Fermentas
LIFE SCIENCES

CERTIFICATE OF ANALYSIS

DNA Marker II for Genomic DNA Analysis

#SM0351 6µg

Concentration: 0.2µg DNA/µl
Lot:
Supplied with: 1ml 10x Loading Dye Solution
Store at –20°C.

Description

DNA Marker II is designed for genomic DNA analysis in Southern blotting experiments. The DNA Marker is prepared with complete digestion of lambda DNA and ΦX174 DNA with appropriate restriction endonucleases and dissolved in storage buffer. The marker contains 30 discrete DNA bands in the range from 702 to 29946bp.

Storage Buffer

10mM Tris-HCl (pH 7.6), 1mM EDTA.

10x Loading Dye Solution

0.5% bromophenol blue, 50% glycerol and 100mM EDTA.

Quality Control Assay Data

The homogeneity of fragmentation patterns has been verified by agarose gel electrophoresis and by performing Southern analysis experiments (agarose gel electrophoresis, blotting, hybridization with the labeled marker and autoradiography).

Quality authorized by:  Jurgita Zilinskiene

ISO 9001 ISO 14001
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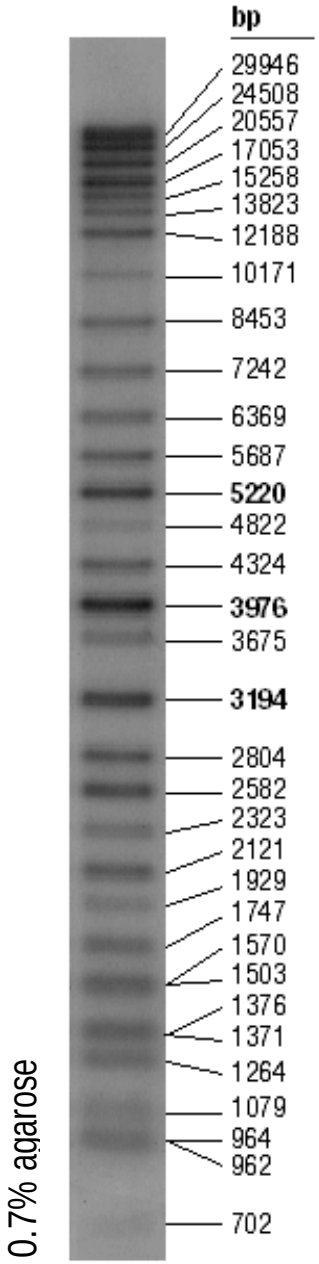
EXPERIMENTAL PROTOCOL

I. Solutions Required:

1. Tris-acetate buffer:
   - 0.04M Tris- acetate,
   - 0.002M EDTA.
2. Denaturation solution:
   - 1.5M NaCl,
   - 0.5M NaOH.
3. Neutralization solution:
   - 1.5M NaCl,
   - 0.5M Tris-HCl (pH7.2),
   - 1mM EDTA.
4. 20x SSC solution (blotting buffer):
   - 3M NaCl,
   - 0.3M sodium citrate,
   - 1mM EDTA.
5. 100x Denhardt's solution:
   - 2% (w/v) BSA (bovine serum albumin),
   - 2% (w/v) Ficoll,
   - 2% (w/v) PVP (polyvinylpyrrolidone).
6. Hybridization solution:
   - 5x SSC,
   - 5x Denhardt's solution,
   - 40% formamide,
   - 0.5% SDS.
7. 1mg/ml Herring sperm DNA solution.
8. 10% (w/v) SDS solution.

DNA Marker II
for Genomic DNA Analysis



50ng of the Marker was run on a 20cm length of 0.7% agarose gel in 1xTAE buffer at 3V/cm 18 hours (until bromophenol blue dye reached the bottom of the gel). Blotting was carried out on Hybond-N^+ membrane, and a [α^{32}P] labeled marker was used as a probe for hybridization. Exposure time - 3 days.

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II. Electrophoresis

Dilute 1µl of this marker with 39µl of deionized water and mix. To 10µl of this mixture (50ng of marker per gel lane or approx 6ng of marker per 1mm of gel lane width) add 1.5µl of 10x Loading Dye Solution and 3.5µl of deionized water and run in a 20cm 0.7% agarose gel in Tris-acetate buffer for 18 hours at 3V/cm (until bromophenol blue marker reaches the bottom of the gel).

Note

The cohesive ends of the 12nt cos site of bacteriophage λ from some fragments may anneal and form the additional bands. Prior to loading on gel, these fragments can be separated by heating at 65°C for 5min and then chilled on ice for 3min.

III. Blotting

1. Before blotting rinse the gel in deionized water and shake in denaturation buffer for 30min at room temperature.
2. Rinse the gel in deionized water and shake in neutralization buffer for 15min at room temperature. Repeat procedure.
3. Fill the glass dish with 20xSSC blotting buffer. Make a platform and cover it with three sheets of Whatman 3MM filter paper, saturated with blotting buffer.
4. Place the gel on the wick and avoid trapping air bubbles beneath it.
5. Cut a sheet of Hybond™ -N^+ membrane to match the size of the gel and place it on the top of the gel. Avoid trapping air bubbles beneath the membrane.
6. Place three sheets of Whatman 3MM filter paper cut to size and wetted with blotting buffer on the top of HybondTM-N^+ membrane.
7. Place a stack of absorbent paper towels on top of the 3MM paper, place a glass plate on the top of the paper towels and put a 1kg weight on the top.
8. Flow to blotting at room temperature for 18 hours in the blotting buffer.
9. Wash the membrane in 2x SSC solution to remove any residual agarose, dry at room temperature and fix for 2min under UV-light.

IV. Radioactive Labeling of Marker

Marker is labeled using HexaLabel™ DNA Labeling Kit, #K0611, #K0612 (Fermentas).

1. Add the following components into a microcentrifuge tube:
   DNA template (50ng) 5µl,
   random primer in 5x reaction buffer 5µl,
   deionized water 10µl.
   Vortex the tube and spin down for 3-5sec. Incubate the tube in a boiling water bath for 5-10min and chill on ice. Spin down quickly.
2. Based on your choise of labeled triphosphate (dATP or dCTP) use Mix A or Mix C, respectively.
3. Add the following components to the same tube:
   Mix A (or Mix C) 1.5µl,
   [α-^{32}P]-dATP (or [α-^{32}P]-dCTP) 3µl,
   (0.925MBq = 25µCi)
   (Klenow fragment (3u/µl) 0.5µl.
   Shake the tube and spin down in a microcentrifuge for 3-5sec.
   Incubate for 10min at 37°C.

4. Add 2µl of the dNTP Mix and incubate for 5min at 37°C.
5. Stop the reaction by addition of 0.5µl of 0.5M EDTA, pH 8.0.
   The labeled marker (specific activity ~1x10^9 dpm/µg) is sufficient for 5 standard hybridization reactions.

V. Hybridization Procedure

1. Prepare 25ml of the hybridization solution.
2. Denature 0.5ml of Herring sperm DNA solution by heating to 100°C for 5min. Chill on ice and add to the hybridization solution.
3. Put the membrane into the hybridization bag and carry out the prehybridization with 12ml of the hybridization solution for 2 hours at 42°C.
4. Denature labeled probe by heating to 100°C for 5min and chill immediately on ice.
5. Add 10-15ml of the hybridization solution and 1/5 of the labeled probe to the hybridization bag.
6. Incubate for at least 12 hours at 42°C.
7. Carry out the following washes of the membrane:
   Twice for 10min in 2x SSC + 0.1% SDS at room temperature
   Twice for 15min in 1x SSC + 0.1% SDS at 65°C
   Twice for 10min in 0.1x SSC + 0.1% SDS at 65°C
8. Dry the membrane using sheets of Whatman 3MM paper.

VI. Autoradiography

Wrap the dried membrane with Saran Wrap™ and expose to a film for 1 day. If necessary, the exposure time can be increased up to 3 days.

Updated July 19, 2002