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DNA Marker 1–10 kb

Introduction

DNA Markers range in size 1 kb to 10 kb for rapid size estimation of PCR[†] products and restriction fragments. Loading 5 μ l per lane yields approximately 50 ng of DNA per band. DNA can be visualized by ethidium bromide staining or kinased with radiolabeled ³²P for detection by autoradiography.

Contents

DNA marker 2 x 250 μl – 100 applications Store at 4°C

6X loading buffer (250 μ l) Contains bromophenol blue Store at 4°C or 25°C

NOTE: Mix small amounts as needed or mix a large amount, aliquot and store at 4°C.

Procedure - Ethidium Bromide Staining

- 1. Mix 5 μ l of DNA marker and 1 μ l of 6X loading buffer.
- 2. Mix 5 parts of your sample to 1 part of 6X loading buffer.
- 3. Load DNA markers and samples onto an agarose gel.
- 4. Electrophorese, stain and photograph following your standard protocol.
- 5. Estimate the size of the sample DNA by reading its relative position to the closest marker.

Procedure for 5' End Radiolabeling

NOTE: The marker can be labeled directly or for more efficient labeling, ethanol precipitate first.

Ethanol Precipitation

- 1. Remove a 100 μ l aliquot of the DNA marker.
- 2. Add 10 µl of 3M potassium acetate, pH 7.4
- 3. Add 300 µl of absolute ethanol.
- 4. Incubate at -70°C for 30 minutes.
- 5. Microcentrifuge at 4°C for 10 minutes.
- 6. Redissolve the pellet in 100 μ l of distilled water.
- Quantitate by reading the absorption at 260 nm and label with [^{γ-32} P]-ATP using T4 polynucleotide kinase and a standard protocol. See Sambrook. 5.68 (1989) or Ausubel, et al., 3.4.3 (1987).

Product Safety:

For details regarding product safety, see Material Safety Data Sheet (MSDS); call (800) 638-8174 for extra copies of the MSDS. Emergency after hours, call collect (303) 595-9048.

Warranty:

Because of the numerous factors affecting results, Lonza DNA markers are sold with the understanding that purchasers will make their own tests to determine the suitability of these markers for their particular purposes. The use suggested by Lonza, is presented only to assist our customers in exploring possible applications for this product. All information and data presented are believed to be accurate and reliable but are presented without the assumption of any liability by Lonza.

References

Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Smith, J.A., Seidman, J.G., and Struhl, K. Current Protocols in Molecular Biology,

John Wiley & Sons, New York 1987. Sambrook, J., Fritsch, E.F., and Maniatis, T. **Molecular Cloning, A Laboratory Manual,** Second Edition, Cold Spring Harbor: Cold Spring Harbor Laboratories 1989

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For more information contact Technical Service at (800) 521-0390 or visit our website at www.Lonza.com.

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[†]The PCR process may be covered by one or more third-party patents.

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