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CloneChecker™ System

Cat. No. 11666-013 **Size: 100 reactions**

Store at room temperature

Description

CloneChecker™ System is a rapid and efficient method for screening recombinant bacterial colonies for the presence of target plasmid DNA. CloneChecker™ System contains reagents for analysis of plasmid DNA directly from a bacterial colony or from liquid culture. Partially purified plasmid DNA obtained by using CloneChecker™ System reagents can be analyzed by agarose gel electrophoresis and verified by direct size comparison, restriction enzyme analysis, or PCR analysis.

Contents

CloneChecker™ System contains sufficient materials for the analysis of 100 bacterial colonies by Restriction Enzyme Analysis and 100 bacterial colonies by Supercoiled DNA Analysis.

Components:	Quantity
Green Solution	800 µL
Yellow Solution	500 µL
Red Solution	500 µL
Loading Buffer	600 µL

Note: Composition of reagents is proprietary and non-hazardous.

Intended Use

For research use only. Not intended for any animal or human therapeutic or diagnostic use.

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Critical Parameters

1. **Clone Age:** Use freshly transformed bacteria; do not use plated colonies more than 3 days old.
2. **Clone Amount:** Use bacterial colonies grown at least 16 to 20 h. Colonies should be at least 1 to 2 mm in diameter. Avoid agar when picking colony. Using more colony in the protocol could lead to difficulties loading the gel.
3. **Electrophoresis:** Avoid using ethidium bromide in the gel or in the gel running buffer. Post-stain the gel using fresh ethidium bromide at a concentration of 0.2 µg/mL.
4. **Photos:** For low yielding plasmid DNA, use a high aperture (4.5) and a longer exposure time (*e.g*., 5 s).
5. **Enzyme Analysis:** Do not digest DNA with restriction enzyme longer than 30 min. Over incubation results in digestion of genomic DNA and an increased background.

Restriction Enzyme Analysis

After overnight culture (16–20 h) of plated transformation reaction:

1. Pick a bacterial colony (1 to 2 mm in diameter) with a pipette tip and suspend it in 6 µL of fresh growth media (*e.g.,* Luria Broth). Mix thoroughly. **Note**: Ensure that the entire colony is picked, while avoiding agar. Agar may cause inhibition of the reaction.
2. Transfer 3 µL of suspended colony to 8 µL of Green Solution. Mix by pipetting up and down 3 times. **Note:** The remaining 3 µL of suspended colony can be used to inoculate an overnight culture for further analysis.
3. Heat the sample at 100°C for 30 s in a boiling water bath or thermal cycler.
4. After sample cools to room temperature, add 2 µL of digestion mix containing 1 µL of 10X reaction buffer and 1 µL of restriction enzyme (1–10 units per reaction).
5. Incubate sample for 30 min at the appropriate temperature for the restriction enzyme used. When using 1 unit of enzyme, incubate for 30 min. When using 10 units of enzyme, incubate for 10 min.

6. Add 2 µL of Blue Loading Buffer to sample. Mix and load into wells of an agarose gel. After electrophoresis, visualize DNA by staining gel in 0.2 µg/mL ethidium bromide for 10 min.

Supercoiled DNA Analysis

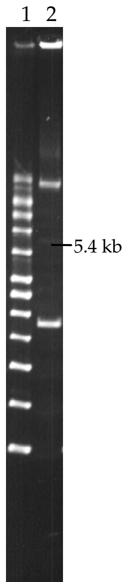
After overnight culture (16–20 h) of plated transformation reaction:

1. Pick a bacterial colony (1 to 2 mm in diameter) with a pipette tip and suspend it in 6 µL of fresh growth media, (*e.g.*, Luria Broth). Mix thoroughly. **Note**: Ensure that the entire colony is picked
2. Transfer 3 µL of suspended colony to 5 µL Red Solution and pipet up and down 3 times. **Note:** The remaining 3 µL of suspended colony can be used to inoculate an overnight culture for further analysis.
3. Add 5 µL of Yellow Solution and pipet up and down 3 times. Vortex 10-15 s.
4. Add 4 µL of Loading Buffer to sample. Vortex 10–15 s immediately before loading into wells of an agarose gel. (Pour electrophoresis buffer into gel box after samples are loaded) For a size marker control, load vector DNA without insert (10–30 ng) mixed in Red and Yellow Solutions as in steps 2 and 3.
5. After electrophoresis, visualize DNA by staining gel in 0.2 µg/mL ethidium bromide for 10 min.

Alternative Protocols

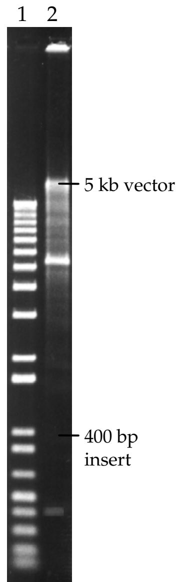
1. Use 3 µL of a bacterial liquid growth culture or glycerol stock instead of a plated colony.
2. For a double restriction digest with incompatible buffers, digest first with the enzyme in the lower salt buffer for 15 min at the appropriate temperature. Then, add salt and the second enzyme and continue incubation for another 15 min.
3. For Supercoiled DNA Analysis, Loading Buffer can be omitted and sample can be loaded directly into the wells before submerging the gel into running buffer. The red color is then used as a tracking dye of mobility equal to ~1.2 kb linear DNA in a 1% agarose gel.

Supercoiled DNA Analysis



Lane 1: Supercoiled DNA Ladder
Lane 2: 5.4 kb Supercoiled DNA Vector

Restriction Endonuclease Analysis



Lane 1: 1Kb Plus DNA Ladder
Lane 2: *Hin*d III digest of 5.4 kb vector DNA

Manufactured by BioChain Institute Inc.

Product Qualification

The Certificate of Analysis provides detailed quality control information for each product. Certificates of Analysis are available at www.invitrogen.com/support.

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