, dilute the enzyme in deionized water just before use.



**Note:** Do not store and reuse diluted enzyme.

**Table 1. Digestion Time Versus Units of Enzyme.**

| Digestion Time                | Units of AgarACE™ Enzyme |
|-------------------------------|--------------------------|
| 15 minutes                    | 1.0                      |
| 1 hour                        | 0.25                     |
| 4 hours                       | 0.1                      |
| 16 hours (overnight at 37°C)* | 0.025                    |

Each reaction used 200µl (200mg) of molten 1% LMP agarose in 1X TBE or 1X TAE at 42–47°C, unless otherwise noted. \*Perform overnight digestions in a 37°C oven.

Because of slow inactivation of AgarACE™ Enzyme at the optimal reaction temperature (42–47°C), a 37°C incubation is highly recommended for overnight digests. Perform long incubations in an oven rather than in a heating block, because a large fraction of the reaction volume can condense on the tube lid in a heating block overnight, concentrating the sample, which may lead to DNA nicking or other deleterious effects. In an oven, condensation is minimized. If you do not have access to a 37°C oven, perform extended digests in tubes that are at least 25% full.

### III.B. Variation in Agarose Type or Concentration

Although AgarACE™ Enzyme has been tested with several agarose types, we recommend using Promega Low Melting Point (LMP), Preparative Grade Agarose [Cat.# V2831 for large fragments (>1,000bp) or Cat.# V3841 for small fragments (10–1,000bp)] or Cambrex SeaPlaque® GTG® or NuSieve® GTG® agarose, for most applications. AgarACE™ Enzyme appears to hydrolyze all agarose types at an equivalent rate and to an equivalent extent, provided the agarose is fully melted and has not regelled before the enzyme is added. This latter consideration is more serious in the case of non-LMP agarose because it tends to gel faster at the digestion temperature. Also, with non-LMP agarose, the high temperatures required to melt the agarose prior to digestion are likely to denature and/or degrade DNA and RNA. Thus, LMP agarose is recommended for most applications.

It is important to use preparative grade agarose (see above for recommendations). Some analytical grade agarose has not been tested for trace nuclease activities and there can be batch-to-batch variability in the melting and gelling temperatures. More importantly, some analytical grade agaroses may contain impurities liberated by hydrolysis that can interfere with

subsequent applications. These interferences might not be observed when the nucleic acid is isolated by other methods (e.g., electroelution) because the interfering agents may not be liberated from the gel under these conditions.

AgarACE™ Enzyme activity is linear with respect to agarose concentration. One unit of AgarACE™ Enzyme will digest 200µl (200mg) of molten 1% agarose in 15 minutes, whereas 2 units of enzyme are required to digest an equivalent volume of 2% agarose in 15 minutes. At higher agarose concentrations, even more care must be taken to ensure complete melting and adequate mixing of the more viscous sample. In general, it is best and least expensive to use the lowest agarose concentration that will adequately resolve the bands of interest.

### III.C. Variation in Buffer Type

AgarACE™ Enzyme performs equally well in TAE (pH 7.3, 7.8 or 8.3) and TBE (pH 8.3), and performs adequately in 20mM phosphate and MOPS buffers across the pH range of 6.5–8.5. However, the buffer selection may affect subsequent applications in which the isolated nucleic acid will be used (discussed in Section IV and VIII.E).

### III.D. Buffer Additives

Various buffer additives have been tested for their effect on the performance of AgarACE™ Enzyme. The results are presented in Table 2.

**Table 2. Effects of Buffer Additives on AgarACE™ Enzyme.**

| <b>Additive</b>              | <b>Effect on AgarACE™ Enzyme</b>                        |
|------------------------------|---|
| ethidium bromide(0.5–5µg/ml) | None  |
| glycerol (0–50%)             | None  |
| DTT (0–10mM)                 | None  |
| NaCl (0–200mM)               | None  |
| NaCl (200–500mM)             | Causes variation in the digestion rate, not recommended |
| EDTA (0–10mM)                | None  |
| SDS (<0.1% w/v)              | None  |
| SDS (>0.3% w/v)              | Inactivates enzyme                                      |
| formaldehyde                 | Inactivates enzyme                                      |

#### IV. Basic AgarACE™ Enzyme Protocol

This protocol can be used for the recovery of either DNA or RNA from agarose gels. Before pouring the gel, consult Section III.C and Section IV to select the appropriate running buffer and prepare any solutions required for subsequent applications. Refer to Section VIII.A for information about preparing agarose gels of accurate percentage.

##### Materials to Be Supplied by the User

(Solution compositions are provided in Section VIII.B.)

- TAE or TBE buffer
- Agarose [LMP, Preparative Grade (Cat.# V2831 or V3841) or Cambrex GTG® LMP agarose]

**Note:** Gelled agarose consists of helical bundles of agarose fibers, and the fibers at the interior of the bundles cannot be attacked by the AgarACE™ Enzyme. If the agarose is not completely melted, the hydrolysis will also be incomplete, resulting in lower recoveries of polynucleotide and possible interference with downstream applications.

#### IV.A. Digestion of Agarose with AgarACE™ Enzyme

1. Load and run the gel using your standard protocol. In general, TAE is the recommended running buffer, although TBE will work in some applications as noted in Section IV.
2. Cut out the desired band from the gel with a clean, nuclease-free razor blade. Many razor blades have a protective oil coating that must be removed with ethanol. Use a long-wavelength UV lamp when visualizing DNA with intercalating dyes, such as ethidium bromide. Irradiate the gel for the absolute minimum time possible; preferably less than 30 seconds. Trim off any excess agarose.
3. Using a nuclease-free spatula, transfer the gel slice to a previously weighed 1.5ml microcentrifuge tube and determine the weight of the slice.
4. Melt LMP agarose by incubating the gel slice at 65–75°C. **Completely melt the agarose.** A typical gel slice of 200µl (200mg) of 1% agarose will take approximately 10 minutes to melt in a 1.5ml microcentrifuge tube and 5 minutes in a glass tube. If the gel slice is more than 300µl in volume, cut it into smaller pieces to aid melting.

Unless the sample contains large (>10kb), shearable DNA, monitor melting by slowly pipetting the sample. If the agarose is completely melted, it should pipette freely and without lumps. For higher molecular weight DNA, tilt the tube and observe the angle at which the agarose begins to move down the side of the tube. Compare this to the angle for a control tube that contains agarose that is completely melted. Avoid agitating the sample. If agarose becomes lodged on the sides of the tube, centrifuge for 1–2 seconds in a room temperature microcentrifuge.

**Note:** The higher the temperature needed to melt non-LMP agarose may denature or degrade the DNA or RNA, so non-LMP-agarose is generally not recommended. If non-LMP agarose is used, melt the gel slice at the temperature recommended by the manufacturer (95–100°C). Equilibrate the melted gel slice to 70°C before adding the AgarACE™ Enzyme and incubating at 42–47°C (Step 5).

5. Transfer the tube to a 42–47°C heating block and add the AgarACE™ Enzyme at a ratio of 1 unit per 200µl (200mg) of 1% gel (Section III.B). When using large volumes of agarose, pre-equilibrate the sample to 42–47°C before adding the AgarACE™ Enzyme. If the volume is greater than 600µl (in a 1.5ml microcentrifuge tube), use multiple tubes. Mix the agarose gently by pipetting or mild agitation, taking care to avoid lodging material high up on the tube walls.
6. Incubate the mixture for 15 minutes at 42–47°C (or longer if desired; refer to Section III.A for instructions regarding longer digestion times).
7. At this point in the protocol, the nucleic acid can be used directly for a number of common procedures as noted in Section IV. Equilibrate the AgarACE™ Enzyme-treated sample to the optimal temperature for the desired application, or the high temperature of the sample may inactivate heat-sensitive enzymes (e.g., ligases). For applications where concentration or further purification of the nucleic acid is necessary, refer to Section VI for suggested protocols.

#### IV.B. Inactivation of AgarACE™ Enzyme

In most cases, inactivation of AgarACE™ Enzyme is not necessary for use of the nucleic acid in subsequent applications. If you suspect that a particular application requires you to inactivate AgarACE™ Enzyme, it can be inactivated by either heat or addition of SDS.

##### Heat Inactivation

1. Place the tube containing the hydrolyzed agarose in a water bath at 65–75°C.
2. After 15 minutes, remove the tube and equilibrate it at the temperature appropriate for the subsequent manipulation.



Do not exceed 75°C, or DNA may be denatured and RNA degraded.

##### SDS Inactivation

Add 1/10 volume of 10% SDS to the tube containing the hydrolyzed agarose and mix gently. For many subsequent applications, the SDS must be removed by phenol:chloroform extraction and ethanol precipitation. Therefore, this method is not generally recommended.

## V. Further Nucleic Acid Purification Protocols

Nucleic acid released from agarose with AgarACE™ Enzyme can be used directly in a number of applications. For some applications, however, you may need to remove interfering substances either before or after AgarACE™ Enzyme digestion. The pH, the borate ions or the EDTA in the gel buffer are the most likely components to interfere with downstream applications. For certain applications, you may need to concentrate the nucleic acid before use. In general, we have found that these requirements can be met by ethanol precipitation after digestion, or by soaking the gel briefly in water before digestion, depending on the application. Refer to Section IV for guidelines on specific applications. Phenol:chloroform extraction before ethanol precipitation can also be performed; however, very few applications require it.

### V.A. Ethanol Precipitation of DNA and RNA

Using this protocol, the final yields are not dependent on size when the nucleic acids are between 200–4,000bp. Good yields of DNA and RNA have been obtained with 100ng total nucleic acid (>90% using NaOAc and >60% using NH<sub>4</sub>OAc), and excellent recoveries are possible with 500ng total nucleic acid (>95% yield using either NaOAc or NH<sub>4</sub>OAc). Ethanol does precipitate some of the AgarACE™ along with the DNA or RNA. We have not observed cases where coprecipitated AgarACE™ interferes with downstream applications, but if this is undesirable for a new application, perform a standard phenol:chloroform extraction before ethanol precipitation.

#### Materials to Be Supplied by the User

(Solution compositions are provided in Section VIII.B.)

- 3M NaOAc (pH 5.2) or 7.5M NH<sub>4</sub>OAc (optional)
- ethanol (95% and 70%)

1. Hydrolyze the agarose completely as described in the standard procedure (Section V.A).
2. For sodium acetate precipitation, add 0.1 volume of 3M NaOAc (pH 5.2), mix, then add 2 volumes of room temperature 95% ethanol (2 times the volume after NaOAc addition). For ammonium acetate precipitation, add 0.5 volume of 7.5M NH<sub>4</sub>OAc, mix, then add 2.5 volumes of room temperature 95% ethanol (2.5 times the volume after NH<sub>4</sub>OAc addition).

Perform ethanol precipitations at room temperature with room temperature ethanol to avoid precipitating traces of oligosaccharides.

3. Allow the mixture to stand 1–2 hours (2 hours for small amounts of DNA in NH<sub>4</sub>OAc) at room temperature, then centrifuge for 15 minutes in a microcentrifuge at maximum speed at room temperature.
4. Immediately decant the supernatant, orienting the tube so that the pellet is on the upper side of the tube. Respin the tube for 1–2 seconds in a

microcentrifuge to bring down residual supernatant. Remove the remaining supernatant by careful pipetting.

5. Wash the pellet by adding 200–500µl of cold (<10°C) 70% ethanol, agitating briefly, respinning for 5 minutes and decanting as above. This step is optional if precipitating with NaOAc.
6. Dry the pellet in a Speed-Vac® if desired, then reconstitute the pellet in an appropriate volume of water or buffer. Allow at least 15 minutes for the nucleic acid to redissolve, with occasional vortexing or flicking of the tube. If in doubt, quantitate the dissolved nucleic acid to make sure the pellet has been completely solubilized. If an insoluble whitish or translucent precipitate remains in the tube after resuspension, refer to Section VII for troubleshooting suggestions.

### V.B. Water Soak Before Digesting Gel

For some applications, components of the running buffer may interfere. We have found that in many cases these effects can be reduced by removing some of the buffer before digesting the gel. The easiest and fastest way to do this is to place the gel in water and allow the osmotic pressure to rapidly reduce the buffer concentration. Use only high-quality, nuclease-free water [Nuclease-Free Water (Cat.# P1193), NANOpure® water or equivalent is recommended].

**Note:** The buffer removal rate varies with the gel type. For LMP, Preparative Grade Agarose (Cat.# V2831) for large fragments and other LMP agaroses, a 10-minute soak is sufficient. High percentage gels (0.2%) of tight LMP agaroses (Cat.# V3841) and all concentrations of non-LMP agarose, require a 15-minute soak.

1. Excise and weigh the gel piece as noted in Section V. Using a clean razor blade, cut the gel into slabs about 1mm thick. Transfer the gel slabs to a centrifuge tube capable of easily holding 10 volumes of water.
2. Add 10 volumes of high-quality, nuclease-free water, cap the tube and agitate moderately (e.g., a rotary shaker at 150–200rpm) for 10–15 minutes at room temperature.
3. Stop the agitation, bring the gel to the bottom of tube with a brief spin, then aspirate off the supernatant.
4. Transfer the gel back to a 1.5ml microcentrifuge tube and proceed with digestion as described in Section V.

### VI. Application Compatibility Guide

Although nucleic acids isolated from agarose using AgarACE™ Enzyme are ready to use in a number of applications, other applications require particular gel running buffers or specific treatments of the nucleic acid or the gel for optimal performance. In general, it is best to use 1X TAE as the running buffer.

This section is designed to help the researcher determine what measures should be taken before running the gel to ensure the best performance of nucleic acids isolated using AgarACE™ Enzyme in subsequent applications. In most cases, we have tested for performance with Promega products and not for compatibility with similar commercially available products. In addition, all of our application compatibility test data have been generated using Promega Low Melting Point (LMP), Preparative Grade Agarose (Cat.# V2831 for large fragments or Cat.# V3841 for small fragments). If you obtain information regarding the compatibility or incompatibility of AgarACE™ Enzyme-isolated nucleic acids with specific kits or agaroses, please feel free to contact our Technical Services Department (1-800-356-9526) so that we can include the information in future revisions of this Technical Bulletin. A summary of the application compatibility information is presented in Section VIII.E.

#### VI.A. Ligation with T4 DNA Ligase

DNA isolated from agarose using AgarACE™ Enzyme can be used directly in ligations with T4 DNA Ligase (Cat.# M1801), if the DNA concentration is within the range normally used by the researcher. If it is necessary to concentrate the DNA, perform an ethanol precipitation (see Section VI.A). Cool the mixture to at least room temperature before adding the ligase buffer or the ligase enzyme to avoid thermal inactivation. To minimize problems arising from endonucleases present in the running buffer or introduced during handling of the gel, use short-term ligations ( $\leq 6$  hours) rather than overnight ligations.

**Note:** Although ligations work well regardless of the running buffer used, transformation efficiencies after ligation vary considerably depending on the type of buffer used and the amount added to the ligation. Refer to Section IV.C for more information.

#### VI.B. Cloning of Isolated Fragments

DNA isolated from agarose using AgarACE™ Enzyme can be used in standard cloning procedures, if the DNA concentration is within the range normally used by the researcher. Although DNA isolated from agarose using AgarACE™ Enzyme works well in the ligation step of cloning, the efficiency of the transformation step is very sensitive to the alkaline pH of the gel running buffers. The buffer used in the ligation step [30mM Tris-HCl (pH 7.8), 10mM MgCl<sub>2</sub>, 10mM DTT, 1mM ATP (final concentration)] moderates this effect significantly. Since the buffer strength in TBE is much higher than TAE, greater negative effects on transformation are seen with TBE buffer than TAE.

When using TAE as the gel running buffer, the ligation reaction should be set up so that  $\leq 5\mu\text{l}$  is used per 100 $\mu\text{l}$  of competent cells in the transformation step (see Section IV.A for general considerations). If it is necessary to concentrate the DNA to meet this requirement, perform an ethanol precipitation (see Section VI.A) after isolating it from the agarose and before adding it to the ligation reaction.

When using TBE buffer, we recommend either running the gel in 0.5X TBE or soaking the gel containing the DNA in water (see Section VI.B) prior to digestion with AgarACE™ Enzyme. Alternatively, ethanol precipitate the DNA after isolation from the agarose (see Section VI.A). The ligation reaction should be set up so that  $\leq 8\mu\text{l}$  is used per 100 $\mu\text{l}$  of competent cells in the transformation step (see Section IV.A for general considerations). With any of these treatments, the maximum number of colonies will be approximately 40% of that observed when transforming with DNA in water. If absolutely necessary, it is possible to ligate isolated DNA and transform cells as described for TAE buffer, but the absolute maximum volume of the ligation reaction that can be added to the competent cells is very low ( $\leq 3\mu\text{l}$  per 100 $\mu\text{l}$  of competent cells), and the transformation efficiency is highly variable.

### VI.C. Transformation of Competent Cells

DNA isolated from agarose using AgarACE™ Enzyme cannot be used directly for transformation of competent cells. As noted in Section IV.B, the pH of the gel running buffers commonly used are too alkaline to obtain good transformation efficiencies, but ligase buffer significantly moderates the effects of the running buffer. For this reason, we recommend adding ligase buffer without ligase to the AgarACE™ Enzyme-isolated DNA (a “pseudoligation” reaction) before transforming competent cells.

When using DNA isolated from a gel cast and run in TAE, prepare a “pseudoligation” reaction by adding 1/10 volume of 10X ligase buffer [300mM Tris-HCl (pH 7.8), 100mM MgCl<sub>2</sub>, 100mM DTT, 10mM ATP] to the DNA. The “pseudoligation” reaction should be set up so that  $\leq 5\mu\text{l}$  is used per 100 $\mu\text{l}$  of competent cells in the transformation step (see Section IV.A). If you need to concentrate the DNA, perform an ethanol precipitation (see Section VI.A) after isolating the DNA from the agarose and before adding it to the “pseudoligation” reaction.

When using TBE buffer, we recommend either running the gel in 0.5X TBE or soaking the gel containing the DNA in water (see Section VI.B) before digestion with AgarACE™ Enzyme. Alternatively, ethanol precipitate the DNA after isolation from agarose (see Section VI.A). The “pseudoligation” reaction should be set up so that  $\leq 8\mu\text{l}$  is used per 100 $\mu\text{l}$  of competent cells in the transformation step. With any of these treatments, the maximum number of colonies will be approximately 40% of that observed when transforming with DNA in water. If necessary, you can transform cells as described above for TAE buffer, but the absolute maximum volume of the “pseudoligation” reaction that can be added to the competent cells is low ( $\leq 3\mu\text{l}$  per 100 $\mu\text{l}$  of competent cells), and the transformation efficiency is highly variable.

### VI.D. Transformation of Bacterial Cells by Electroporation

DNA isolated from agarose using AgarACE™ Enzyme cannot be used directly for transformation by electroporation because the ionic strength of the buffers and the hydrolyzed agarose cause electrical arcing in the electroporation

cuvette. Before using the DNA for electroporation, ethanol precipitate it using sodium acetate and wash the DNA pellet at least once with cold (<10°C) 70% ethanol to remove traces of the salt (see Section VI.A). The total number of colonies observed will be approximately two- to threefold lower than observed for DNA in water.

#### **VI.E. Cloning into the pGEM<sup>®</sup>-T Vector Systems**

PCR-amplified DNA obtained from agarose using AgarACE<sup>™</sup> Enzyme can be used directly for cloning into the pGEM<sup>®</sup>-T Vector Systems (Cat.# A3600 and A3610), if the DNA concentration is within the range normally used by the researcher. If you need to concentrate the DNA, perform an ethanol precipitation (see Section VI.A). TAE is the preferred running buffer. The number of colonies obtained using the pGEM<sup>®</sup>-T Vector is variable for both AgarACE<sup>™</sup> Enzyme-isolated DNA and control DNA in water. The percentage of clones containing inserts will be approximately the same for both AgarACE<sup>™</sup> Enzyme-isolated DNA and control DNA in water. If TBE is used as the running buffer, soak the gel as described in Section VI.B because TBE decreases the transformation efficiency.

#### **VI.F. 5'-End Labeling with T4 Polynucleotide Kinase**

DNA isolated from agarose using AgarACE<sup>™</sup> Enzyme can be phosphorylated directly by T4 polynucleotide kinase (Cat.# M4101), if the DNA concentration is within the range normally used by the researcher. If you need to concentrate the DNA, perform an ethanol precipitation (see Section VI.A). Do not use 7.5M NH<sub>4</sub>OAc for the precipitation because T4 Polynucleotide Kinase is inhibited by ammonium ions. If no special precautions are taken, the rate of labeling is about 3–4 times slower, and the eventual specific activity of the DNA will be 2–3 times lower than for control DNA in water. There are two ways to obtain better kinetics and a higher specific activity label. The first approach is to add twice as many units of kinase as normal. This results in labeling kinetics and specific activities indistinguishable from those obtained for DNA in water. Alternatively, soak the gel in water before labeling (Section VI.B). This results in labeling to a slightly lower specific activity than obtained by adding twice the kinase but has the merit of costing less.

#### **VI.G. Dephosphorylation with Alkaline Phosphatase, Calf Intestinal**

Nucleic acid isolated from agarose using AgarACE<sup>™</sup> Enzyme can be directly dephosphorylated using calf intestinal alkaline phosphatase (CIAP; Cat.# M1821), if TAE is used and the DNA concentration is within the range normally used by the researcher. If it is necessary to concentrate the DNA, perform an ethanol precipitation (see Section VI.A).

If TBE is used as the running buffer, CIAP will be completely inhibited by the borate ions, which act as competitive inhibitors of the enzyme. If the gel must be run in TBE, ethanol precipitate the nucleic acid before treating with CIAP (Section VI.A). Alternatively, the nucleic acid may be dephosphorylated before running the gel.

#### VI.H. Digestion with Restriction Enzymes

In general, more units of restriction enzyme are required to cut DNA isolated from agarose with AgarACE™ Enzyme than for DNA in water. The magnitude of this effect differs with each enzyme and appears to be a combination of the effects of the hydrolyzed agarose, the buffer and, in some instances, the AgarACE™ Enzyme itself. Since in many cases the majority of the inhibition cannot be traced specifically to AgarACE™ Enzyme, we believe that this problem has existed for some time with other agaroses as well. In some cases, such as digests with *Pst* I, you may need to purify further the DNA using the Wizard® PCR Preps DNA Purification System (Cat.# A7170) to eliminate this problem. When purifying the DNA with Wizard® PCR Preps, you need to use TAE as the running buffer because TBE buffer is not compatible with this purification system. To avoid the potential difficulties associated with restriction of agarose-isolated DNA, digest the DNA before electrophoresis. Table 3 lists the effects of agarose and AgarACE™ Enzyme treatment on the activity of various restriction enzymes.

**Table 3. Effect of AgarACE™ Enzyme Digestion on Restriction Enzyme Activity.**

| Enzyme          | *Units Required if Isolated from 1X TBE Gel** | *Units Required if Isolated from 1X TAE Gel | Notes                 |
|-----------------|---|---|-----------------------|
| <i>Acc</i> I    | 3.5X  | 7X  | Use Wizard® PCR Preps |
| <i>Apa</i> I    | 3X  | 3X  |                       |
| <i>Bam</i> HI   | 4X  | 4X  |                       |
| <i>Eco</i> R I  | 6X  | 6X  | Use Wizard® PCR Preps |
| <i>Hind</i> III | 8.5X  | 8.5X  | Use Wizard® PCR Preps |
| <i>Kpn</i> I    | 4X  | 4X  |                       |
| <i>Not</i> I    | 3.5X  | 2X  |                       |
| <i>Pst</i> I    | 7X  | 17X   | Use Wizard® PCR Preps |
| <i>Sac</i> I    | 1X  | 1.5X  |                       |
| <i>Sal</i> I    | 1.5X  | 2X  |                       |
| <i>Sph</i> I    | 1X  | 1X  |                       |

\*The amount of restriction enzyme required (X-fold) to digest the DNA relative to a control digestion containing DNA in water. The DNA was isolated from Promega LMP, Preparative Grade agarose. Other agaroses may give different results.\*\*The use of TBE buffer is not recommended with the Wizard® PCR Preps DNA Purification System.

## VI.I. Random Hexamer Labeling with Prime-a-Gene® Labeling System

DNA isolated from agarose using AgarACE™ Enzyme can be used directly in random hexamer labeling reactions using the Prime-a-Gene® Labeling System (Cat.# U1100), if the DNA concentration is within the range normally used by the researcher. If you need to concentrate the DNA, perform an ethanol precipitation (see Section VI.A).

**Note:** Cool the mixture to at least room temperature before adding the components of the Prime-a-Gene® Labeling System to avoid thermal inactivation.

## VI.J. *fmo*® DNA Cycle Sequencing System

DNA isolated from agarose using AgarACE™ Enzyme can be used directly in the *fmo*® DNA Cycle Sequencing System (Cat.# Q4100), if the DNA concentration is within the range normally used by the researcher. If you need to concentrate the DNA, perform an ethanol precipitation (see Section VI.A; be certain that the DNA is completely resolubilized). TAE is the recommended running buffer. In some cases, “hard stops” have been observed at various locations when the DNA was not ethanol precipitated. In such instances, ethanol precipitation of the test DNA eliminated the problem. If TAE is used as the running buffer, the pH should be 8.3. A recipe is provided in Section VIII.B. If TBE buffer is used, an ethanol precipitation must be performed because TBE inhibits the sequencing reactions.

## VI.K. PCR from Isolated Fragment

DNA isolated from agarose using AgarACE™ Enzyme can be used directly in PCR<sup>(b)</sup>, provided the DNA concentration is within the ranges normally used by the researcher. If it is necessary to concentrate the DNA, perform an ethanol precipitation (see Section VI.A). TAE is highly recommended as the running buffer. When TAE is used, the yield of amplified product is similar to that obtained from control DNA in water alone. When TBE is used, the yield of amplification product can decrease by two- to threefold relative to a control DNA in water. This low yield does not appear to be remedied by increasing the number of thermal cycles. However, ethanol precipitation (see Section VI.A) of the template DNA does eliminate the problem.

## VII. Troubleshooting

| Symptom   | Causes and Comments  |
|---|--|
| Large quantities of white or translucent precipitate in tube when ethanol precipitated; pellet resuspends poorly. | Incomplete digestion of agarose before precipitation due to loss of enzyme activity. Resuspend sample, remelt residual agarose and retreat with AgarACE™ Enzyme. |
|   | Incomplete digestion of agarose because of high agarose concentration. Resuspend sample, remelt residual agarose and retreat with AgarACE™ Enzyme.               |
|   | Incomplete digestion of agarose because of buffer incompatibility. Repeat using a different buffer or longer incubation.   |

## VIII. Appendix

### VIII.A. Gel Preparation

To accurately determine how much AgarACE™ Enzyme is needed to digest the gel, you must know the exact agarose percentage in the gel. Carefully weigh the agarose into the buffer solution. Also, when melting agarose, a significant amount of water can evaporate. If this volume is not replaced, the agarose gel will be of a higher percentage than expected, and more AgarACE™ Enzyme will be required to obtain complete digestion. This effect tends to be more pronounced when a microwave oven is used to melt the agarose. We recommend weighing the container of agarose/buffer both before and after melting the agarose. If the volume has been reduced by evaporation, add deionized water to the molten agarose until the solution has been restored to its original weight. Alternatively, use a 10–20% excess of AgarACE™ Enzyme to compensate for potential differences in agarose concentration.

### VIII.B. Composition of Buffers and Solutions

#### 7.5M NH<sub>4</sub>OAc (100ml)

57.81g ammonium acetate

Dissolve the ammonium acetate in 100ml water (final volume). Sterilize by filtration (0.45µm filter).

#### 3M NaOAc (pH 5.2; 100ml)

24.61g sodium acetate

Dissolve the sodium acetate in water, adjust the pH to 5.2 with acetic acid and bring the final volume to 100ml with water. Sterilize by filtration (0.45µm filter).

#### 1X TAE buffer

40mM Tris acetate  
(with respect to Tris)

1mM EDTA

Adjust the pH to 8.3 with glacial acetic acid.

#### 1X TBE buffer

89mM Tris-borate (pH 8.3)

2mM EDTA

### VIII.C. Characteristics of AgarACE™ Enzyme

**Molecular Weight:** 42kDa.

**Usable pH Range:** 6.5–8.5; less than 20% activity variation over this range.

**Optimal Temperature Range:** 42–47°C.

**Method of Inactivation:** >65°C for 15 minutes.

**Stability:** The expiration date is indicated on the product label for product stored at –15 to –25°C. The enzyme is stable for 3 weeks at 4–8°C and >1 day at room temperature. Stable to at least 10 freeze-thaw cycles.

### VIII.D. Related Products

#### Agarose

| Product  | Size | Cat. # |
|--|------|--------|
| Agarose, Low Melting Point, Preparative Grade for Large Fragments (>1,000bp)   | 25g  | V2831  |
| Agarose, Low Melting Point, Preparative Grade for Small Fragments (10–1,000bp) | 25g  | V3841  |

#### Buffers and Reagents

| Product               | Size    | Cat. # |
|-----------------------|---------|--------|
| TAE Buffer, 10X       | 1,000ml | V4271  |
| TAE Buffer, 40X       | 1,000ml | V4281  |
| TBE Buffer, 10X       | 1,000ml | V4251  |
| SDS Solution (10%w/v) | 100ml   | V6551  |
|                       | 500ml   | V6553  |
| Nuclease-Free Water*  | 50ml    | P1193  |

\*For Laboratory Use.

#### Competent *E. coli* Strains

| Product  | Size            | Cat.# |
|--|-----------------|-------|
| HB101 Competent Cells >10 <sup>8</sup> cfu/μg  | 1ml (5 × 200μl) | L2011 |
| HB101 Competent Cells >10 <sup>7</sup> cfu/μg  | 1ml (5 × 200μl) | L1011 |
| JM109 Competent Cells >10 <sup>8</sup> cfu/μg* | 1ml (5 × 200μl) | L2001 |
| JM109 Competent Cells >10 <sup>7</sup> cfu/μg  | 1ml (5 × 200μl) | L1001 |

\*For Laboratory Use.

**Enzymes**

| <b>Product</b>                        | <b>Size</b>  | <b>Conc. (u/μl)</b> | <b>Cat.#</b> |
|---------------------------------------|--------------|---------------------|--------------|
| Alkaline Phosphatase, Calf Intestinal | 1,000u       | 1                   | M1821        |
| T4 DNA Ligase                         | 100u (Weiss) | 1-3                 | M1801        |
|                                       | 500u (Weiss) | 1-3                 | M1804        |
|                                       | 500u (Weiss) | 10-20               | M1794        |
| T4 Polynucleotide Kinase              | 100u         | 5-10                | M4101        |
|                                       | 1,000u       | 5-10                | M4103        |

\*For Laboratory Use.

**Miscellaneous**

| <b>Product</b>                                      | <b>Size</b>   | <b>Cat. #</b> |
|---|---------------|---------------|
| DNA 5' End-Labeling System                          | 10 reactions  | U2010         |
| <i>fmo</i> <sup>®</sup> DNA Cycle Sequencing System | 100 reactions | Q4100         |
| Prime-a-Gene <sup>®</sup> Labeling System           | 30 reactions  | U1100         |

\*For Laboratory Use.

**pGEM<sup>®</sup> T-Vector Systems**

| <b>Product</b>                             | <b>Size</b>  | <b>Cat.#</b> |
|--|--------------|--------------|
| pGEM <sup>®</sup> -T Vector System I       | 20 reactions | A3600        |
| pGEM <sup>®</sup> -T Vector System II      | 20 reactions | A3610        |
| pGEM <sup>®</sup> -T Easy Vector System I  | 20 reactions | A1360        |
| pGEM <sup>®</sup> -T Easy Vector System II | 20 reactions | A1380        |

\*For Laboratory Use.

**DNA Purification System**

| <b>Product</b>  | <b>Size</b>   | <b>Cat.#</b> |
|---|---------------|--------------|
| Wizard <sup>®</sup> PCR Preps DNA Purification System         | 50 preps*     | A7170        |
| Wizard <sup>®</sup> SV Gel and PCR Clean-Up System            | 50 preps*     | A9281        |
| Wizard <sup>®</sup> MagneSil <sup>®</sup> PCR Clean-Up System | 4 × 96 preps* | A1930        |
| Wizard <sup>®</sup> SV 96 PCR Clean-Up System                 | 1 × 96 preps* | A9340        |
| Wizard <sup>®</sup> DNA Clean-Up System                       | 100 preps*    | A7280        |

For Laboratory Use. \*Additional sizes available.

## VII.E. Compatibility of AgarACE® Enzyme-Isolated Nucleic Acid with Downstream Applications

| Application (running buffer)             | Directly Usable in Application?                    | Required Pretreatment                      | Comments  |
|--|--|--|---|
| <b>Ligation</b>                          |  |  |   |
| TAE                                      | Yes  | None                                       | TBE lowers transformation efficiency.   |
| TBE                                      | Yes  | None                                       | See Sections IV.A and B.  |
| <b>Cloning DNA fragment</b>              |  |  |   |
| TAE                                      | Yes  | None                                       | TBE can greatly lower transformation efficiency.  |
| TBE                                      | Yes  | None                                       | See Section IV.B.   |
| <b>Transformation of <i>E. coli</i></b>  |  |  |   |
| TAE                                      | No   | Add ligase buffer to samples.              | For TBE, DNA volume is important. See Section IV.C.   |
| TBE                                      | No   |  |   |
| <b>Electroporation of <i>E. coli</i></b> |  |  |   |
| TAE                                      | No   | Ethanol precipitation                      | Slightly fewer colonies obtained. See Section IV.D.   |
| TBE                                      | No   |  |   |
| <b>pGEM®-T Vector Cloning</b>            |  |  |   |
| TAE                                      | Yes  | None for TAE gels. Soak TBE gels in water. | The number of colonies is variable. See Section IV.E.   |
| TBE                                      | Yes  |  |   |
| <b>Labeling with PNK</b>                 |  |  |   |
| TAE                                      | Yes for TAE and TBE, but lower labeling efficiency | Add 2X kinase (optional)                   | Soaking the gel in water can improve labeling See Section IV.F.                               |
| TBE                                      |  |  |   |
| <b>Dephosphorylation (AP)</b>            |  |  |   |
| TAE                                      | Yes  | None                                       | Borate ions inhibit CIAP. See Section IV.G.   |
| TBE                                      | No   | Ethanol precipitation                      |   |
| <b>Restriction Digestions</b>            |  |  |   |
| TAE                                      | Yes for both, but more enzyme will be needed.      | Pretreatment will depend on enzyme used.   | Important! Varies for enzyme used in restriction digest. See Section IV.H.                    |
| TBE                                      |  |  |   |
| <b>Prime-a-Gene® Labeling</b>            |  |  |   |
| TAE                                      | Yes  | None                                       | See Section IV.I.   |
| TBE                                      | Yes  | None                                       |   |
| <b><i>fmo</i>® DNA sequencing</b>        |  |  |   |
| TAE                                      | Yes  | Ethanol precipitation                      | Ethanol precipitation before sequencing is useful for eliminating "hard stops." Section IV.J. |
| TBE                                      | No   |  |   |
| <b>PCR</b>                               |  |  |   |
| TAE                                      | Yes  | None                                       | See Section IV.L  |
| TBE                                      | Yes, but 40–60% yield                              | Ethanol precipitation                      |   |

<sup>(a)</sup>U.S. Pat. Nos. 5,814,487, 5,869,310 and 6,001,636 have been issued to Promega Corporation for using agarase to isolate DNA from high melting agarose, for the purified AgarACE™ Enzyme and for the cloning and expression thereof.

<sup>(b)</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.

\*In Europe, effective March 28, 2006, European Pat. Nos. 201,184 and 200,362 will expire. In the U.S., the patents covering the foundational PCR process expired on March 29, 2005.

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