Molecular cloning

Fast and easy preparation of vectors for cloning experiments using FastDigest restriction enzymes and the E-Gel Power Snap Plus Electrophoresis System

Introduction

Typical cloning preparation processes for inserts and vectors are lengthy and tedious. Time is often wasted through long incubation periods and gel preparation procedures that may include handling of toxic chemicals like ethidium bromide. Selecting excellent techniques and tools that work efficiently together not only gives you the most accurate and reproducible results but also helps make the most of your time.

Thermo Scientific™ FastDigest™ restriction enzymes and the Invitrogen™ E-Gel™ Power Snap Plus Electrophoresis System are advanced molecular biology products that help simplify restriction digestion and agarose gel electrophoresis, respectively. Use of up-to-date products like these means less time spent on trivial tasks, faster data generation for inclusion in critical grant proposals or publications, and more time available to explore new ideas.

Here we demonstrate the speed and convenience of FastDigest restriction enzymes when used in tandem with the E-Gel Power Snap Plus Electrophoresis System (Figure 1). When used together, they provide:

- **A streamlined DNA digestion and analysis workflow**—up to six times faster than conventional methods (Table 1)
- **Simplified restriction digestion**—all restriction enzymes work in one universal buffer
- **A safer work environment for you and your sample**—no ethidium bromide or UV light is used for sample visualization, resulting in improved cloning efficiency

Table 1. Comparison of restriction digestion times for FastDigest enzymes and E-Gel agarose gels vs. the conventional method.

	FastDigest enzymes and E-Gel agarose gels	Conventional method
Hands-on time	10 min	23 min
Total time	37 min	3 hr 49 min

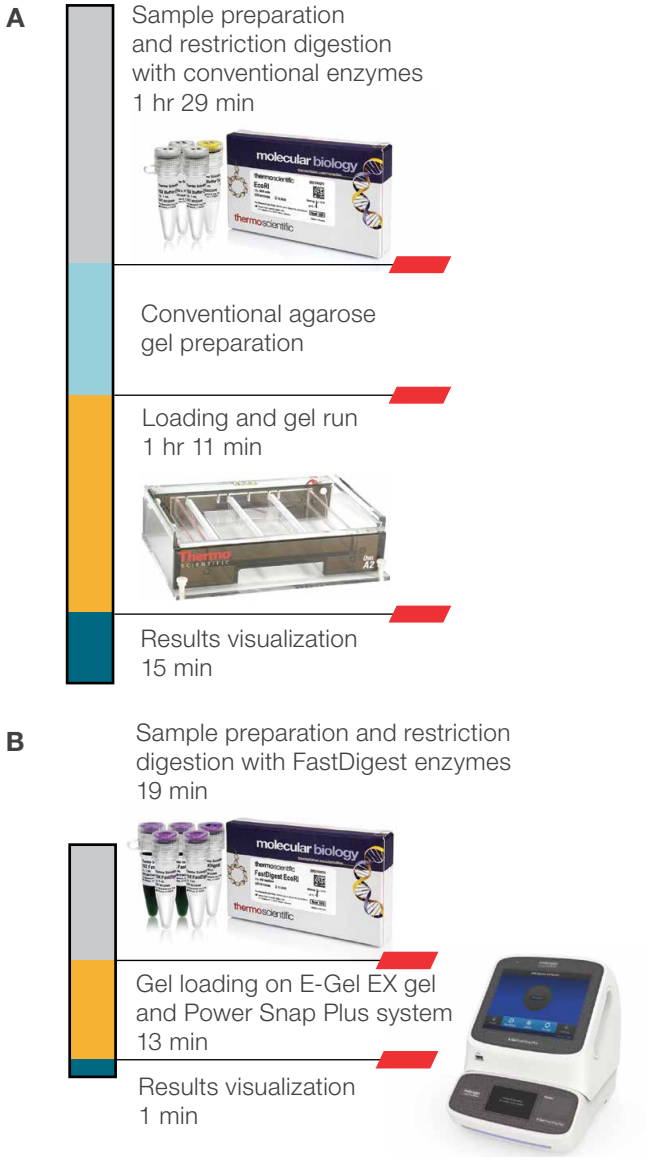

Figure 1. Comparison of restriction digestion workflows using conventional enzymes vs. FastDigest enzymes. (A) Restriction digestion and electrophoresis using conventional enzymes, agarose gel, and electrophoresis rig. **(B)** Restriction digestion and electrophoresis using FastDigest enzymes, Invitrogen™ E-Gel™ EX Agarose Gels (1%), and the E-Gel Power Snap Plus Electrophoresis System.

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Rapid vector preparation and analysis using FastDigest restriction enzymes and the E-Gel Power Snap Plus Electrophoresis System

FastDigest restriction enzymes are designed to work quickly and, when used in tandem with E-Gel EX Agarose Gels and the E-Gel Power Snap Plus Electrophoresis System, can streamline vector preparation and analysis. The following is an example protocol for restriction digestion and visualization of fragments.

Materials

1. Sterile, nuclease-free tubes (1.5 mL) and pipette tips (P10 or P20, P200)
2. Pipettes (P10 or P20, P200)
3. Heat block or water bath
4. E-Gel EX Agarose Gels (1%)
5. E-Gel Power Snap Plus Electrophoresis System
6. FastDigest enzyme and 10X buffer
7. Invitrogen™ E-Gel™ 1 Kb Plus Express DNA Ladder

Table 2. Composition of restriction digestion reaction mixture.

Component	Volume
Water, nuclease-free	15 µL
10X FastDigest Buffer	2 µL
Plasmid DNA	2 µL (up to 1 µg)
FastDigest enzyme	1 µL
Total volume	**20 µL**

Protocol

1. Prepare the restriction digestion reaction mixture at room temperature according to Table 2.
2. Mix gently and spin down.
3. Incubate at 37°C in a heat block or water bath for 5–15 min.*
4. Inactivate the enzyme. Refer to the product data sheet for the enzyme-specific conditions.
5. Prepare the E-Gel 1 Kb Plus Express DNA Ladder by adding 0.6 µL of ladder to 20 µL of nuclease-free water. Gently mix and briefly spin down.
6. Prepare the sample:
   a. Dilute the restriction reaction mixture up to 80x (Table 3).
   b. Optional: Add Invitrogen™ E-Gel™ Sample Loading Buffer to a final concentration of 0.1X.

Table 3. Composition of restriction digestion reaction mixture for electrophoresis.

Component	Volume
Restriction reaction	1 µL
Water, nuclease-free	79 µL
Total volume	**80 µL**

7. Transfer 20 µL of prepared ladder and samples into the wells of the E-Gel agarose gel.
8. Select the dedicated protocol and start the electrophoresis run.
9. Analyze the results using the Invitrogen™ E-Gel™ Power Snap Plus Camera (Figure 2).

* See the product information sheet for enzyme- and substrate-specific incubation times and enzyme inactivation conditions.

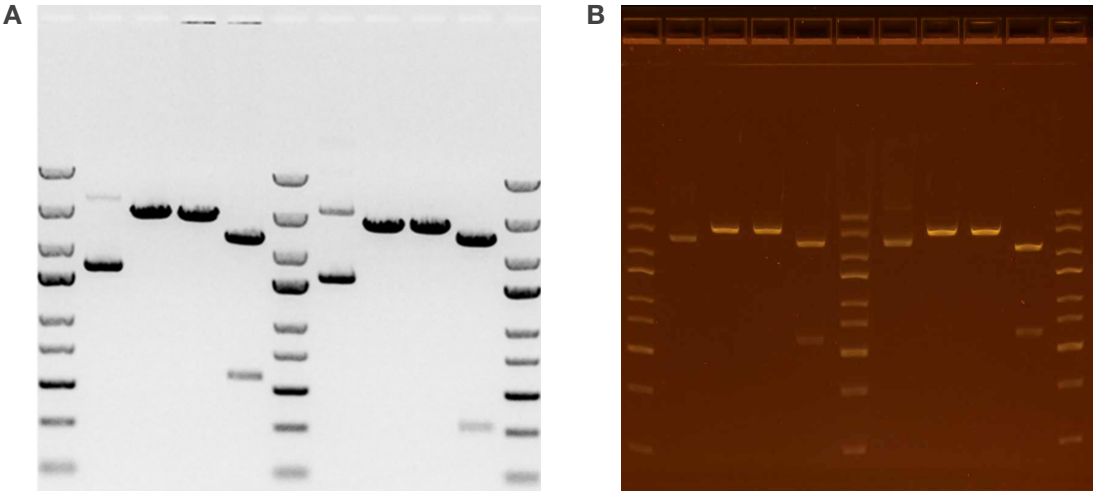

Figure 2. Gel electrophoresis images of restriction digests using (A) conventional methods and (B) FastDigest enzymes with the E-Gel Power Snap Plus Electrophoresis System.

Ordering information

Description	Cat. No.
FastDigest Value Pack	K1991
E-Gel EX Agarose Gels, 1%	G401001
E-Gel 1 Kb Plus Express DNA Ladder	10488091
E-Gel Sample Loading Buffer, 1X	10482055
E-Gel Power Snap Plus Electrophoreis System	G9301, G9311
FastDigest DpnI	FD1704
FastDigest Esp3I (IIs class)	FD0454
FastDigest BshTI	FD1464
FastDigest BpiI (IIs class)	FD1014
FastDigest NheI	FD0974
FastDigest BcuI	FD1254
FastDigest NotI	FD0594
FastDigest BamHI	FD0054
FastDigest SalI	FD0644
FastDigest EcoRI	FD0274
FastDigest KpnI	FD0524
FastDigest PacI	FD2204
FastDigest XhoI	FD0694
FastDigest LguI (IIs class)	FD1934
FastDigest NcoI	FD0574
FastDigest Eco31I (IIs class)	FD0294

Explore our advanced line of 176 FastDigest restriction enzymes offering fast and complete digestion of DNA in a single universal buffer at thermofisher.com/fastdigest.

Learn more at **thermofisher.com/powersnapplus**

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