Lonza Rockland, Inc.
www.lonza.com
scientific.support@lonza.com
Scientific Support: 800-521-0390
Customer Service: 800-638-8174
Document # 18672-0111-10
Rockland, ME 04841 USA

DNA Standards in InCert™ Agarose Gel Plugs

Introduction

For size estimation of megabase DNA separated by pulsed field gel (PFG) electrophoresis, Lonza offers 2 DNA standards in InCert™ Agarose Gel Plugs:

- Lambda DNA ladders (c1857S7)
- *Saccharomyces cerevisiae* chromosomal DNA (YPH80)

These DNA standards are of consistent high quality. The agarose used to make the gel plugs is InCert™ Agarose, specifically developed and quality assured for preparation of chromosomal DNA in agarose gel plugs. InCert™ Agarose, having twice the gel strength of other low melting temperature agaroses, forms strong gel plugs which are easier to handle and cut.

Like other Lonza Genetic Technology Grade™ Products, each lot of standard is tested and certified. The standards are quality assured to yield consistent molecular weights, sharp bands, and low background in pulsed field gel electrophoresis. A Certificate of Performance is included with each standard.

Contents

Each standard is prepared in a ready-to-use gel plug format measuring 2 mm x 5 mm x 10 mm. There are five plugs per vial of standard and each plug has sufficient DNA to load at least 4 to 6 lanes as determined on the Pulsaphor® Apparatus.

Description	Size Range	# of bands	cells/plug	µg DNA/plug
Lambda Ladder	48.5 kb- at least 873 kb	18	N.A.	10 ± 2
S. cerevisiae	220 kb- at least 1 Mb	16	5×10^8	5 ± 2

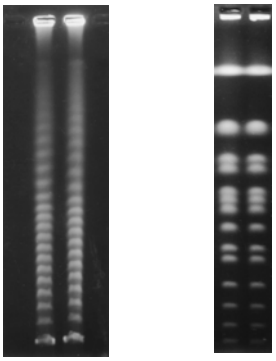
Storage

Store DNA Standards at 4°C.
The standards are supplied in the following buffers:
S. cerevisiae
0.5 M Ethylenediaminetetraacetic acid (EDTA), pH 9
1 mg/ml Proteinase K
1% N-lauroyl sarcosine
Lambda ladders
50 mM EDTA
10 mM [Tris(hydroxymethyl)aminoethane], pH 8.0
5 µg/ml Proteinase K
0.01% N-lauroyl sarcosine

NOTE: Care should be taken in handling the storage buffer and plugs as proteinase K is a powerful protease.
Store removed gel plugs in another sterile tube with fresh, filter-sterilized storage buffer.

Performance of Megabase DNA Standards in PFGE

Lambda Ladders *S. cerevisiae*



Lambda DNA Ladders and *S. cerevisiae* DNA Standards were run on the Bio-Rad® CHEF-DR® III System.

Running Conditions:
Lambda Ladders: 1% SeaKem® GTG™ Agarose, 0.5X TBE, switch angle 120º, 6 V/cm, ramped switch time from 50-90 seconds over 22 hours.

S. cerevisiae: 1% SeaKem® GTG™ Agarose, switch angle 120º, 6 V/cm, ramped 0.5X TBE, switch time from 40-100 seconds over 24 hours.

Handling Standards

When handling the DNA Standards please follow the guidelines below

- Use sterile technique for removing plugs.
- Decant the storage buffer until the top of the plugs are visible.
- Use a sterile, flame-sealed, bent-tipped Pasteur pipette to remove the gel plugs.
- Store removed gel plugs in another sterile tube with fresh, filter-sterilized storage buffer.
- Care should be taken in handling the storage buffer and plugs as proteinase K is a powerful protease.

Equilibration of Gel Plug or Slice Prior to Loading

Before loading, note that the conductivity of the buffer in the agarose gel plug should be equivalent to or less than that of the running buffer and running gel. If the running buffer is 1X TBE, there is no need to dialyze DNA standards prior to loading. However, if the running buffer is TAE, dialyze the gel slice containing the DNA standard by washing in sterile TAE or TE buffer for two – 30 min washes prior to loading and electrophoresing.

Gel Slice Loading

Using a glass cover slip, slice the desired size plug.
We routinely load 1/6 to 1/4 of the gel plug per lane for electrophoresis on the Pulsaphor® System. This amount is sufficient for visualization of standards after ethidium bromide staining. Less plug may be sufficient for other alternating field gel electrophoresis equipment, such as CHEF and OFAGE, where wells can be as narrow as 0.5 mm to 1.0 mm.

Loading Methods

Method I

- Gently push the gel slice into a well with an alcohol-sterilized glass rod, keeping the slice intact until it touches the bottom of the well. Avoid trapping air bubbles or mashing the gel slice.
- Overlay the gel slice with liquid agarose of the same type and concentration as the running gel.
- Electrophorese once the agarose in the wells has set.

Method II

This method saves time and requires a different set-up in gel casting. Gel plugs are applied to the comb prior to casting the gel.

1. Apply gel plugs to the comb teeth with the slice placed level with the bottom of the tooth.
2. Once all of the sample slices are placed on the individual teeth of the comb, the comb is placed in the gel casting tray with the slices facing the anode (in the direction of migration). Both the comb teeth and the gel slices must touch the bottom of the gel tray.
3. Cast the gel as usual. Allow the agarose solution to cool to 65ºC prior to casting the gel.
4. Gently remove the comb from the gel.
5. Fill the wells with liquid agarose of the same type and concentration as the running gel.
6. Electrophorese once the agarose ins the wells has set.

Liquid Gel Loading

Liquid loading is not advised for the Lambda DNA Ladder.
The DNA standard will degrade when heated above 65ºC.
For liquid loading of *S. cerevisiae*:

1. Wash the plug with four 30 minute washes of sterile 50 mM EDTA pH 8.0.
2. Slice and re-melt the slice at 65ºC.
3. Use a wide-bored, plastic pipette or glass capillary to load.

Guidelines for Electrophoresis Conditions

Pulsaphor® System

DNA ranging from 50 kb to 1 Mb,
Lambda ladders and *S. cerevisiae* chromosomal DNA

	Agarose	
	SeaKem® Gold	SeaKem® GTG™ or LE
Agarose conc:	1%	1%
Buffer:	0.5X TAE	1X TBE (PFG)
Temperature	10ºC	10ºC
Voltage:	10 V/cm,	10 V/cm
(33 x 33 cm box)	330 volts	330 volts
Pulse time:	50 sec.	110 sec.
Ramping:	—	—
Length of Run:	20 hr.	40 hr.

0.5X Tris Acetate EDTA Buffer (TAE buffer)	**PFGE-Tris Borate EDTA Buffer (PFG-TBE buffer)**
20 mM Tris-acetate	100 mM Tris
0.5 mM EDTA, pH 8.3	100 mM Boric Acid
	0.2 mM EDTA, pH 8.0

CHEF-DR® III System

	DNA Standard		
	S.cerevisiae ~220 kb to 1,900 kb	*Lambda ladders* 50 kb to 873 kb	S.cerevisiae ~220 kb to 1,900 kb
Agarose:	SeaKem® GTG™ or LE	SeaKem® GTG™ or LE	SeaKem® HGT*
Agarose conc:	1.0%	1.0%	1.0%
Buffer:	0.5X TBE	0.5X TBE	1X TBE
Temperature:	14ºC	14ºC	14ºC
Voltage:	6V/cm	6V/cm	6V/cm
Switch time:	60-120 sec.	50-90 sec.	60-120 sec.
Angle:	120º	120º	120º
Length of Run:	24 hr.	22 hr.	24 hr.

CHEF protocols are derived from Bio-Rad Laboratories, Inc. Instruction Manual and Applications Guide for the CHEF-DR III apparatus.
*SeaKem HGT Agarose replaces FastLane™ Agarose in Pulsed Field Gels.

FIGE system

	DNA Standard	
	S.cerevisiae ~220 kb to 1,900 kb	*Lambda ladders* 50 kb to 873 kb
Agarose:	SeaKem® GTG(M) or SeaKem® LE	SeaKem® GTG™ or SeaKem® LE
Agarose conc:	1.0%	1.0%
Buffer:	0.5X TBE	0.5X TBE
Temperature:	14ºC	14ºC
Voltage & Forward:	6V/cm, 9-60 sec.	6V/cm, 9-60 sec.
Switch time: Backward:	6V/cm, 3-20 sec.	6V/cm, 3-20 sec.
Length of Run:	20 hr.	20 hr.

FIGE protocol – Lai, E. (1991) **RESOLUTIONS®** 7, #2, 4. Buffers listed above are derived from Sambrook, *et al.* Stock TAE and TBE buffers. Alternatively Lonza offers ready-to-use AccuGENE™ 5X and 10X TBE solutions.

Troubleshooting Guide

1. Problem

The DNA standard streaks upon electrophoresis.

Solution

Be certain that the buffer of the DNA standard is of equal or lower conductivity than the buffer used in the running gel. If not, dialyze the plug or slice in the same buffer.

2. Problem

The DNA standard does not remelt.

Solution

The melting temperature of the InCert™ Agarose gel plug may be affected by the buffer in which it is stored. Dialyze the plug or gel slice in 0.5 mM EDTA, pH 8.0 prior to melting.

3. Problem

The Lambda DNA Ladders look compressed with the larger rungs of the ladder having disappeared.

Solution

Be certain not to remelt this standard and not to run this standard at high voltages as the running gel can heat excessively. Exposure of lambda ladders to high temperatures will cause the larger oligomers to fall apart.

4. Problem

DNA banding pattern of the standard is too intense or too faint.

Solution

Try loading different widths of the gel plug to determine the right amount of DNA standard for your system.

References

Schwartz, D.C. and Cantor, C.R. (1984) *Cell* 37: 67-75.

Schwartz, D.C., Saffran, W., Welsh, J., Haas, R., Goldenberg, N., and Cantor, C. (1983) *Cold Spring Harbor Symposia on Quant. Biol.* XLVII: 189-195.

Carle, G.F., and Olson, M.V. (1984) *Nucl. Acids Res*. 12: 5647-5664, 1984.

Bernards, A., Kooter, J.M., Michels, P.A.M., Moberts, R.M.P. and Borst P. (1986) *Gene* 42*:* 313-322.

Southern, E.M., Anand, R., Brown, W.R.A., and Fletcher, D.D. (1987) *Nucl. Acids Res.* 15: 5925-5943.

Carle, G.F., and Olson, M.V. (1985) *Proc. Natl. Acad. Sci. USA* 87*:* 3756.

Mathew, K.M., Smith, C.L., and Cantor, C.R. (1988*) Biochem* 27: 9204-9210.

Smith, C.L., Matsumoto, T., Niwa, O., Fan, J.B., Yanagida, M., and Cantor, C.R. (1987) *Nucl.Acids Res.* 15: 4481-4489

Birren, B., Lai, E., Clark, S.M., Hood, L., and Simon, M.I. (1988) *Nucl. Acids Res.* 16: 7563-7582

Vollrath, D., and Davis, R.W. (1987) *Nucl. Acids Res.* 15: 7865-7876.

Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Smith, J.A., Seidman, J.G., and Struhl, K. (1987) *Current Protocols in Molecular Biology*, John Wiley & Sons, New York.

Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989) *Molecular Cloning, A Laboratory Manual,* Second Edition, Cold Spring Harbor: Cold Spring Harbor Laboratories.

Birren, B., and Lai, E. (1983) *Pulsed Field Gel Electrophoresis, A Practical Guide,* Academic Press, Inc., San Diego.

Patent Position

Lonza does not warrant against infringements of other patents of third parties by reason of any uses made of the product in combination with other material or in the operation of any process, and purchasers assume all risks of patent infringement by reason of any such use, combination, or operation.

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

For Research Use Only.
Not for Use in Diagnostic Procedures.