The E-Gel® CloneWell™ method: a faster, safer and more efficient way to purify DNA fragments for cloning.

A new method to isolate DNA from agarose gels using E-Gel® CloneWell™ SYBR® Safe Gels, E-Gel® iBase™ Power System, and E-Gel® Safe Imager™ Real-Time Transilluminator.

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Recovering DNA fragments from agarose gels using traditional methods is a cumbersome, time-consuming procedure involving hazardous ethidium bromide and UV light. We have developed the E-Gel® CloneWell™ method, the easiest, most effective method for isolating DNA bands separated by agarose gel electrophoresis. This method takes advantage of the fundamental improvements provided by the E-Gel® CloneWell™ SYBR® Safe gels, E-Gel® iBase™ Power System, and E-Gel® Safe Imager™ Real-Time Transilluminator. To extract a DNA fragment, load the DNA sample into the top row, run until the band moves into the bottom row of wells, and remove the purified DNA band with a pipette. This will:

• Save time - no additional gel purification steps required after electrophoresis
• Simplify DNA recovery - remove purified DNA directly from the well
• Improve cloning - reduced DNA damage results in more colonies

DNA extracted using the E-Gel® CloneWell™ method is compatible and more efficient with common cloning methods, such as restriction cloning, TOPO® cloning, and Gateway® recombination cloning.

Introduction

Purification of DNA fragments from agarose gels is an important step in many cloning efforts. In traditional methods, DNA bands are excised from the gel and then extracted using resin binding, organic solvents, electro-elution devices, "freeze and squeeze" methods, "crush and soak" methods, or spin methods. These techniques are time consuming, and may create problems in follow-up enzymatic reactions due to contaminants that inhibit ligase and other enzymes1, 2.

Other methods let DNA fragments run out of the gel onto a support, dialysis tubing, or into a dead space in the gel containing a high salt concentration. Though based on a more elegant principle, these methods have their own major disadvantages, including extra elution steps (which reduce the yield), the presence of liquid buffer above the gel (which can dilute the sample), and the need to repeatedly monitor the gel over a UV transilluminator (which damages the DNA, affects cloning efficiency, poses hazard to the user, and is damaging to skin and eyes)1-3.

To overcome these problems we have developed the E-Gel® CloneWell™ method using our E-Gel® agarose gel electrophoresis platform and the non-mutagenic SYBR® Safe DNA gel stain. We have dramatically improved the purification of DNA fragments from agarose gels, using a system to purify DNA fragments by running them out of a dedicated agarose gel, with the following major improvements:

• The use of SYBR® Safe DNA gel stain and non-UV visualization dramatically reduces DNA damage.
• No contaminants are present that inhibit down-stream applications.
• The method offers a fast, simple protocol.
• The integrated system for DNA purification is easy to use.

Below we describe the E-Gel® CloneWell™ method, which provides superior cloning results (10 – 1,000 fold) compared to traditional purification methods.
Integrated system for DNA purification

The E-Gel® CloneWell™ method combines the advantage of three new components, which form an integrated system for DNA purification (Fig. 1):

- **The E-Gel® CloneWell™ SYBR® Safe Gel** is a closed and dry cassette with two rows of wells, and does not need a liquid buffer to run. It contains the SYBR® Safe DNA gel stain, which can be visualized with blue light.

- **The E-Gel® iBase™ Power System** is an easy-to-use, programmable, automated device designed to simplify electrophoresis of single comb or double comb E-Gel® gels from Invitrogen. The E-Gel® iBase™ is ideally suited for the E-Gel® CloneWell™ method, as it has an open design enabling visualization of gels while they are running. In addition, the E-Gel® iBase™ can reverse current, so bands that have run too far can be run back into the collection well.

- **The E-Gel® Safe Imager™ Real-Time Transilluminator** visualizes the SYBR® Safe stained DNA with minimal damage.

The E-Gel® CloneWell™ SYBR® Safe Gel, E-Gel® iBase™ Power System, and E-Gel® Safe Imager™ Real-Time Transilluminator all fit seemlessly together, and can remain in place during the entire E-Gel® CloneWell™ method, adding significantly to the ease-of-use of the entire system.

The E-Gel® CloneWell™ method offers a simplified workflow

The E-Gel® CloneWell™ method has considerably simplified the workflow for DNA purification, compared to traditional methods. Simply load the sample into the wells in the first row and run using the E-Gel® iBase™ Power System. Monitor progress using visible light from the E-Gel® Safe Imager™ Real-Time Transilluminator. When the band of interest enters the water-filled well, collect it directly from the well using a pipette (Fig. 2).

More than one band per lane can be collected in this way, as long as the empty well is refilled with water between collections. The E-Gel® CloneWell™ method has a wide range of separation – bands ranging from 200 bp to 5,000 bp have been successfully purified using this system. You can use the isolated DNA without any further purification.

**Figure 1: Integrated system for DNA purification.** The E-Gel® CloneWell™ SYBR® Safe gels, E-Gel® iBase™ Power System, and E-Gel® Safe Imager™ Real-Time Transilluminator form an integrated system for DNA fragment purification.

**Figure 2: Illustration of the E-Gel® CloneWell™ method.** Purifying DNA fragments using the E-Gel® CloneWell™ method takes only a few simple steps.

**Downstream applications**, such as:
- Restriction cloning
- TOPO® cloning
- Gateway® cloning
Superior quality DNA

The stain in the gel, SYBR® Safe DNA gel stain can be visualized using visible light from the E-Gel® Safe Imager™ Real-Time Transilluminator. Using SYBR® Safe and blue light over ethidium bromide and UV light significantly reduces DNA damage (nicked DNA; Fig. 3A). Experimentation with a lacZ construct across a range of exposure times demonstrated that the SYBR® Safe / blue light method did not cause the DNA damage (nicking, mutations, and deletions resulting in white colonies) that was induced with EtBr / UV light exposure (Fig. 3B). Apart from delivering superior quality DNA, using SYBR® Safe DNA gel stain and visible light also enables constant monitoring of the migration without affecting downstream processes.

Better cloning results with E-Gel® CloneWell™

The efficiency of restriction enzyme cloning and high-throughput recombination cloning has been shown to be enhanced 10–1,000 fold by using the SYBR® Safe / blue light method instead of EtBr/UV light. After just 10 seconds of visualization, cloning efficiency goes down noticeably for EtBr/UV sample (Fig. 4A). In sharp contrast to the EtBr/UV samples, the cloning efficiency from SYBR® Safe/blue light does not go down, even after several minutes of blue-light exposure (Fig. 4B). Clearly, for cloning, using SYBR® Safe / blue light is vastly superior to traditional methods with ethidium bromide gels.

Moreover, when the E-Gel® CloneWell™ method is compared to a competitor gel extraction kit used with SYBR® Safe stained gels (thus eliminating ethidium bromide and UV induced DNA damage as a discriminating factor), the E-Gel® CloneWell™ method also comes out on top for all common cloning methods (Figure 4C). This is probably due to the absence of harsh downstream purification steps, and the relative purity of the extracted DNA. Hence, the E-Gel® CloneWell™ method eliminates the safety and environmental hazards of the standard EtBr / UV procedure while simultaneously enhancing restriction and recombinational cloning efficiency.

References