PureExtreme®

Highest quality & performance

We guarantee that these products are free of contaminating activities.
Our stringent quality control with the most advanced tests guarantees you pure products for your experiments. ISO9001 and ISO14001 is your assurance of consistency and lot-to-lot reproducibility.
PureExtreme® Quality will provide the performance you need for your most demanding experiments.

DNA ELECTROPHORESIS

DNA Electrophoresis: Introduction

Introduction

Agarose and polyacrylamide gel electrophoresis are rapid techniques used to identify, quantify and purify nucleic acids.

DNA molecules are negatively charged due to dissociation of the phosphate backbone. During electrophoresis they migrate towards the positively charged electrode. Small DNA fragments migrate more rapidly in the gel matrix compared to large fragments, resulting in molecule separation based on size.

Agarose Gels

Agarose is a non-toxic polysaccharide extracted from seaweed. It is easy to use and is relatively inexpensive if compared to polyacrylamide. But the overall resolving power is relatively low (*see* p.441) compared to polyacrylamide.

- Standard high melting point agarose is used in routine DNA electrophoresis for separation of a wide range of DNA fragments (**TopVision™ LE GQ Agarose** (#R0491)). Separated fragments can be extracted from the gel by a silica powder-based DNA purification using Fermentas **DNA Gel Extraction Kit** (#K0513).
- Low melting/gelling temperature agarose (**TopVision™ LM GQ Agarose** (#R0801)) is recommended for rapid DNA gel extraction with the agarose digesting enzyme **Agarase** (#E00461) or with the **DNA Gel Extraction Kit** (#K0513). Low melting temperature agarose is also commonly used for "in-gel" DNA treatment with enzymes or for bacterial transformation with nucleic acids directly after re-melting the gel. Low melting point agarose has a lower resolving power than that of standard agarose.

Polyacrylamide Gels

Polyacrylamide is a cross-linked polymer of acrylamide. Polyacrylamide gels have a relatively small range of separation, but they provide very high resolution of DNA molecules. They are normally used for the separation of nucleic acids less than 500 bp (*see* p.441). Under the appropriate conditions, molecules differing in size by a single base pair can be resolved.

Protocols and Recommendations

Separation of Double-stranded and Single-stranded DNA

Both agarose and polyacrylamide gels can be used for separation of double-stranded and single-stranded DNA molecules.

- Double-stranded DNA molecules can be separated on agarose or polyacrylamide gels under non-denaturing conditions (protocols on pp.442, 443). The size and approximate quantity of the DNA molecule is determined by comparing the DNA band of interest with the bands of an appropriate Fermentas DNA ladder on the same gel. For the majority of our ladders we provide data on the quantity of each DNA fragment allowing for quick sample quantification.
- Single-stranded DNA molecules are separated on polyacrylamide gels in the presence of strong denaturants (7-8 M urea or formamide) and at high temperature (protocol on p.444) or in agarose gels at alkaline pH (protocol on p.443).

Denaturing Polyacrylamide Gel Electrophoresis (PAGE)

Commonly, dsDNA markers are used for denaturing PAGE, even though none of them is specially designed for this application. Under denaturing conditions, dsDNA size standards frequently give unrecognizable banding patterns. To address this problem, we offer precise data about the migration of several of our DNA ladders (**GeneRuler™ Ultra Low Range** (#SM1211/2) and **Low Range** (#SM1191/2)) under denaturing conditions, as well as a protocol for denaturing PAGE (p.444). When used with our **2X RNA Loading Dye** (#R0641), these ladders achieve excellent separation under denaturing conditions and allow for a precise sizing of ssDNA molecules.

DNA Loading Dyes

Prior to loading the DNA sample on a gel, it is necessary to mix it with an appropriate loading buffer or loading dye solution.

DNA loading dyes normally contain:

- Glycerol to ensure that the sample easily sinks into the well.
- EDTA, which binds divalent metal ions that may interfere with electrophoresis. By complexing metal ions, EDTA also stops metal-dependent enzymatic reactions such as DNA degradation by nucleases.
- Tracking dyes (bromophenol blue, xylene cyanol FF, orange G) to monitor the progress of electrophoresis by the migration of the dyes (Tables 9.4, 9.5 on p.441). Loading dyes are supplied with each DNA ladder/marker and are also available separately:
- **6X DNA Loading Dye** (#R0611)
- **6X MassRuler™ DNA Loading Dye** (#R0621)
- **6X Orange DNA Loading Dye** (#R0631)
- **6X DNA Loading Dye & SDS Solution** (#R1151)
- **6X TriTrack™ DNA Loading Dye** (#R1161)
- **2X RNA Loading Dye** (#R0641).

Please refer to p.438 to choose loading dye for your application.

Electrophoresis Conditions

The electrophoretic mobility of DNA molecules depends on the voltage and the composition of the electrophoresis buffer. Fermentas offers concentrated solutions of Tris-borate and Tris-acetate electrophoresis buffers (p.437):

- **10X TBE Buffer** (#B52)
- **50X TAE Buffer** (#B49).

Depending on the size of DNA molecules, appropriate buffer and voltage should be chosen. For detailed information please *see* **Protocols and Recommendations for DNA Electrophoresis** on p.441.

Gel Staining

Intercalating dyes such as ethidium bromide or SYBR® Green I allow for DNA visualization directly on a gel under UV light.

Up to 10 ng of DNA can be detected on a gel with ethidium bromide. The detection threshold on gels stained with SYBR® Green I is about 60 pg of DNA. For detection of femtograms quantities of DNA, Fermentas offers a number of DNA labeling and detection techniques (*see* Molecular Labeling & Detection on p.373).

Discrepancies in DNA Mobility

The electrophoretic mobility of DNA is influenced by primary, secondary and tertiary DNA structures. These structures may appear as artifacts in electrophoresis. During high resolution electrophoresis, DNA molecules of the same size can have different migration patterns due to variations in their sequence.

Gel-shifts or diffused bands can appear due to DNA/protein interactions (*see* Fig.9.10 on p.446). The presence of salts and other components in the DNA solutions can also result in DNA mobility shifts. Ethidium bromide present in the electrophoresis buffer and the gel can change the mobility of large DNA molecules resulting in incomplete separation of high molecular weight DNA fragments.

Secondary and tertiary DNA structures present in nicked, supercoiled and dimeric molecules will always have different mobilities on a gel compared to linear DNA standards of the same size (*see* p.450).

For further details about discrepancies in DNA mobility please *see* the **Troubleshooting Guide for DNA Electrophoresis** (p.450).

Detailed **Protocols and Recommendations** on p.441 contains information and technical assistance to ensure success in DNA electrophoresis.

DNA Ladders and Markers

Fermentas offers a broad selection of DNA ladders/markers ranging from 10 bp to 48.5 kb for accurate analysis of linear double-stranded DNA in agarose or polyacrylamide gels. DNA ladders/markers are manufactured and quality controlled to supersede all industry standards, making Fermentas the quality leader for DNA standards. DNA ladders are made of chromatography-purified individual DNA fragments using a proprietary patent-pending technology for preparation of pharmaceutical grade plasmid DNA.

Fermentas PureExtreme® restriction enzymes are used for the production of DNA fragments for our ladders/markers. These products are stable during prolonged incubations at room temperature and multiple freeze-thaw cycles.

Typical data regarding the purity of Fermentas DNA ladders and markers is presented in Fig.9.1 below.

In addition to our standard DNA ladders/markers, Fermentas also offers a **NoLimits™ Bulk Custom DNA Ladder Service**. Using the **Collection of Individual NoLimits™ DNA Fragments** covering a 10-20,000 bp range (p.419.), we can create a custom DNA ladder based on customer-specified range and composition. For more details about **NoLimits™ Custom DNA Ladders** *see* p.419.

Distinguishing features

- Bright, sharp bands for highly accurate sizing.
- Ready-to-use DNA ladders/markers are stable at room temperature for at least 6 months.
- Supplied with loading dye for sample DNA.
- Large packages are provided in small aliquots to minimize the possibility of contamination during usage.
- Available in bulk quantities.

The main features of our DNA ladders and markers and their applications are listed on p.414.

Fermentas carries the following groups of DNA ladders/markers:

- **NoLimits™ Custom DNA ladders** produced upon request. DNA ladders of any fragment size and quantity combination for a variety of applications (p.419).
- **GeneRuler™ and O'GeneRuler™ DNA ladders** are designed for sizing and quantification of broad range of DNA fragments (p.420).
- **MassRuler™ DNA ladders** are designed for sizing and highly accurate DNA quantification on gels (p.424).
- **FastRuler™ DNA ladders** are designed for fast high throughput electrophoresis (p.426).
- **O'RangeRuler™ DNA ladders** are step ladders for precise sizing of DNA fragments differing in 5, 10, 20, 50, 100, 200, 500 bp increments (p.428).
- **ZipRuler™ Express DNA ladders** are designed for fast and accurate sizing of broad range DNA fragments (p.430).
- **Conventional Lambda DNA Markers** are designed for determining the size of large DNA fragments (p.432).
- **Conventional Phage and Plasmid DNA Markers** are classical markers for small DNA fragment analysis (p.434).
- **Markers for Genomic DNA Analysis**, are specifically designed for genomic DNA analysis in Southern blots. These ladders are prepared from lambda and ΦX174 DNA and do not contain any plasmid sequence (p.436).

Unique Fermentas **Express DNA ladders** are specifically designed for fast fragment separation (e.g. in 5-15 min at 23 V/cm) under a wide range of electrophoresis conditions. Those ladders supplement GeneRuler™, O'GeneRuler™ and MassRuler™ ladder groups.

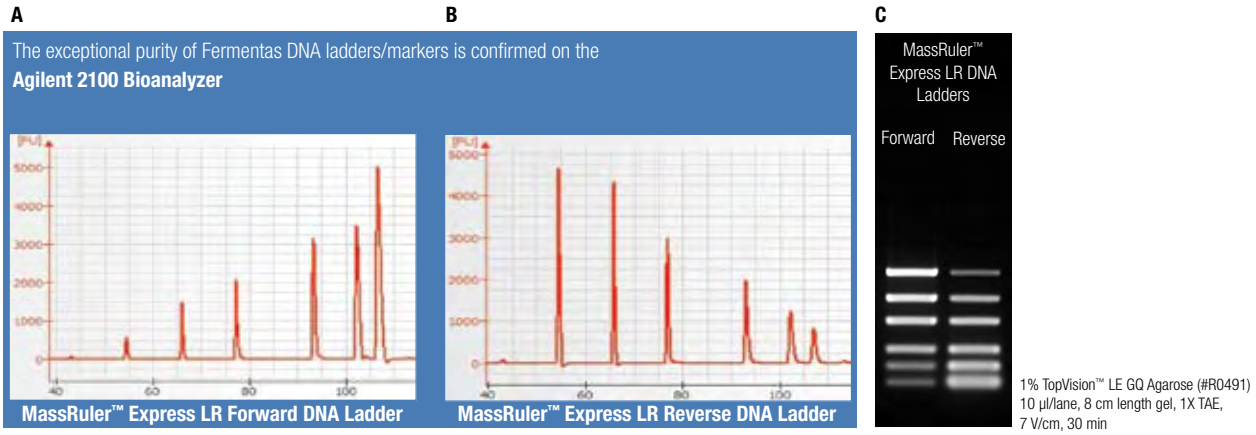


Figure 9.1. Analysis of the MassRuler™ Express LR Forward and Reverse DNA Ladders (#SM1263 and #SM1273) using the Agilent 2100 Bioanalyzer DNA 1000 LabChip® Kit and agarose gel electrophoresis.
A – analysis of MassRuler™ Express LR Forward DNA Ladder
B – analysis of MassRuler™ Express LR Reverse DNA Ladder
C – agarose gel analysis of MassRuler™ Express LR Forward and Reverse DNA Ladders

DNA Ladders and Markers: Group Selection Guide

DNA ladder/marker group	Features						Applications						Page
							Electrophoresis			Quanti-fication	Labeling* with		
	Formulation	Origin	Range, bp	Separation time on agarose	Main feature	Loading dyes supplied	Agarose	PAGE	Fast		T4 Polynucleotide Kinase (radio-active)	Klenow Frag.exo- (filling in 3′ recessed termini)	
NoLimits™ Custom DNA Ladders	In TE buffer or ready-to-use	Individual, chromato-graphy-purified DNA fragments	10-20,000	Composition dependent	Formulated according to client's specifications. Bulk orders	Any Fermentas Loading Dye upon request	✓	✓	✓	✓	✓	–	419
GeneRuler™ and O'GeneRuler™ DNA Ladders	In TE buffer or ready-to-use	Mixture of individual, chromato-graphy-purified DNA fragments	10-48,502	10 min-1.5 h	Sizing of broad range DNA fragments	6X DNA Loading Dye or 6X Orange Loading Dye	✓	✓	–	✓	✓	–	420
MassRuler™ DNA Ladders	Ready-to-use	Mixture of individual, chromato-graphy-purified DNA fragments	80-10,000	10-45 min	Accurate DNA quantification	6X MassRuler™ Loading Dye	✓	–	✓	✓	–	–	424
FastRuler™ DNA Ladders	Ready-to-use	Mixture of individual, chromato-graphy-purified DNA fragments	10-10,000	8-14 min	Fast high throughput short distance electrophoresis	6X MassRuler™ Loading Dye, 6X Orange Loading Dye	✓	✓	✓	✓	✓	–	426
O'RangeRuler™ DNA Ladders	Ready-to-use	Ligation products of 10, 15, 20, 50, 100, 200, 500 bp DNA fragments	10-6,000	45 min-1.5 h	Step ladders of 5, 10, 20, 50, 100, 200, 500 bp increments	6X Orange Loading Dye	✓	✓	–	–	–	–	428
ZipRuler™ Express DNA Ladders	Ready-to-use	Mixture of individual, chromato-graphy-purified DNA fragments	100-20,000	10-20 min	Fast and precise sizing of broad range DNA fragments	6X Orange Loading Dye	✓	–	✓	✓	–	–	430
Conventional Lambda DNA Markers	In TE buffer or ready-to-use	Lambda DNA digested with restriction enzymes	15-48,502	45 min-18 h	Determination of the size of large DNA fragments	6X DNA Loading Dye	✓	–	–	✓	✓	✓	432
Conventional Phage and Plasmid DNA Markers	In TE buffer or ready-to-use	Phage and plasmid DNA digested with restriction enzymes	8-1,353	45 min-1.5 h	Classical markers for small DNA analysis	6X DNA Loading Dye	✓	✓	–	✓	✓	✓	434
Markers for Genomic DNA Analysis	In TE buffer	Lambda DNA and ΦX174 DNA digested with restriction enzymes	702-29,946	18 hours	Plasmid DNA-free, used for genomic DNA analysis in Southern blots	10X DNA Loading Dye	✓	–	–	–	✓	**	436

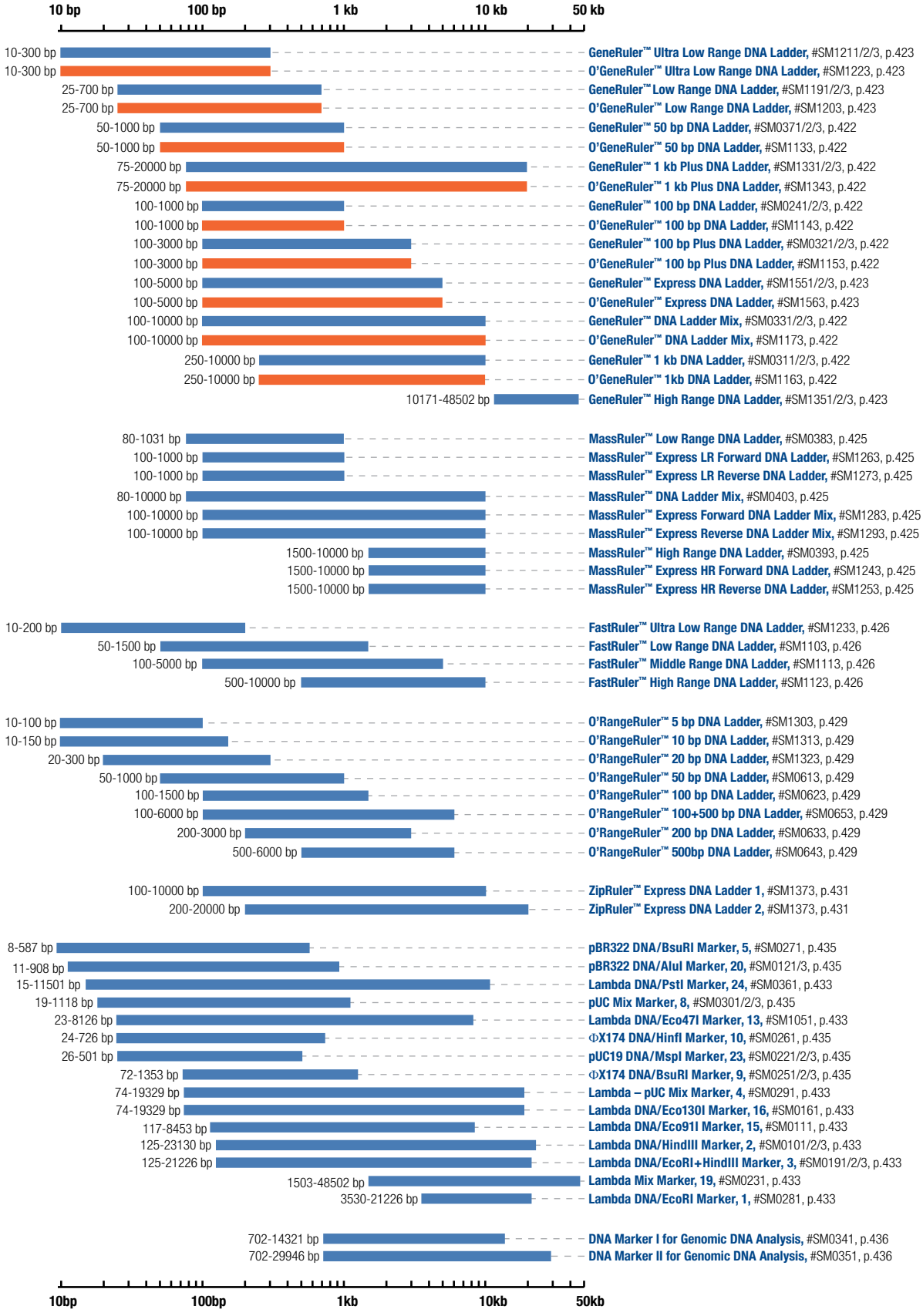
Note

Not all the DNA ladders/markers of the group are suitable for the indicated application.
For more details, *see* description of the DNA ladder/marker group on the indicated pages.

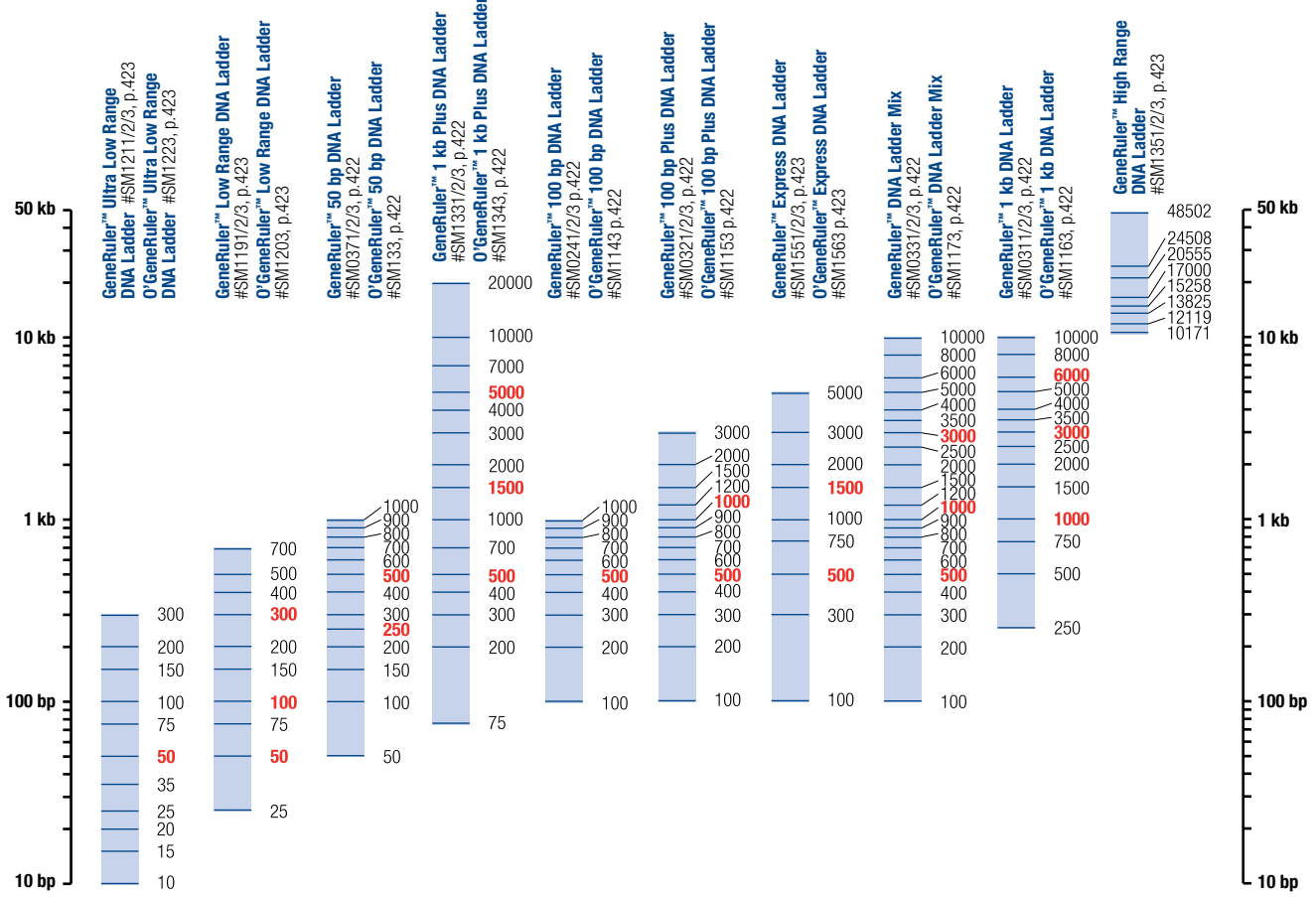
* Only DNA ladders/markers supplied in TE buffer can be labeled.

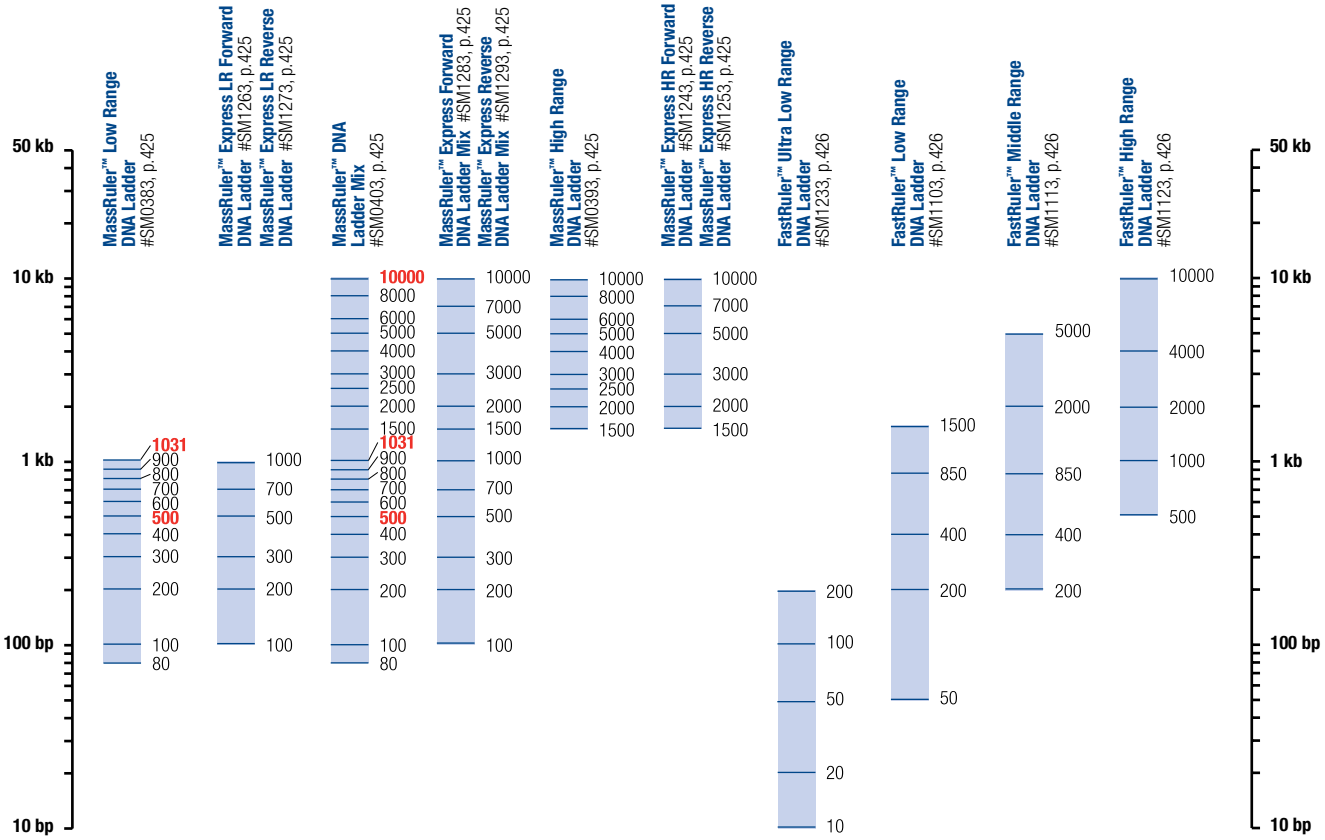
** Markers for Genomic DNA analysis are detected on Southern blots (*see* protocol on p.388) with the radioactive probe of the marker generated by a random-primed labeling with Klenow Fragment, exo⁻ (#EP0421) or DecaLabel™ DNA Labeling Kit (#K0621).

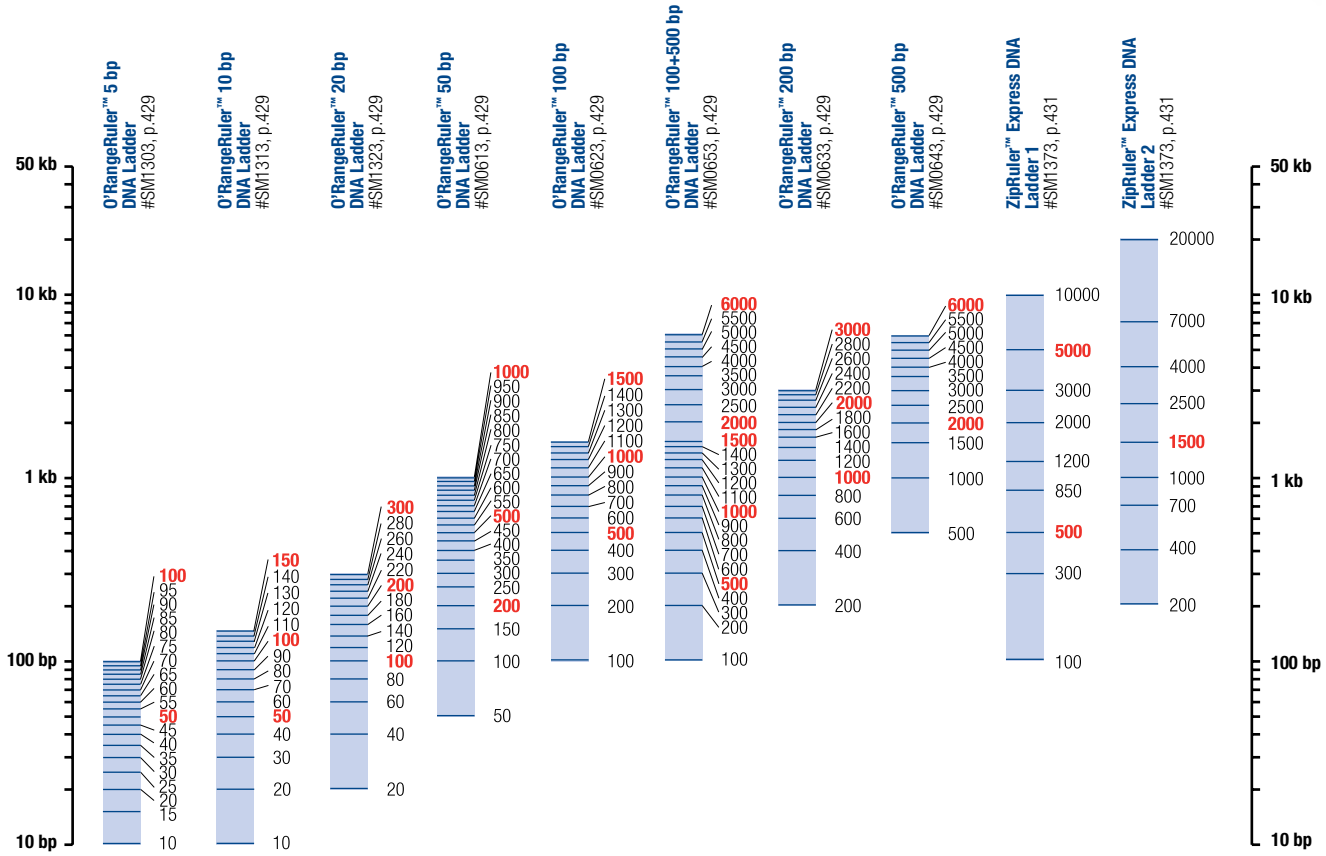
DNA Ladders and Markers: Range Selection Guide

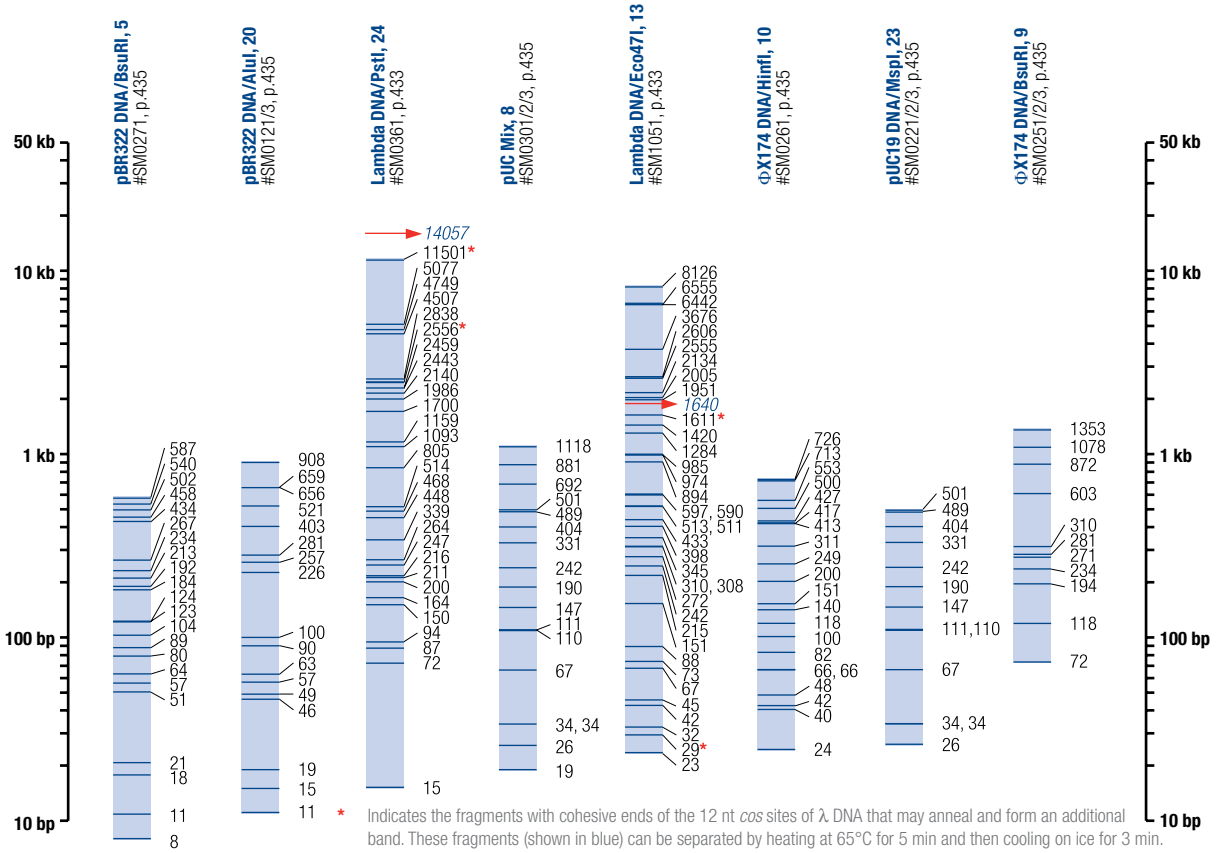


DNA Ladders and Markers: Reference Guide



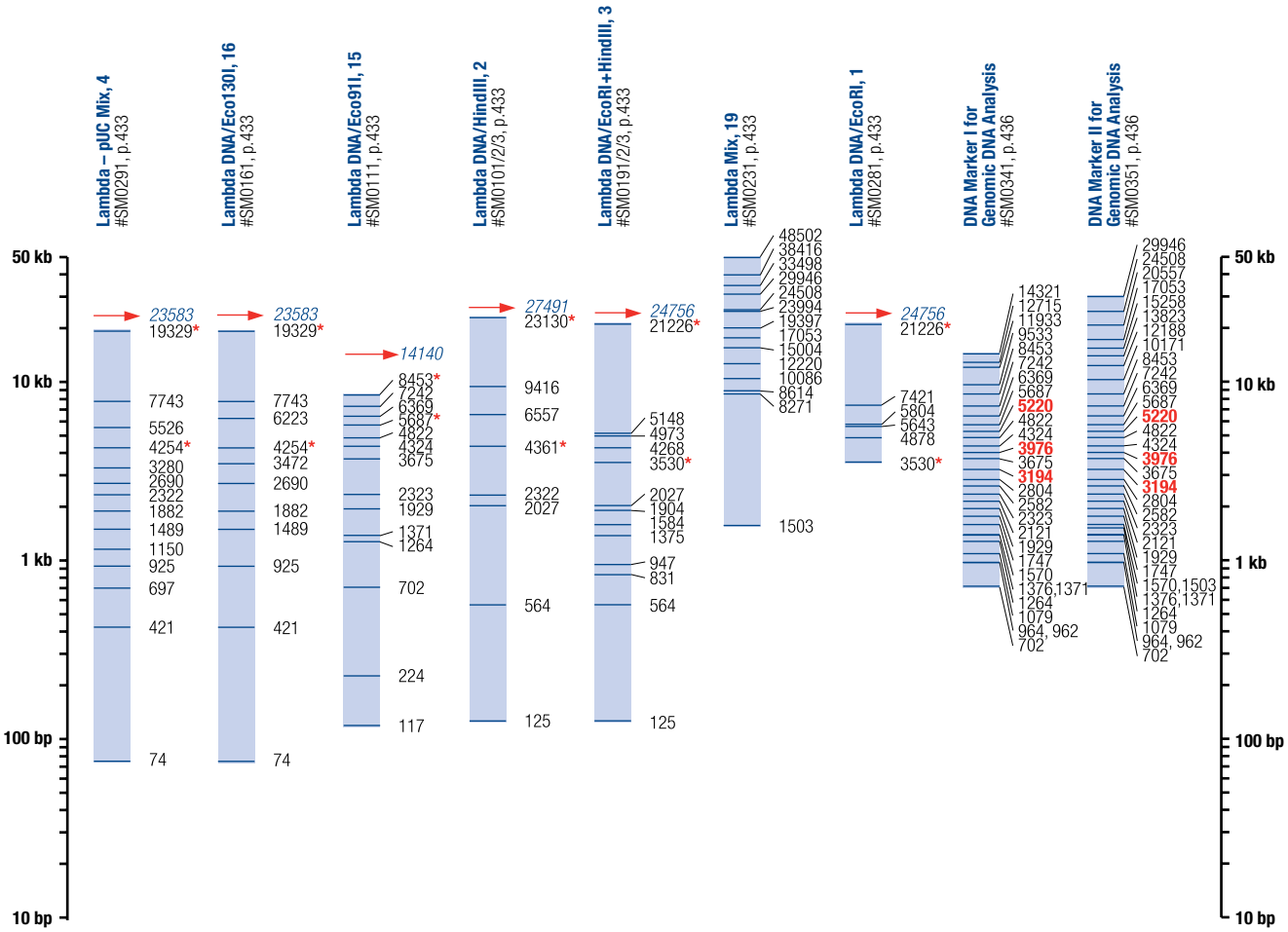






* Indicates the fragments with cohesive ends of the 12 nt *cos* sites of λ DNA that may anneal and form an additional band. These fragments (shown in blue) can be separated by heating at 65°C for 5 min and then cooling on ice for 3 min.

→ Indicates a possible additional band due to annealing of cohesive ends of the fragments from 12 nt *cos* sites of λ DNA. Reference bands appear in red.



Note

* Indicates the fragments with cohesive ends of the 12 nt *cos* sites of λ DNA that may anneal and form an additional band. These fragments (shown in blue) can be separated by heating at 65°C for 5 min and then cooling on ice for 3 min.

→ Indicates a possible additional band due to annealing of cohesive ends of the fragments from 12 nt *cos* sites of λ DNA. Reference bands appear in red.

NoLimits™ Individual DNA Fragments and Custom DNA Ladders -20°

DNA fragment size	Catalog #	Amount, µg
10 bp	SM1391	10
15 bp	SM1381	10
20 bp	SM1401	10
25 bp	SM1761	10
35 bp	SM1411	10
50 bp	SM1421	10
75 bp	SM1431	10
100 bp	SM1441	10
150 bp	SM1601	10
200 bp	SM1611	10
250 bp	SM1451	10
300 bp	SM1621	10
400 bp	SM1631	10
500 bp	SM1641	10
600 bp	SM1461	10
700 bp	SM1651	10
750 bp	SM1471	10
800 bp	SM1481	10
850 bp	SM1661	10
900 bp	SM1491	10
1000 bp	SM1671	10
1200 bp	SM1681	10
1500 bp	SM1691	10
2000 bp	SM1701	10
2500 bp	SM1571	10
3000 bp	SM1711	10
3500 bp	SM1501	10
4000 bp	SM1721	10
5000 bp	SM1731	10
6000 bp	SM1511	10
7000 bp	SM1741	10
8000 bp	SM1521	10
10000 bp	SM1751	10
15000 bp	SM1531	10
17000 bp	SM1771	10
20000 bp	SM1541	10

Collection of Individual DNA Fragments

Description

NoLimits™ is a collection of 36 highly-purified individual DNA fragments ranging from 10-20,000 bp.
NoLimits™ DNA fragments are not produced by PCR like custom standards from other vendors.
Fermentas DNA fragments are produced using specifically designed plasmids purified by a proprietary patent-pending technology. Plasmid DNA is digested with PureExtreme® restriction enzymes and the individual DNA fragments are chromatography-purified from the digestion mixture. As a result, we offer exceptionally pure DNA fragments that are free of any truncated or degraded molecules. Our custom DNA fragments are the best choice for applications ranging from electrophoresis (both gel and capillary) to HPLC and beyond.
Detailed information about the NoLimits™ collection can be found on www.fermentas.com.

Features

- Chromatography-purified individual DNA fragments.
- Broad range: 10-20,000 bp.
- Sharp peaks during capillary electrophoresis, sharp bands during gel electrophoresis.
- Precise DNA concentration.

Applications

- Gel electrophoresis.
- Capillary electrophoresis.
- DNA quantification.
- HPLC analysis.
- Applications where a custom DNA size standard is required.

Concentration

0.5 µg/µl

Storage Buffer (*TE buffer*)

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

Quality Control

Tested on an Agilent 2100 bioanalyzer and by appropriate gel electrophoresis. DNA concentration determined spectrophotometrically. The absence of nucleases confirmed by appropriate tests.

Storage

Store at -20°C.

1.1% TopVision™ LE GQ Agarose (#R0491), 1X TAE, 7 V/cm, 30 min.
NoLimits™ DNA fragments from 300 bp to 10 kb.

NoLimits™ Custom DNA Ladders Service

Bulk orders can be customized to fit your needs:

- Individual DNA fragments or mixtures.
- Custom ladders of any combination and concentration.
- Reference bands in a custom-made DNA ladder.
- Flexible formulations.
- Ready-to-use versions premixed with any loading dye (*see* p.438).
- From microgram to gram quantities.

How to Order?

Contact your local Fermentas representative:
see Contacts on www.fermentas.com

Bulk quantities and custom formulations available upon request

GeneRuler™ and O'GeneRuler™ DNA Ladders (10-20,000 bp)

Related Products

- TopVision™ Agaroses p.440
- Loading Dyes p.438
- 50X TAE Buffer p.437
- 10X TBE Buffer p.437
- Agarase p.348
- DNA Gel Extraction Kit p.347
- T4 Polynucleotide Kinase p.246
- ATP p.481
- 0.5 M EDTA, pH 8.0 p.491
- Water, nuclease-free p.490

Description

GeneRuler™ DNA ladders are recommended for sizing and approximate quantification of a wide range of double-stranded DNA fragments in agarose or polyacrylamide gels. These ladders are mixtures of chromatography-purified individual DNA fragments.

The GeneRuler™ group features our most popular ladders such as the **GeneRuler™ 100 bp** and **1 kb DNA Ladder**. Broader versions of these ladders – **100 bp Plus**, **1 kb Plus** and **Ladder Mix** – are also available.

GeneRuler™ DNA ladders are available in two formats: conventional (TE buffer), and a convenient ready-to-use format premixed with 6X DNA Loading Dye for direct loading onto agarose or polyacrylamide gels.

Conventional versions can be labeled radioactively with T4 Polynucleotide Kinase (#EK0031), *see* protocol on p.381.

O'GeneRuler™ DNA ladders are another ready-to-use version of GeneRuler™ DNA ladders. They are premixed with 6X Orange DNA Loading Dye, which contains xylene cyanol FF and orange G. In a 1% agarose gel orange G dye migrates at 50 bp. Therefore O'GeneRuler™ DNA ladders are ideal when visualization of small DNA fragments is important.

GeneRuler™ Ultra Low Range and Low Range DNA ladders are ideal for denaturing polyacrylamide gel electrophoresis. For more details, please *see* page 444.

GeneRuler™ and O'GeneRuler™ Ultra Low Range DNA ladders are ideal for analysis of siRNA (*see* Fig. 10.2 on p.456 in RNA electrophoresis chapter).

GeneRuler™ High Range DNA Ladder is ideal for fast sizing of high molecular weight DNA fragments (in 1.5 h, in 0.4% agarose gel).

GeneRuler™ and O'GeneRuler™ Express DNA ladders are designed for fast separation (in 5-15 min at 23 V/cm) under a wide range of electrophoresis conditions (Table 9.1 on p.423). The band pattern of express ladders is not affected by the composition of electrophoresis buffer, voltage or gel percentage.

The ladders are supplied (depending on the version) either with 6X DNA Loading Dye or with 6X Orange DNA Loading Dye.

Features

- Ideal for both DNA sizing and approximate quantification.
- Sharp bands.
- Bright reference bands (given in red).
- Ready-to-use ladders can be directly loaded and are stable at room temperature for 6 months.
- Supplied with loading dye for sample DNA.

Storage Buffer *(TE buffer)*

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

GeneRuler™ Storage and Loading Buffer (for ready-to-use ladders)

10 mM Tris-HCl (pH 7.6), 10 mM EDTA, 0.005% bromophenol blue, 0.005% xylene cyanol FF and 10% glycerol.

6X DNA Loading Dye

10 mM Tris-HCl (pH 7.6), 0.03% bromophenol blue, 0.03% xylene cyanol FF, 60% glycerol and 60 mM EDTA.

O'GeneRuler™ Storage and Loading Buffer

10 mM Tris-HCl (pH 7.6), 10 mM EDTA, 0.025% orange G, 0.005% xylene cyanol FF and 10% glycerol.

6X Orange DNA Loading Dye

10 mM Tris-HCl (pH 7.6), 0.15% orange G, 0.03% xylene cyanol FF, 60% glycerol and 60 mM EDTA.

Quality Control

Tested in appropriate gel electrophoresis applications. Concentration of each DNA fragment and of the complete ladder determined spectrophotometrically. The absence of nucleases confirmed by appropriate tests.

Storage

Store at -20°C.

Ready-to-use versions can be stored at room temperature or at 4°C for 6 months. For longer periods, store at -20°C.

Protocols and Recommendations

» General Recommendations p.441
» Preparation of Gels for DNA Electrophoresis p.442
» DNA Ladder/Sample Preparation p.445
» Labeling 5'-termini of DNA by T4 Polynucleotide Kinase p.381
» Troubleshooting Guide p.447

Supplied with:
6X DNA Loading Dye 1 ml/2 ml/10 ml
6X Orange DNA Loading Dye 1 ml/2 ml/10 ml

For sizing and approximate quantification of a wide range of DNA fragments on agarose and polyacrylamide gels.

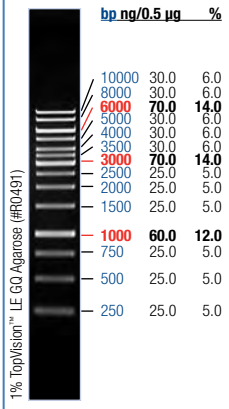
DNA ladder	Catalog #	Concentration, µg/µl	Amount, µg	Applications, 0.5µg/lane	Loading, µg(µl)/lane	Range, bp	Fragments	Agarose, %	PAGE, %
GeneRuler™ 1 kb DNA Ladder	SM0311 SM0312	0.5	250 (5x50) 1250 (25x50)	500 2500	0.5 (1)	250-10000	14	0.7-1.2	–
GeneRuler™ 1 kb DNA Ladder, ready-to-use	SM0313	0.1	250 (5x50)	500	0.5 (5)				
O'GeneRuler™ 1 kb DNA Ladder, ready-to-use	SM1163								
GeneRuler™ 1 kb Plus DNA Ladder	SM1331 SM1332	0.5	250 (5x50) 1250 (25x50)	500 2500	0.5 (1)	75-20000	15	0.7-1.2	–
GeneRuler™ 1 kb Plus DNA Ladder, ready-to-use	SM1333	0.1	250 (5x50)	500	0.5 (5)				
O'GeneRuler™ 1 kb Plus DNA Ladder, ready-to-use	SM1343								
GeneRuler™ DNA Ladder Mix	SM0331 SM0332	0.5	250 (5x50) 1250 (25x50)	500 2500	0.5 (1)	100-10000	21	0.7-1.2	–
GeneRuler™ DNA Ladder Mix, ready-to-use	SM0333	0.1	250 (5x50)	500	0.5 (5)				
O'GeneRuler™ DNA Ladder Mix, ready-to-use	SM1173								
GeneRuler™ 100 bp DNA Ladder	SM0241 SM0242	0.5	50 250 (5x50)	100 500	0.5 (1)	100-1000	10	1.7-2.5	4-8
GeneRuler™ 100 bp DNA Ladder, ready-to-use	SM0243	0.1	50	100	0.5 (5)				
O'GeneRuler™ 100 bp DNA Ladder, ready-to-use	SM1143								
GeneRuler™ 100 bp Plus DNA Ladder	SM0321 SM0322	0.5	50 250 (5x50)	100 500	0.5 (1)	100-3000	14	1.7-2.5	–
GeneRuler™ 100 bp Plus DNA Ladder, ready-to-use	SM0323	0.1	50	100	0.5 (5)				
O'GeneRuler™ 100 bp Plus DNA Ladder, ready-to-use	SM1153								
GeneRuler™ 50 bp DNA Ladder	SM0371 SM0372	0.5	50 250 (5x50)	100 500	0.5 (1)	50-1000	13	1.7-2.5	4-8
GeneRuler™ 50 bp DNA Ladder, ready-to-use	SM0373	0.1	50	100	0.5 (5)				
O'GeneRuler™ 50 bp DNA Ladder, ready-to-use	SM1133								
GeneRuler™ Ultra Low Range DNA Ladder	SM1211 SM1212	0.5	50 250 (5x50)	50-100 250-500	0.5-1 (1-2)	10-300	11	4.5-5.0	8-10
GeneRuler™ Ultra Low Range DNA Ladder, ready-to-use	SM1213	0.1	50	100	0.5-1 (5-10)				
O'GeneRuler™ Ultra Low Range DNA Ladder, ready-to-use	SM1223								
GeneRuler™ Low Range DNA Ladder	SM1191 SM1192	0.5	50 250 (5x50)	50-100 250-500	0.5-1 (1-2)	25-700	10	2.5-3.0	8-10
GeneRuler™ Low Range DNA Ladder, ready-to-use	SM1193	0.1	50	100	0.5-1 (5-10)				
O'GeneRuler™ Low Range DNA Ladder, ready-to-use	SM1203								
GeneRuler™ High Range DNA Ladder	SM1351 SM1352	0.5	50 250 (5x50)	100 500	0.5 (1)	10171-48502	8	0.3-0.5	–
GeneRuler™ High Range DNA Ladder, ready-to-use	SM1353	0.1	50	100	0.5 (5)				
GeneRuler™ Express DNA Ladder	SM1551 SM1552	0.5	50 250 (5x50)	100 500	0.5 (1)	100-5000	9	1.7-2.5	–
GeneRuler™ Express DNA Ladder, ready-to-use	SM1553	0.1	50	100	0.5 (5)				
O'GeneRuler™ Express DNA Ladder, ready-to-use	SM1563								

(continued on next page)

Bulk quantities and custom formulations available upon request

GeneRuler™ 1 kb DNA Ladder
#SM0311/2/3

O'GeneRuler™ 1 kb DNA Ladder, ready-to-use
#SM1163

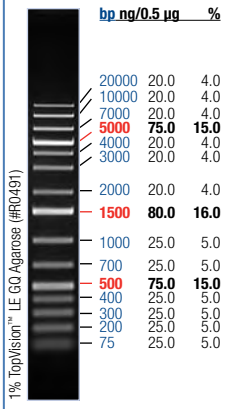


bp	ng/0.5 µg	%
10000	30.0	6.0
8000	30.0	6.0
6000	**70.0**	**14.0**
5000	30.0	6.0
4000	30.0	6.0
3500	30.0	6.0
3000	**70.0**	**14.0**
2500	25.0	5.0
2000	25.0	5.0
1500	25.0	5.0
1000	**60.0**	**12.0**
750	25.0	5.0
500	25.0	5.0
250	25.0	5.0

0.5 µg/lane, 8 cm length gel,
1X TAE, 7 V/cm, 45 min

GeneRuler™ 1 kb Plus DNA Ladder
#SM1331/2/3

O'GeneRuler™ 1 kb Plus DNA Ladder, ready-to-use
#SM1343

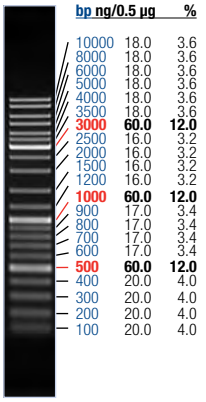


bp	ng/0.5 µg	%
20000	20.0	4.0
10000	20.0	4.0
7000	20.0	4.0
5000	**75.0**	**15.0**
4000	20.0	4.0
3000	20.0	4.0
2000	20.0	4.0
1500	**80.0**	**16.0**
1000	25.0	5.0
700	25.0	5.0
500	**75.0**	**15.0**
400	25.0	5.0
300	25.0	5.0
200	25.0	5.0
75	25.0	5.0

0.5 µg/lane, 8 cm length gel,
1X TAE, 7 V/cm, 45 min

GeneRuler™ DNA Ladder Mix
#SM0331/2/3

O'GeneRuler™ DNA Ladder Mix, ready-to-use
#SM1173

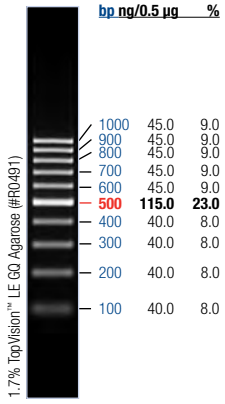


bp	ng/0.5 µg	%
10000	18.0	3.6
8000	18.0	3.6
6000	18.0	3.6
5000	18.0	3.6
4000	18.0	3.6
3500	18.0	3.6
3000	**60.0**	**12.0**
2500	16.0	3.2
2000	16.0	3.2
1500	16.0	3.2
1200	16.0	3.2
1000	**60.0**	**12.0**
900	17.0	3.4
800	17.0	3.4
700	17.0	3.4
600	17.0	3.4
500	**60.0**	**12.0**
400	20.0	4.0
300	20.0	4.0
200	20.0	4.0
100	20.0	4.0

0.5 µg/lane, 8 cm length gel,
1X TAE, 7 V/cm, 45 min

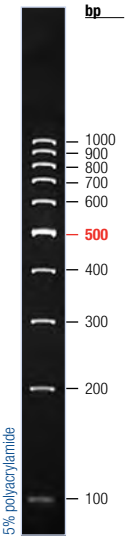
GeneRuler™ 100 bp DNA Ladder
#SM0241/2/3

O'GeneRuler™ 100 bp DNA Ladder, ready-to-use
#SM1143



bp	ng/0.5 µg	%
1000	45.0	9.0
900	45.0	9.0
800	45.0	9.0
700	45.0	9.0
600	45.0	9.0
500	**115.0**	**23.0**
400	40.0	8.0
300	40.0	8.0
200	40.0	8.0
100	40.0	8.0

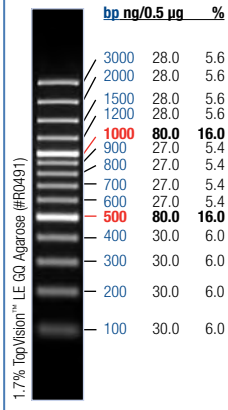
0.5 µg/lane, 8 cm length gel,
1X TBE, 5 V/cm, 1 h



0.5 µg/lane, 20 cm length gel,
1X TAE, 8 V/cm, 3 h

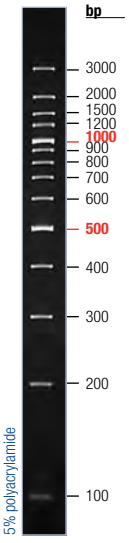
GeneRuler™ 100 bp Plus DNA Ladder
#SM0321/2/3

O'GeneRuler™ 100 bp Plus DNA Ladder, ready-to-use
#SM1153



bp	ng/0.5 µg	%
3000	28.0	5.6
2000	28.0	5.6
1500	28.0	5.6
1200	28.0	5.6
1000	**80.0**	**16.0**
900	27.0	5.4
800	27.0	5.4
700	27.0	5.4
600	27.0	5.4
500	**80.0**	**16.0**
400	30.0	6.0
300	30.0	6.0
200	30.0	6.0
100	30.0	6.0

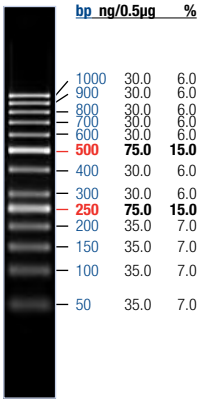
0.5 µg/lane, 8 cm length gel,
1X TBE, 5 V/cm, 1 h



0.5 µg/lane, 20 cm length gel,
1X TAE, 8 V/cm, 3 h

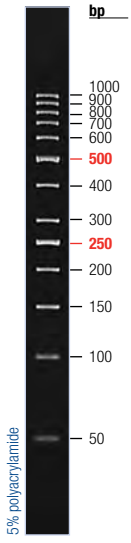
GeneRuler™ 50bp DNA Ladder
#SM0371/2/3

O'GeneRuler™ 50bp DNA Ladder, ready-to-use
#SM1133



bp	ng/0.5µg	%
1000	30.0	6.0
900	30.0	6.0
800	30.0	6.0
700	30.0	6.0
600	30.0	6.0
500	**75.0**	**15.0**
400	30.0	6.0
300	30.0	6.0
250	**75.0**	**15.0**
200	35.0	7.0
150	35.0	7.0
100	35.0	7.0
50	35.0	7.0

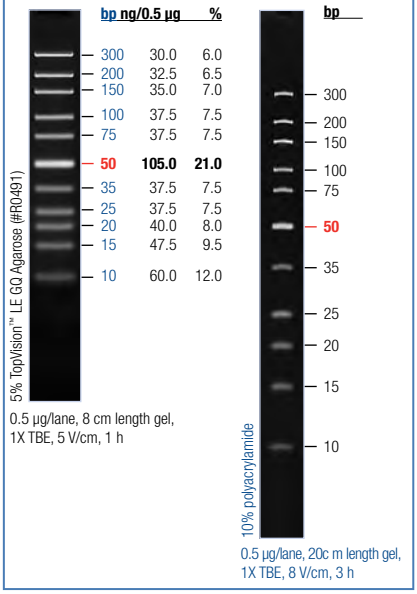
0.5 µg/lane, 8 cm length gel,
1X TBE, 5 V/cm, 1 h



0.5 µg/lane, 20 cm length gel,
1X TAE, 8 V/cm, 3 h

GeneRuler™ Ultra Low Range DNA Ladder
#SM1211/2/3

O'GeneRuler™ Ultra Low Range DNA Ladder, ready-to-use
#SM1223

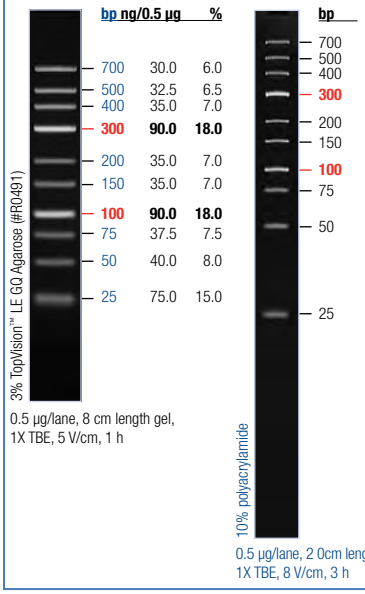


0.5 µg/lane, 8 cm length gel, 1X TBE, 5 V/cm, 1 h

0.5 µg/lane, 20c m length gel, 1X TBE, 8 V/cm, 3 h

GeneRuler™ Low Range DNA Ladder
#SM1191/2/3

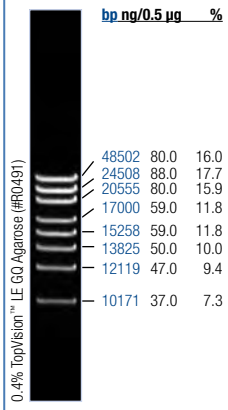
O'GeneRuler™ Low Range DNA Ladder, ready-to-use
#SM1203



0.5 µg/lane, 8 cm length gel, 1X TBE, 5 V/cm, 1 h

0.5 µg/lane, 2 0cm length gel, 1X TBE, 8 V/cm, 3 h

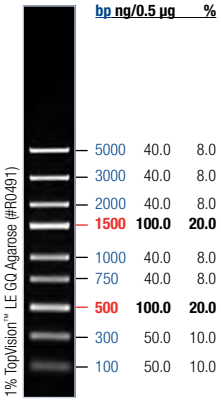
GeneRuler™ High Range DNA Ladder
#SM1351/2/3



0.4 µg/lane, 8 cm length gel, 1X TAE, 5 V/cm, 1.5 h

GeneRuler™ Express DNA Ladder
#SM1551/2/3

O'GeneRuler™ Express DNA Ladder, ready-to-use
#SM1563



0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 40 min

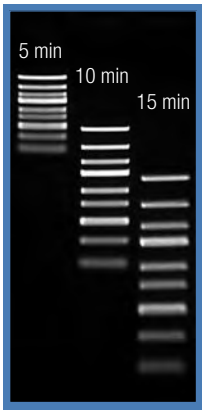


Figure 9.2. Fast separation of GeneRuler™ Express DNA Ladder at high voltage (23 V/cm). Electrophoresis conditions: 0.5 µg/lane, 1% TopVision™ LE GQ Agarose (#R0491), 1X TAE, 23 V/cm, 5-15 min.

Table 9.1. GeneRuler™ Express DNA Ladder Separation Guide for Various Electrophoresis Conditions.

GeneRuler™ Express DNA Ladder												
Duration of electrophoresis 23 V/cm	0.8% agarose		1% agarose		1.2% agarose		1.5% agarose		2% agarose		2.5% agarose	
	TAE	TBE	TAE	TBE	TAE	TBE	TAE	TBE	TAE	TBE	TAE	TBE
5 min												
10 min												
15 min												
20 min												

Excellent separation of all bands
Incomplete separation of the two closest bands
No separation
Lowest bands run off of gel (8 cm)

Bulk quantities and custom formulations available upon request

MassRuler™ DNA Ladders, ready-to-use (80-10,000 bp) RT

Supplied with:
6X MassRuler™ DNA Loading Dye 1 ml

For accurate quantification and sizing of DNA fragments on agarose gels

DNA ladder, ready-to-use	Catalog #	Concentration, ng/µl	Volume, µl	Applications	Loading, µl/lane	Range, bp	Fragments	Agarose, %
MassRuler™ Low Range DNA Ladder	SM0383	60.8	2x500	50-200	5-20	80-1031	11	1.7-2.5
MassRuler™ Express LR Forward DNA Ladder	SM1263	28				100-1000	6	0.7-2.0
MassRuler™ Express LR Reverse DNA Ladder	SM1273							
MassRuler™ High Range DNA Ladder	SM0393	42.2	2x500	50-200	5-20	1500-10000	9	0.7-1.2
MassRuler™ Express HR Forward DNA Ladder	SM1243	28.5					6	0.7-1.5
MassRuler™ Express HR Reverse DNA Ladder	SM1253							
MassRuler™ DNA Ladder Mix	SM0403	103	2x500	50-200	5-20	80-10000	20	1.0-1.2
MassRuler™ Express Forward DNA Ladder Mix	SM1283	56.5				100-10000	12	0.7-1.5
MassRuler™ Express Reverse DNA Ladder Mix	SM1293							

Description

MassRuler™ DNA ladders are specially designed for accurate quantification and sizing of DNA fragments by agarose gel electrophoresis. The intensity of the fragment in each ladder is calibrated against a standard that guarantees the precise quantity of each band. The ladders are mixtures of chromatography-purified, individual DNA fragments.

MassRuler™ Express Forward and Reverse DNA Ladders allow fast and reliable DNA quantification in short separation distances under variuos electrophoresis conditions (Table 9.2 below). In the forward versions, the mass of each DNA fragment is directly proportional to the fragment size, whereas in the reverse versions, the mass is inversely proportional to fragment's size.

Features

- Accurate DNA sizing and quantification – DNA ladder fragments are quantified spectrophotometrically.
- Sharp bands.
- Easy-to-remember fragment sizes and quantities.
- Ready-to-use – premixed with 6X MassRuler™ DNA Loading Dye for direct loading and room temperature storage.
- Supplied with loading dye for sample DNA.

Storage and Loading Buffer

10 mM Tris-HCl (pH 7.6), 10 mM EDTA, 0.005% bromophenol blue, 10% glycerol.

6X MassRuler™ DNA Loading Dye

10 mM Tris-HCl (pH 7.6), 0.03% bromophenol blue, 60% glycerol and 60 mM EDTA.

Quality Control

Tested in appropriate gel electrophoresis applications. Concentration of each DNA fragment and of the complete ladder determined spectrophotometrically. The absence of nucleases confirmed by appropriate tests.

Storage

Store at room temperature or at 4°C for 6 months. For longer periods, store at -20°C.

Related Products

- TopVision™ Agaroses p.440
- Loading Dyes p.438
- 50X TAE Buffer p.437
- 10X TBE Buffer p.437
- Agarase p.348
- DNA Gel Extraction Kit p.347
- 0.5 M EDTA, pH 8.0 p.491
- Water, nuclease-free p.490

Table 9.2. MassRuler™ Express DNA Ladder Separation Guide for Various Electrophoresis Conditions.

MassRuler™ Express LR Forward & Reverse DNA Ladders										
Duration of electrophoresis 7 V/cm	0.7% agarose		1% agarose		1.3% agarose		1.5% agarose		2% agarose	
	TAE	TBE	TAE	TBE	TAE	TBE	TAE	TBE	TAE	TBE
10 min										
20 min										
30 min										
40 min										
50 min										

MassRuler™ Express HR Forward & Reverse DNA Ladders								
Duration of electrophoresis 7V/cm	0.7% agarose		1% agarose		1.3% agarose		1.5% agarose	
	TAE	TBE	TAE	TBE	TAE	TBE	TAE	TBE
10 min								
20 min								
30 min								
40 min								
50 min								

MassRuler™ Express Forward & Reverse DNA Ladders Mix								
Duration of electrophoresis 7 V/cm	0.7% agarose		1% agarose		1.3% agarose		1.5% agarose	
	TAE	TBE	TAE	TBE	TAE	TBE	TAE	TBE
10 min								
20 min								
30 min								
40 min								
50 min								

Excellent separation of all bands
Incomplete separation of the two closest bands
No separation

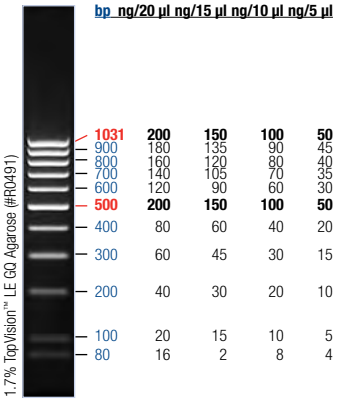
Protocols and Recommendations

» General Recommendations p.441
» Preparation of Gels for DNA Electrophoresis p.442
» DNA Ladder/Sample Preparation p.445
» Troubleshooting Guide p.447

Ready-to-use | 4° Store at 4°C | RT Store at Room Temperature | -20° Store at -20°C

MassRuler™ Low Range DNA Ladder, ready-to-use

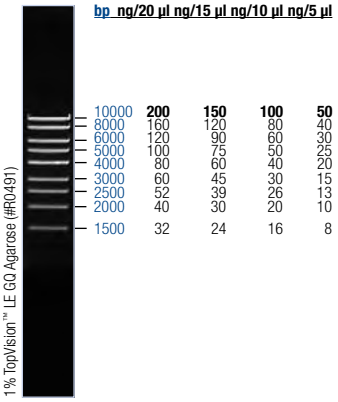
#SM0383



10 µl/lane, 8 cm length gel,
1X TBE, 5 V/cm, 1.5 h

MassRuler™ High Range DNA Ladder, ready-to-use

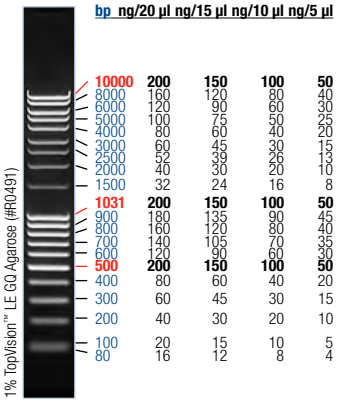
#SM0393



10 µl/lane, 8 cm length gel,
1X TAE, 7 V/cm, 45 min

MassRuler™ DNA Ladder Mix, ready-to-use

#SM0403



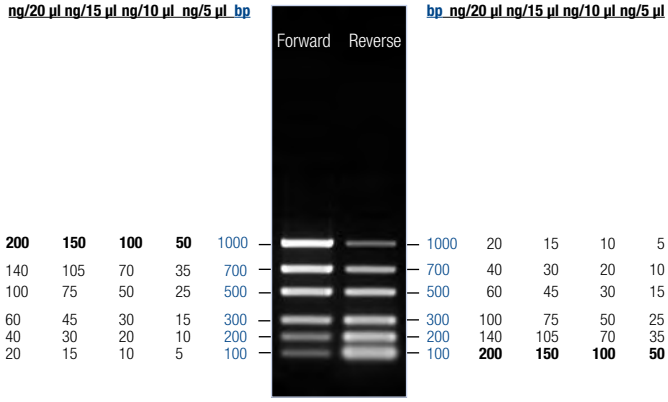
10 µl/lane, 8 cm length gel,
1X TAE, 7 V/cm, 45 min

MassRuler™ Express LR Forward DNA Ladder, ready-to-use

#SM1263

MassRuler™ Express LR Reverse DNA Ladder, ready-to-use

#SM1273



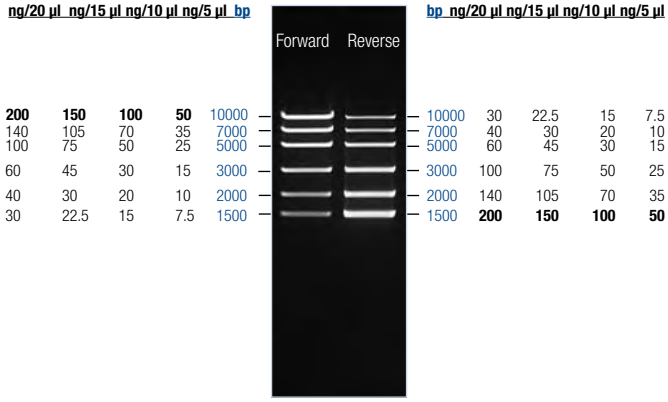
1% TopVision™ LE GQ Agarose (#R0491)
10 µl/lane, 8 cm length gel, 1X TAE, 7 V/cm, 30 min

MassRuler™ Express HR Forward DNA Ladder, ready-to-use

#SM1243

MassRuler™ Express HR Reverse DNA Ladder, ready-to-use

#SM1253



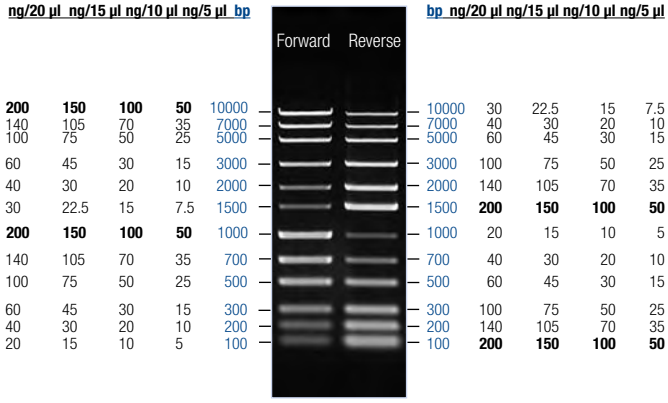
1% TopVision™ LE GQ Agarose (#R0491)
10 µl/lane, 8 cm length gel, 1X TAE, 7 V/cm, 30 min

MassRuler™ Express Forward DNA Ladder Mix, ready-to-use

#SM1283

MassRuler™ Express Reverse DNA Ladder Mix, ready-to-use

#SM1293



1% TopVision™ LE GQ Agarose (#R0491)
10 µl/lane, 8 cm length gel, 1X TAE, 7 V/cm, 30 min

Bulk quantities and custom formulations available upon request

FastRuler™ DNA Ladders, ready-to-use (10-10,000 bp) RT

Supplied with:
6X MassRuler™ DNA Loading Dye 1 ml
6X Orange DNA Loading Dye 1 ml

For fast (8-14 min) DNA sizing and quantification in high throughput (48-96-well) short run agarose gels

DNA ladder, ready-to-use	Catalog #	Concentration, ng/µl	Volume, µl	Applications	Loading, µl/lane	Range, bp	Fragments	Agarose, %
FastRuler™ Ultra Low Range DNA Ladder	SM1233	22.2	2x500	50-333	3-20	10-200	5	4.0
FastRuler™ Low Range DNA Ladder	SM1103	20				50-1500		2.0
FastRuler™ Middle Range DNA Ladder	SM1113	20				100-5000		1.0
FastRuler™ High Range DNA Ladder	SM1123	20				500-10000		1.0

Related Products

- TopVision™ Agaroses p.440
- Loading Dyes p.438
- 50X TAE Buffer p.437
- 10X TBE Buffer p.437
- Agarase p.348
- DNA Gel Extraction Kit p.347
- T4 Polynucleotide Kinase p.246
- ATP p.481
- 0.5 M EDTA, pH 8.0 p.491
- Water, nuclease-free p.490

Description

FastRuler™ DNA ladders are specifically designed for fast sizing and approximate quantification of double-stranded DNA in 48 well (or 96-well) high throughput gels, as well as in conventional agarose gels. These ladders are mixtures of five blunt-ended chromatography-purified individual DNA fragments that are easily resolved in a short separation distance (10-20 mm) after an 8-14 min run on the appropriate agarose gel. **FastRuler™ Ultra Low Range DNA Ladder, ready-to-use** contains dephosphorylated DNA fragments and is ideal for forward 5'-end labeling with T4 Polynucleotide Kinase (#EK0031) (*see* the Certificate of Analysis for the detailed labeling protocol).

Features

- Fast separation (8-14 min).
- Short separation distance (10-20 mm).
- Sharp bands, easy-to-remember fragment sizes and quantities.
- Ready-to-use – premixed with loading dye.
- Supplied with loading dye solution for sample DNA.

Storage and Loading Buffer

10 mM Tris-HCl (pH 7.6), 10 mM EDTA, 0.005% bromophenol blue, 10% glycerol.

Storage and Loading Buffer (for FastRuler™ Ultra Low Range)

10 mM Tris-HCl (pH 7.6), 10 mM EDTA, 0.025% orange G, 0.005% xylene cyanol FF, 10% glycerol.

6X MassRuler™ DNA Loading Dye

10 mM Tris-HCl (pH 7.6), 0.03% bromophenol blue, 60% glycerol and 60 mM EDTA.

6X Orange DNA Loading Dye

10 mM Tris-HCl (pH 7.6), 0.15% orange G, 0.03% xylene cyanol FF, 60% glycerol and 60 mM EDTA.

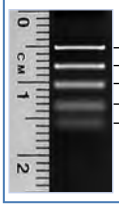
Quality Control

Tested in appropriate gel electrophoresis applications. Concentration of each DNA fragment and of the complete ladder determined spectrophotometrically. The absence of nucleases confirmed by appropriate tests.

Storage

Store at room temperature or at 4°C for 6 months. For longer periods, store at -20°C.

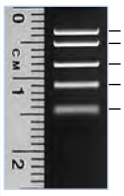
FastRuler™ Ultra Low Range DNA Ladder, ready-to-use
#SM1233



bp	ng/20 µl	ng/15 µl	ng/10 µl	ng/5 µl	ng/3 µl
200	80	60	40	20	12
100	80	60	40	20	12
50	80	60	40	20	12
20	80	60	40	20	12
10	124	93	62	31	19

4% TopVision™ LE GQ Agarose (#R0491)
20 µl/lane, 1X TBE, 7 V/cm, 14 min

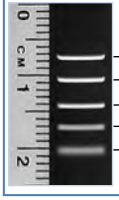
FastRuler™ Low Range DNA Ladder, ready-to-use
#SM1103



bp	ng/20 µl	ng/15 µl	ng/10 µl	ng/5 µl	ng/3 µl
1500	80	60	40	20	12
850	80	60	40	20	12
400	80	60	40	20	12
200	80	60	40	20	12
50	80	60	40	20	12

2% TopVision™ LE GQ Agarose (#R0491)
20 µl/lane, 1X TBE, 7 V/cm, 14 min

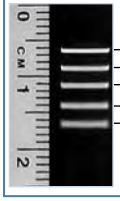
FastRuler™ Middle Range DNA Ladder, ready-to-use
#SM1113



bp	ng/20 µl	ng/1 5µl	ng/10 µl	ng/5 µl	ng/3 µl
5000	80	60	40	20	12
2000	80	60	40	20	12
850	80	60	40	20	12
400	80	60	40	20	12
100	80	60	40	20	12

1% TopVision™ LE GQ Agarose (#R0491)
20 µl/lane, 1X TAE, 7 V/cm, 14 min

FastRuler™ High Range DNA Ladder, ready-to-use
#SM1123



bp	ng/20 µl	ng/15 µl	ng/10 µl	ng/5 µl	ng/3 µl
10000	80	60	40	20	12
4000	80	60	40	20	12
2000	80	60	40	20	12
1000	80	60	40	20	12
500	80	60	40	20	12

1% TopVision™ LE GQ Agarose (#R0491)
20 µl/lane, 1X TAE, 7 V/cm, 14 min

Protocols and Recommendations

» General Recommendations p.441
» Preparation of Gels for DNA Electrophoresis p.442
» DNA Ladder/Sample Preparation p.445
» Troubleshooting Guide p.447

Ready-to-use | 4° Store at 4°C | RT Store at Room Temperature | -20° Store at -20°C

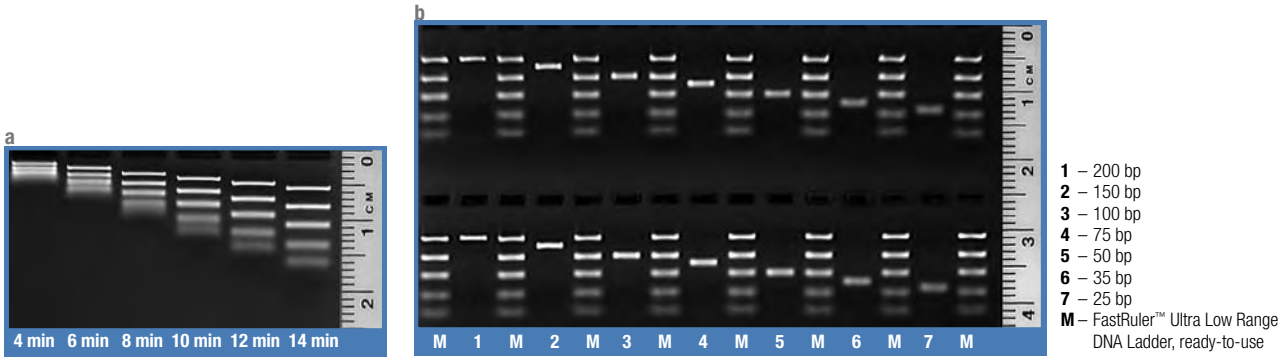


Figure 9.3. FastRuler™ Ultra Low Range DNA Ladder, ready-to-use.

a. Time course of band separation. Electrophoresis conditions: 20 µl/lane, 4% TopVision™ LE GQ Agarose (#R0491), 1X TBE, 7 V/cm.
b. Performance in 96-well format gel electrophoresis. Electrophoresis conditions: 10 µl/lane, 4% TopVision™ LE GQ Agarose (#R0491), 1X TBE, 7 V/cm, 14 min.

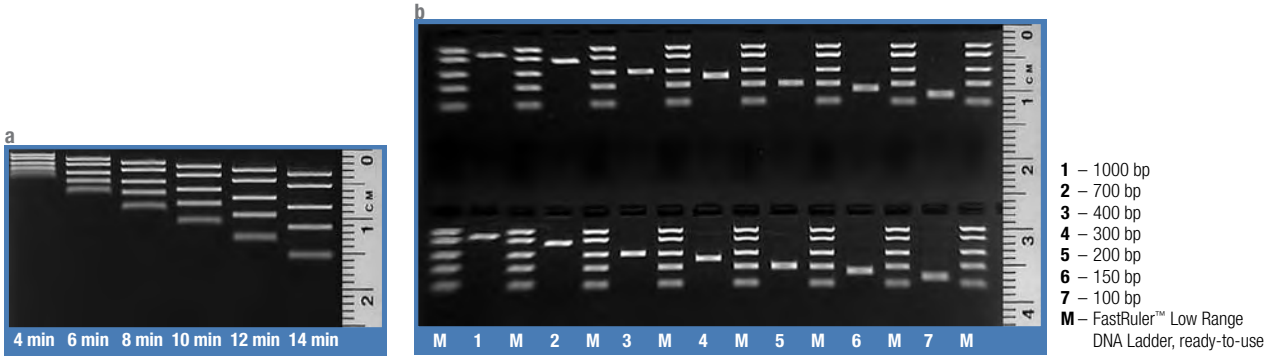


Figure 9.4. FastRuler™ Low Range DNA Ladder, ready-to-use.

a. Time course of band separation. Electrophoresis conditions: 20 µl/lane, 2% TopVision™ LE GQ Agarose (#R0491), 1X TBE, 7 V/cm.
b. Performance in 96-well format gel electrophoresis. Electrophoresis conditions: 10 µl/lane, 2% TopVision™ LE GQ Agarose (#R0491), 1X TBE, 7 V/cm, 14 min.

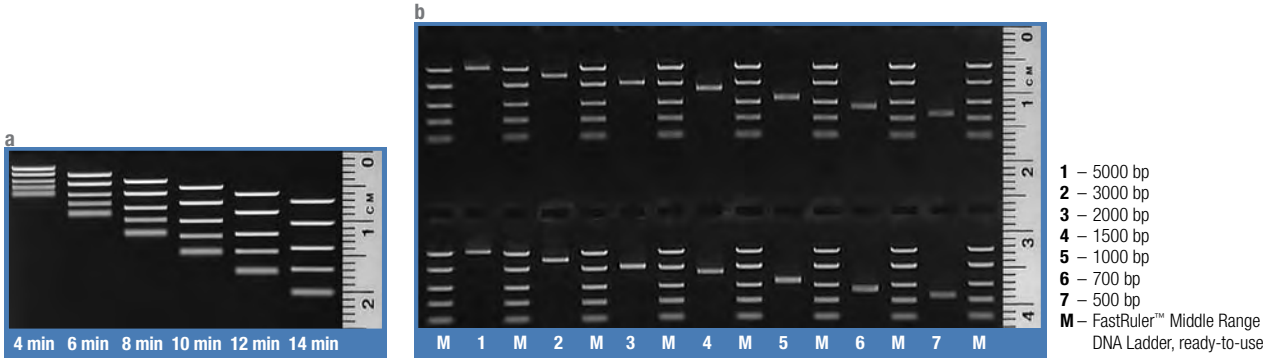


Figure 9.5. FastRuler™ Middle Range DNA Ladder, ready-to-use.

a. Time course of band separation. Electrophoresis conditions: 20 µl/lane, 1% TopVision™ LE GQ Agarose (#R0491), 1X TAE, 7 V/cm.
b. Performance in 96-well format gel electrophoresis. Electrophoresis conditions: 10 µl/lane, 1% TopVision™ LE GQ Agarose (#R0491), 1X TAE, 7 V/cm, 14 min.

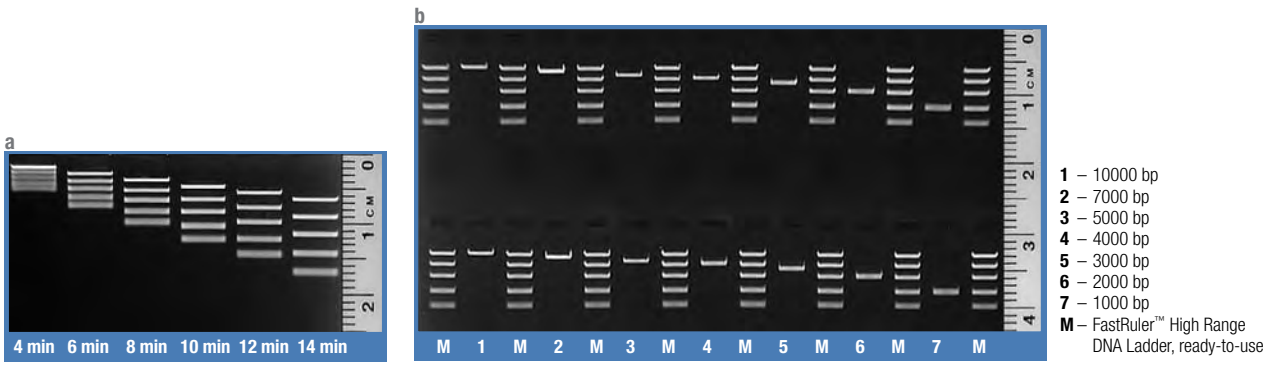


Figure 9.6. FastRuler™ High Range DNA Ladder, ready-to-use.

a. Time course of band separation. Electrophoresis conditions: 20 µl/lane, 1% TopVision™ LE GQ Agarose (#R0491), 1X TAE, 7 V/cm.
b. Performance in 96-well format gel electrophoresis. Electrophoresis conditions: 10 µl/lane, 1% TopVision™ LE GQ Agarose (#R0491), 1X TAE, 7 V/cm, 14 min.

O'RangeRuler™ DNA Ladders, ready-to-use (10-6000 bp) RT

Supplied with:
6X Orange DNA Loading Dye 1 ml

Step ladders with 5, 10, 20, 50, 100, 200 or 500 bp increments. Not suitable for quantification

DNA ladder, ready-to-use	Catalog #	Concentration, µg/µl	Amount, µg	Applications	Loading, µg(µl)/lane	Recommended range, bp	Fragments	Agarose, %	PAGE, %
O'RangeRuler™ 5 bp DNA Ladder	SM1303	0.1	50	50-100	0.5-1 (5-10)	10-100	19	5.0	8-10
O'RangeRuler™ 10 bp DNA Ladder	SM1313					10-150	15	4.5-5.0	
O'RangeRuler™ 20 bp DNA Ladder	SM1323					20-300	15	3.5-4.0	
O'RangeRuler™ 50 bp DNA Ladder	SM0613	0.05	25	100	0.25 (5)	50-1000	20	1.7-2.5	4-8
O'RangeRuler™ 100 bp DNA Ladder	SM0623					100-1500	15	1.7-2.5	4-8
O'RangeRuler™ 200 bp DNA Ladder	SM0633					200-3000	15	0.8-1.2	–
O'RangeRuler™ 500 bp DNA Ladder	SM0643					500-6000	12	0.8-1.2	
O'RangeRuler™ 100+500 bp DNA Ladder	SM0653					100-6000	32	1.0-1.2	

Related Products

- TopVision™ Agaroses p.440
- Loading Dye p.438
- 50X TAE Buffer p.437
- 10X TBE Buffer p.437
- Agarase p.348
- DNA Gel Extraction Kit p.347
- 0.5 M EDTA, pH 8.0 p.491
- Water, nuclease-free p.490

Description

O'RangeRuler™ DNA ladders are designed for precise sizing of PCR products and other double-stranded DNA fragments in agarose or non-denaturing polyacrylamide gels. They are step ladders with 5, 10, 20, 50, 100, 200 or 500 bp differences between the DNA fragments. These ladders consist of purified and ligated blunt ended basic unit repeats of 10, 15, 20, 50, 100, 200 or 500 bp. They are not recommended for DNA quantification.

O'RangeRuler™ DNA ladders are supplied with 6X Orange DNA Loading Dye for sample DNA. Orange G dye can be used to monitor DNA migration in agarose or polyacrylamide gels and migrates at 50 bp in a 1% agarose gel. It is the preferable dye when visualization of small DNA fragments is important.

Features

- Step ladders with 5, 10, 20, 50, 100, 200 or 500 bp increments.
- Sharp bands, easy-to-remember fragment sizes.
- Evenly spaced reference bands (given in red).
- Ready-to-use – premixed with 6X Orange Loading Dye for direct loading and room temperature storage.
- Supplied with loading dye for sample DNA.

Storage and Loading Buffer

10 mM Tris-HCl (pH 7.6), 10 mM EDTA, 0.025% orange G, 0.005% xylene cyanol FF, 10% glycerol.

6X Orange DNA Loading Dye

10 mM Tris-HCl (pH 7.6), 0.15% orange G, 0.03% xylene cyanol FF, 60% glycerol and 60 mM EDTA.

Quality Control

Tested in appropriate gel electrophoresis applications. The DNA concentration of the complete ladder determined spectrophotometrically. The absence of nucleases confirmed by appropriate tests.

Storage

Store at room temperature or at 4°C for 6 months. For longer periods, store at -20°C.

Protocols and Recommendations

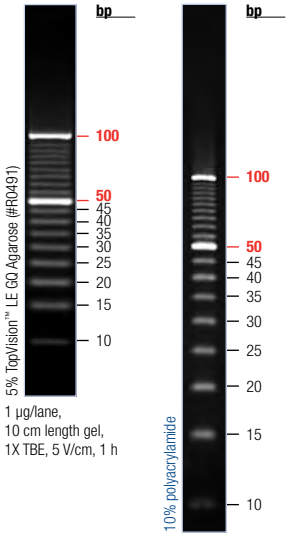
» General Recommendations p.443
» Preparation of Gels for DNA Electrophoresis p.442
» DNA Ladder/Sample Preparation p.445
» Troubleshooting Guide p.447

www.fermentas.com

Ready-to-use | 4° Store at 4°C | RT Store at Room Temperature | -20° Store at -20°C

O'RangeRuler™ 5 bp DNA Ladder, ready-to-use

#SM1303

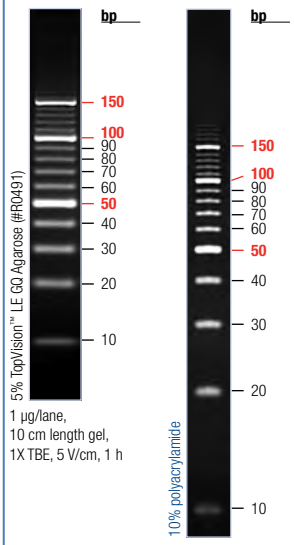


1 µg/lane,
10 cm length gel,
1X TBE, 5 V/cm, 1 h

0.5 µg/lane,
20 cm length gel,
1X TBE, 8 V/cm, 3 h

O'RangeRuler™ 10 bp DNA Ladder, ready-to-use

#SM1313

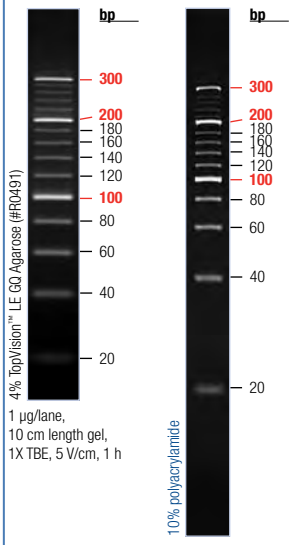


1 µg/lane,
10 cm length gel,
1X TBE, 5 V/cm, 1 h

0.5 µg/lane,
20 cm length gel,
1X TBE, 8 V/cm, 3 h

O'RangeRuler™ 20 bp DNA Ladder, ready-to-use

#SM1323

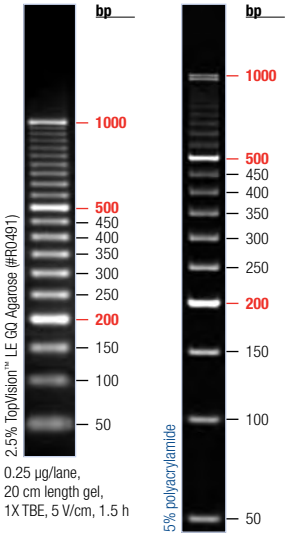


1 µg/lane,
10 cm length gel,
1X TBE, 5 V/cm, 1 h

0.5 µg/lane,
20 cm length gel,
1X TBE, 8 V/cm, 3 h

O'RangeRuler™ 50 bp DNA Ladder, ready-to-use

#SM0613

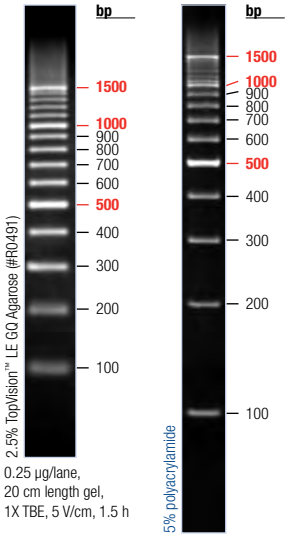


0.25 µg/lane,
20 cm length gel,
1X TBE, 5 V/cm, 1.5 h

0.25 µg/lane,
20 cm length gel,
1X TAE, 8 V/cm, 3 h

O'RangeRuler™ 100 bp DNA Ladder, ready-to-use

#SM0623

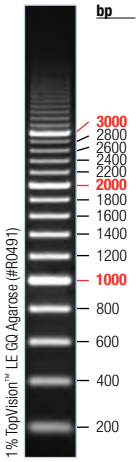


0.25 µg/lane,
20 cm length gel,
1X TBE, 5 V/cm, 1.5 h

0.25 µg/lane,
20 cm length gel,
1X TAE, 8 V/cm, 3 h

O'RangeRuler™ 200 bp DNA Ladder, ready-to-use

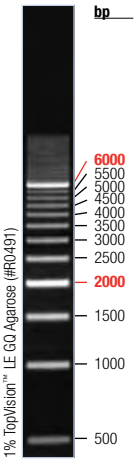
#SM0633



0.25 µg/lane,
20 cm length gel,
1X TAE, 7 V/cm, 1 h

O'RangeRuler™ 500 bp DNA Ladder, ready-to-use

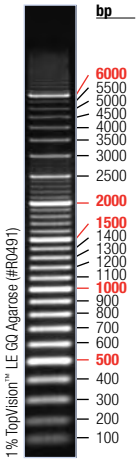
#SM0643



0.25 µg/lane,
20 cm length gel,
1X TAE, 7 V/cm, 1 h

O'RangeRuler™ 100+500 bp DNA Ladder, ready-to-use

#SM0653



0.25 µg/lane,
20 cm length gel,
1X TAE, 7 V/cm,1 h

ZipRuler™ Express DNA Ladder, ready-to-use (100-20,000 bp) RT

Supplied with:
6X Orange DNA Loading Dye 1 ml

For fast sizing of wide range (100-20,000 bp) of DNA fragments

DNA ladder, ready-to-use	Catalog #	Concentration, µg/µl	Amount, µg	Applications	Loading, µg(µl)/lane	Range, bp	Fragments	Agarose, %
ZipRuler™ Express DNA Ladder Set ZipRuler™ Express DNA Ladder 1 ZipRuler™ Express DNA Ladder 2	**SM1373**	0.1	50	100-200	0.25-0.5 (2.5-5)	100-10000 200-20000	9	5.0

Related Products

- TopVision™ Agaroses p.440
- Loading Dye Solutions p.438
- 50X TAE Buffer p.437
- 10X TBE Buffer p.437
- Agarase p.348
- DNA Gel Extraction Kit p.347
- 0.5 M EDTA, pH 8.0 p.491
- Water, nuclease-free p.490

Description

The ZipRuler™ Express DNA Ladder Set is specifically designed for fast and highly accurate sizing of a broad range (100-20,000 bp) of DNA fragments and their approximate quantification under a variety of electrophoresis conditions. The band pattern is not affected by the composition of electrophoresis buffer, voltage or gel percentage (*see* Table 9.3 on p.431).

The ZipRuler™ Express DNA Ladder Set contains two ladders: ZipRuler™ Express DNA Ladder 1 and ZipRuler™ Express DNA Ladder 2. Both are mixtures of chromatography-purified individual DNA fragments.

For fast results, we recommend loading the ZipRuler™ Express DNA Ladder 1 and 2 into two different wells in the same gel to evaluate a wide range of DNA bands during short electrophoresis runs.

Alternatively, load equal amounts of both ladders into the same gel well. Three reference bands will then be formed during electrophoresis of the mixed ZipRuler™ ladder. Longer electrophoresis run times are needed to achieve full separation of complex ladder.

ZipRuler™ Express DNA Ladder 1 is premixed with the 6X Orange DNA Loading Dye (orange G and xylene cyanol), whereas ZipRuler™ Express DNA Ladder 2 is premixed with the 6X MassRuler™ DNA Loading Dye.

When both ladders are loaded in the same gel lane, the migration of DNA is monitored by three electrophoresis tracking dyes (bromophenol blue, xylene cyanol FF and orange G).

The set is supplied with 6X Orange DNA Loading Dye for sample DNA.

Features

- Fast and robust when used in two different lanes of the same gel and run under fast (10-15 min) electrophoresis conditions.
- Precise when used in one lane and run with conventional electrophoresis conditions.
- Sharp bands, easy-to-remember fragment sizes, bright reference bands.
- Ready-to-use – premixed with a loading dye for direct loading and room temperature storage.
- Supplied with loading dye for sample DNA.

Storage and Loading Buffer (for ZipRuler™ Express DNA Ladder 1)

10 mM Tris-HCl (pH 7.6), 10 mM EDTA, 0.025% orange G, 0.005% xylene cyanol FF, 10% glycerol.

Storage and Loading Buffer (for ZipRuler™ Express DNA Ladder 2)

10 mM Tris-HCl (pH 7.6), 10 mM EDTA, 0.005% bromophenol blue, 10% glycerol.

6X Orange DNA Loading Dye

10 mM Tris-HCl (pH 7.6), 0.15% orange G, 0.03% xylene cyanol FF, 60% glycerol and 60 mM EDTA.

Quality Control

Tested in appropriate gel electrophoresis applications. Concentration of each DNA fragment and of the complete ladder determined spectrophotometrically. The absence of nucleases confirmed by appropriate tests.

Storage

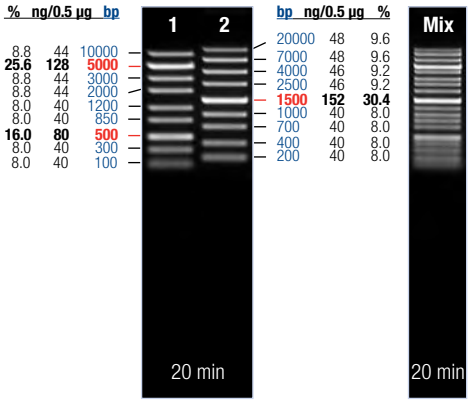
Store at room temperature or at 4°C for 6 months. For longer periods, store at -20°C.

Protocols and Recommendations

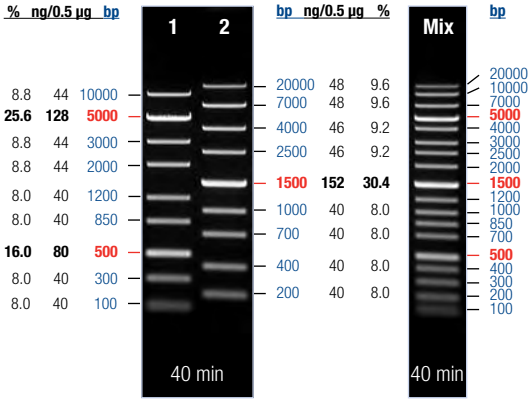
» General Recommendations p.441
» Preparation of Gels for DNA Electrophoresis p.442
» DNA Ladder/Sample Preparation p.445
» Troubleshooting Guide p.447

Ready-to-use | 4° Store at 4°C | RT Store at Room Temperature | -20° Store at -20°C

ZipRuler™ Express DNA Ladder Set, ready-to-use
#SM1373



1% TopVision™ LE GQ Agarose (#R0491)
5 µl/lane, 8 cm length gel, 1X TAE, 7 V/cm, **20 min**



1% TopVision™ LE GQ Agarose (#R0491)
5 µl/lane, 8 cm length gel, 1X TAE, 7 V/cm, **40 min**

Table 9.3. ZipRuler™ Express DNA Ladder Separation Guide for Various Electrophoresis Conditions.

ZipRuler™ Express DNA Ladders																																				
Duration of electrophoresis 10 V/cm	0.8% agarose						1% agarose						1.2% agarose						1.5% agarose						1.7% agarose						2% agarose					
	TAE			TBE			TAE			TBE			TAE			TBE			TAE			TBE			TAE			TBE			TAE			TBE		
	Ladder						Ladder						Ladder						Ladder						Ladder						Ladder					
	1	2	Mix	1	2	Mix	1	2	Mix	1	2	Mix	1	2	Mix	1	2	Mix	1	2	Mix	1	2	Mix	1	2	Mix	1	2	Mix	1	2	Mix	1	2	Mix
5 min																																				
10 min																																				
15 min																																				
20 min																																				
25 min																																				

Excellent separation of all bands
Incomplete separation of the two closest bands
No separation

Conventional Lambda DNA Markers (15-48,502 bp)

Supplied with:
6X DNA Loading Dye 2 ml / 10 ml

For analysis of linear double-stranded large DNA fragments in agarose gels

DNA marker, marker #	Catalog #	Concentration, µg/µl	Amount, µg	Applications, 0.5 µg/lane	Loading, µg(µl)/lane	Range, bp	Fragments	Agarose, %
Lambda DNA/EcoRI Marker, 1	SM0281	0.5	250 (5x50)	500	0.5 (1)	3530-21226	6	0.7
Lambda DNA/HindIII Marker, 2	SM0101 SM0102	0.5	250 (5x50) 1250 (25x50)	500 2500	0.5 (1)	125-23130	8	1.0
Lambda DNA/HindIII Marker, 2, ready-to-use	SM0103	0.1	250 (5x50)	500	0.5 (5)			
Lambda DNA/EcoRI+HindIII Marker, 3	SM0191 SM0192	0.5	250 (5x50) 1250 (25x50)	500 2500	0.5 (1)	125-21226	13	1.0
Lambda DNA/EcoRI+HindIII Marker, 3, ready-to-use	SM0193	0.1	250 (5x50)	500	0.5 (5)			
Lambda – pUC MIx Marker, 4	SM0291	0.5	50	100	0.5 (1)	74-19329	6	1.0
Lambda DNA/Eco47I (AvaII) Marker, 13	SM1051	0.5	250 (5x50)	500	0.5 (1)	23-8126	36	1.5
Lambda DNA/Eco91I (BstEII) Marker, 15	SM0111	0.5	250 (5x50)	500	0.5 (1)	117-8453	14	1.0
Lambda DNA/Eco130I (StyI) Marker, 16	SM0161	0.5	250 (5x50)	500	0.5 (1)	74-19329	11	1.0
Lambda Mix Marker, 19	SM0231	0.5	250 (5x50)	500	0.5 (1)	1503-48502	14	0.7
Lambda DNA/PstI Marker, 24	SM0361	0.5	250 (5x50)	500	0.5 (1)	15-11501	29	1.5

Related Products

- TopVision™ Agaroses p.440
- Loading Dye p.438
- 50X TAE Buffer p.437
- 10X TBE Buffer p.437
- Agarase p.348
- DNA Gel Extraction Kit p.347
- Klenow Fragment p.252
- Klenow Fragment, exo⁻ p.253
- dNTP Set p.478
- Modified dNTPs p.481
- T4 Polynucleotide Kinase p.246
- ATP p.481
- 0.5 M EDTA, pH 8.0 p.491
- Water, nuclease-free p.490

Protocols and Recommendations

» General Recommendations p.441
» Preparation of Gels for DNA Electrophoresis p.442
» DNA Ladder/Sample Preparation p.445
» Labeling 5'-termini of DNA by T4 Polynucleotide Kinase p.381
» Labeling 5'-overhangs of dsDNA including DNA Markers with Klenow Fragment p.383
» Troubleshooting Guide p.447

Description

Conventional Lambda DNA markers are recommended for sizing of linear double-stranded large DNA fragments in agarose gels. Lambda DNA is digested to completion with the appropriate Fermentas PureExtreme® restriction enzyme(s), purified and dissolved in storage buffer. DNA fragments contain various sticky ends depending on the restriction enzyme used for preparation of the marker.

Lambda DNA/HindIII Marker and **Lambda DNA/EcoRI+HindIII Marker** are available in ready-to-use versions – premixed with 6X DNA Loading Dye for direct loading (after heating) onto agarose gels.

Conventional versions (supplied in storage (TE) buffer) can be labeled radioactively with T4 Polynucleotide Kinase (#EK0031). Alternatively, they can be labeled radioactively or non-radioactively with Klenow Fragment (#EP0051) or Klenow Fragment, exo⁻ (#EP0421) with the fill-in reaction (with the exception of Lambda DNA/PstI Marker), *see* protocols on pp.383-384.

Features

- Sizing and approximate quantification of large DNA fragments.
- Sharp bands.
- Ready-to-use versions are premixed with 6X DNA Loading Dye for a direct loading (after heating) and for room temperature storage.
- Supplied with loading dye for sample DNA.

Storage Buffer (TE buffer)

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

Storage and Loading Buffer (for ready-to-use markers)

10 mM Tris-HCl (pH 7.6), 10 mM EDTA, 0.005% bromophenol blue, 0.005% xylene cyanol FF and 10% glycerol.

6X DNA Loading Dye

10 mM Tris-HCl (pH 7.6), 0.03% bromophenol blue, 0.03% xylene cyanol FF, 60% glycerol and 60 mM EDTA.

Quality Control

Tested in appropriate gel electrophoresis applications. The DNA concentration of the complete marker determined spectrophotometrically. The absence of nucleases confirmed by appropriate tests.

Note

- The cohesive ends (the 12 nt *cos* site of lambda DNA) of fragments indicated * may anneal and form additional bands. These fragments can be separated by heating at 65°C for 5 min and then cooling on ice for 3 min (*see* p.433).
- The shortest fragments (oblique) are not visible in standard electrophoresis. Fragment lengths are predicted by computer analysis of respective DNA sequences (*see* pp.416-418).

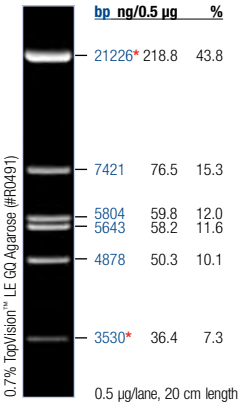
Storage

Store at -20°C.

Ready-to-use versions can be stored at room temperature or at 4°C for 6 months. For longer periods, store at -20°C.

Lambda DNA/EcoRI Marker, 1

#SM0281



bp	ng/0.5 µg	%
21226*	218.8	43.8
7421	76.5	15.3
5804	59.8	12.0
5643	58.2	11.6
4878	50.3	10.1
3530*	36.4	7.3

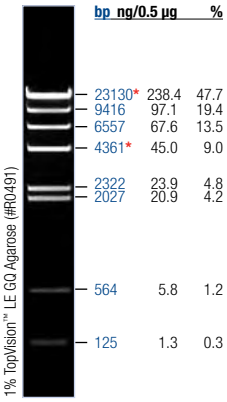
0.5 µg/lane, 20 cm length gel, 1X TAE buffer, 3 V/cm, 18 h (until bromophenol blue dye reached the bottom of the gel)

Range

6 fragments (in bp): 21226*, 7421, 5804, 5643, 4878, 3530*.

Lambda DNA/HindIII Marker, 2

#SM0101/2/3



bp	ng/0.5 µg	%
23130*	238.4	47.7
9416	97.1	19.4
6557	67.6	13.5
4361*	45.0	9.0
2322	23.9	4.8
2027	20.9	4.2
564	5.8	1.2
125	1.3	0.3

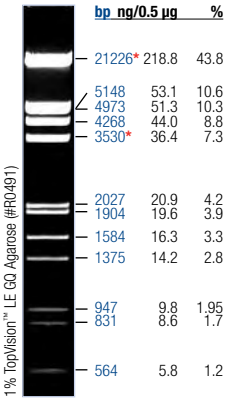
0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min

Range

8 fragments (in bp): 23130*, 9416, 6557, 4361*, 2322, 2027, 564, 125.

Lambda DNA/EcoRI+HindIII Marker, 3

#SM0191/2/3



bp	ng/0.5 µg	%
21226*	218.8	43.8
5148	53.1	10.6
4973	51.3	10.3
4268	44.0	8.8
3530*	36.4	7.3
2027	20.9	4.2
1904	19.6	3.9
1584	16.3	3.3
1375	14.2	2.8
947	9.8	1.95
831	8.6	1.7
564	5.8	1.2

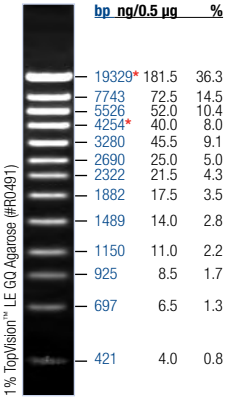
0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min

Range

13 fragments (in bp): 21226*, 5148, 4973, 4268, 3530*, 2027, 1904, 1584, 1375, 947, 831, 564, *125 (it comprises 0.3%)*.

Lambda – pUC Mix Marker, 4

#SM0291



bp	ng/0.5 µg	%
19329*	181.5	36.3
7743	72.5	14.5
5526	52.0	10.4
4254*	40.0	8.0
3280	45.5	9.1
2690	25.0	5.0
2322	21.5	4.3
1882	17.5	3.5
1489	14.0	2.8
1150	11.0	2.2
925	8.5	1.7
697	6.5	1.3
421	4.0	0.8

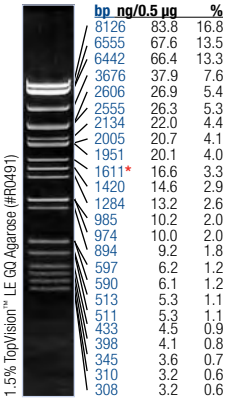
0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min

Range

6 fragments (in bp): 19329*, 7743, 5526, 4254*, 3280, 2690, 2322, 1882, 1489, 1150, 925, 697, 421, *74 (it comprises 0.1%)*.

Lambda DNA/Eco47I (AvaII) Marker, 13

#SM1051



bp	ng/0.5 µg	%
8126	83.8	16.8
6555	67.6	13.5
6442	66.4	13.3
3676	37.9	7.6
2606	26.9	5.4
2555	26.3	5.3
2134	22.0	4.4
2005	20.7	4.1
1951	20.1	4.0
1611*	16.6	3.3
1420	14.6	2.9
1284	13.2	2.6
985	10.2	2.0
974	10.0	2.0
894	9.2	1.8
597	6.2	1.2
590	6.1	1.2
513	5.3	1.1
511	5.3	1.1
433	4.5	0.9
398	4.1	0.8
345	3.6	0.7
310	3.2	0.6
308	3.2	0.6

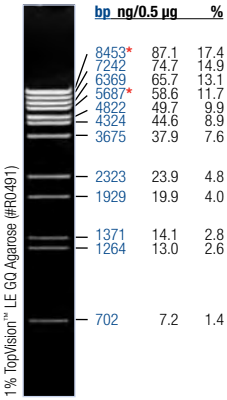
0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 1 h

Range

36 fragments (in bp): 8126, 6555, 6442, 3676, 2606, 2555, 2134, 2005, 1951, 1611*, 1420, 1284, 985, 974, 894, 597, 590, 513, 511, 433, 398, 345, 310, 308, *272, 242, 215, 151, 88, 73, 67, 45, 42, 32, 29*, 23 (oblique fragments comprise 2.7%)*.

Lambda DNA/Eco91I (BstEII) Marker, 15

#SM0111



bp	ng/0.5 µg	%
8453*	87.1	17.4
7242	74.7	14.9
6369	65.7	13.1
5687*	58.6	11.7
4822	49.7	9.9
4324	44.6	8.9
3675	37.9	7.6
2323	23.9	4.8
1929	19.9	4.0
1371	14.1	2.8
1264	13.0	2.6
702	7.2	1.4

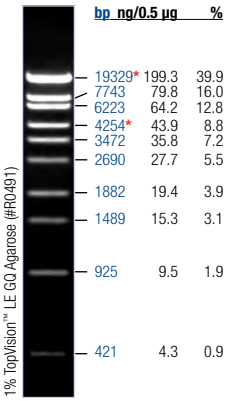
0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min

Range

14 fragments (in bp): 8453*, 7242, 6369, 5687*, 4822, 4324, 3675, 2323, 1929, 1371, 1264, 702, *224, 117 (oblique fragments comprise 0.7%)*.

Lambda DNA/Eco130I (StyI) Marker, 16

#SM0161



bp	ng/0.5 µg	%
19329*	199.3	39.9
7743	79.8	16.0
6223	64.2	12.8
4254*	43.9	8.8
3472	35.8	7.2
2690	27.7	5.5
1882	19.4	3.9
1489	15.3	3.1
925	9.5	1.9
421	4.3	0.9

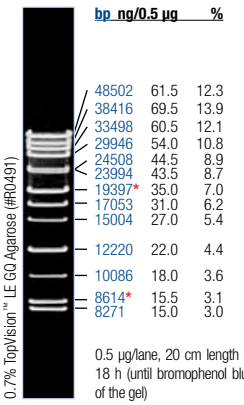
0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min

Range

11 fragments (in bp): 19329*, 7743, 6223, 4254*, 3472, 2690, 1882, 1489, 925, 421, *74 (it comprises 0.2%)*.

Lambda Mix Marker, 19

#SM0231



bp	ng/0.5 µg	%
48502	61.5	12.3
38416	69.5	13.9
33498	60.5	12.1
29946	54.0	10.8
24508	44.5	8.9
23994	43.5	8.7
19397*	35.0	7.0
17053	31.0	6.2
15004	27.0	5.4
12220	22.0	4.4
10086	18.0	3.6
8614*	15.5	3.1
8271	15.0	3.0

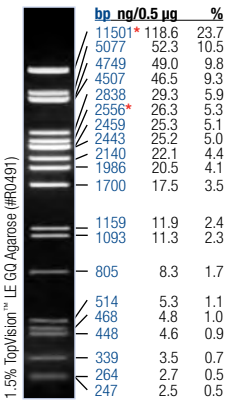
0.5 µg/lane, 20 cm length gel, 1X TAE buffer, 3 V/cm, 18 h (until bromophenol blue dye reached the bottom of the gel)

Range

14 fragments (in bp): 48502, 38416, 33498, 29946, 24508, 23994, 19397*, 17053, 15004, 12220, 10086, 8614*, 8271, *1503 (it comprises 0.6%)*.

Lambda DNA/PstI Marker, 24

#SM0361



bp	ng/0.5 µg	%
11501*	118.6	23.7
5077	52.3	10.5
4749	49.0	9.8
4507	46.5	9.3
2838	29.3	5.9
2556*	26.3	5.3
2459	25.3	5.1
2443	25.2	5.0
2140	22.1	4.4
1986	20.5	4.1
1700	17.5	3.5
1159	11.9	2.4
1093	11.3	2.3
805	8.3	1.7
514	5.3	1.1
468	4.8	1.0
448	4.6	0.9
339	3.5	0.7
264	2.7	0.5
247	2.5	0.5

0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm,1 h

Range

29 fragments (in bp): 11501*, 5077, 4749, 4507, 2838, 2556*, 2459, 2443, 2140, 1986, 1700, 1159, 1093, 805, 514, 468, 448, 339, 264, 247, *216, 211, 200, 164, 150, 94, 87, 72, 15 (oblique fragments comprise 2.3%)*.

Conventional Phage and Plasmid DNA Markers (8-1353 bp)

Supplied with:
6X DNA Loading Dye 2 ml / 10 ml

For analysis of linear double-stranded small DNA fragments in agarose and polyacrylamide gels

DNA marker, marker #	Catalog #	Concentration, µg/µl	Amount, µg	Applications, 0.5 µg/lane	Loading, µg(µl)/lane	Range, bp	Fragments	Agarose, %	PAGE, %
pBR322 DNA/BsuRI (HaeIII) Marker, 5	SM0271	0.5	50	100	0.5 (1)	8-587	22	2.5	5.0
pUC Mix Marker, 8	SM0301 SM0302	0.5	50 250 (5x50)	100 500	0.5 (1)	19-1118	17	1.7	
pUC Mix Marker, 8, ready-to-use	SM0303	0.1	50	100	0.5 (5)				
ΦX174 DNA /BsuRI (HaeIII) Marker, 9	SM0251 SM0252	0.5	50 250 (5x50)	100 500	0.5 (1)	72-1353	11	1.7	
ΦX174 DNA /BsuRI (HaeIII) Marker, 9, ready-to-use	SM0253	0.1	50	100	0.5 (5)				
ΦX174 DNA /HinfI Marker, 10	SM0261	0.5	50	100	0.5 (1)	24-726	21	2.5	
pBR322 DNA/AluI Marker, 20	SM0121	0.5	50	100	0.5 (1)	11-908	17	1.7	–
pBR322 DNA/AluI Marker, 20, ready-to-use	SM0123	0.1			0.5 (5)				
pUC19 DNA/MspI (HpaII) Marker, 23	SM0221 SM0222	0.5	50 250 (5x50)	100 500	0.5 (1)	26-501	13	1.7	5.0
pUC19 DNA/MspI (HpaII) Marker, 23, ready-to-use	SM0223	0.1	50	100	0.5 (5)				

Related Products

- TopVision™ Agaroses p.440
- Loading Dye p.438
- 50X TAE Buffer p.437
- 10X TBE Buffer p.437
- Agarase p.348
- DNA Gel Extraction Kit p.347
- Klenow Fragment p.252
- Klenow Fragment, exo⁻ p.253
- dNTP Set p.478
- Modified dNTPs p.481
- T4 Polynucleotide Kinase p.246
- ATP p.481
- 0.5 M EDTA, pH 8.0 p.491
- Water, nuclease-free p.490

Protocols and Recommendations

- » General Recommendations p.441
- » Preparation of Gels for DNA Electrophoresis p.442
- » DNA Ladder/Sample Preparation p.445
- » Labeling 5'-termini of DNA by T4 Polynucleotide Kinase p.381
- » Labeling 5'-overhangs of dsDNA including DNA Markers with Klenow Fragment p.383
- » Troubleshooting Guide p.447

Description

Conventional Phage and Plasmid DNA markers are recommended for sizing and approximate quantification of small linear double-stranded DNA fragments in agarose and non-denaturing polyacrylamide gels. Phage or plasmid DNA is digested to completion with the appropriate Fermentas PureExtreme® restriction enzyme, then purified and dissolved in storage buffer. The DNA fragments contain blunt or sticky ends, depending on the restriction enzyme used for the marker's preparation.

pUC Mix, ΦX174 DNA/BsuRI, pBR322 DNA/AluI and pUC19 DNA/MspI markers are also available in ready-to-use versions – premixed with 6X DNA Loading Dye for direct loading onto agarose and polyacrylamide gels.

All conventional versions (supplied in storage (TE) buffer) can be labeled radioactively with T4 Polynucleotide Kinase (#EK0031). Alternatively pUC Mix, ΦX174 DNA/HinfI and pUC19 DNA/MspI markers can be labeled radioactively or non-radioactively with Klenow Fragment (#EP0051) or Klenow Fragment, exo⁻ (#EP0421) with the fill-in reaction, *see* protocols on pp.383-384.

Features

- Sizing and approximate quantification of small DNA fragments.
- Sharp bands.
- Ready-to-use versions are premixed with 6X DNA Loading Dye for direct loading and room temperature storage.
- Supplied with loading dye for sample DNA.

Storage Buffer *(TE buffer)*

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

Storage and Loading Buffer (for ready-to-use markers)

10 mM Tris-HCl (pH 7.6), 10 mM EDTA, 0.005% bromophenol blue, 0.005% xylene cyanol FF and 10% glycerol.

6X DNA Loading Dye

10 mM Tris-HCl (pH 7.6), 0.03% bromophenol blue, 0.03% xylene cyanol FF, 60% glycerol and 60 mM EDTA.

Quality Control

Tested in appropriate gel electrophoresis applications. The DNA concentration of the complete marker determined spectrophotometrically. The absence of nucleases confirmed by appropriate tests.

Note

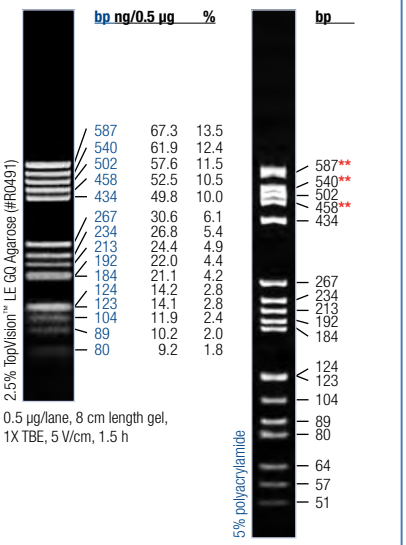
- Bands indicated ** form an anomalous pattern on polyacrylamide gels (*see* p.437).
- The shortest fragments (oblique) are not visible in standard electrophoresis. Fragment lengths are predicted by computer analysis of the respective DNA sequences.

Storage

Store at -20°C.

Ready-to-use versions can be stored at room temperature or at 4°C for 6 months. For longer periods, store at -20°C.

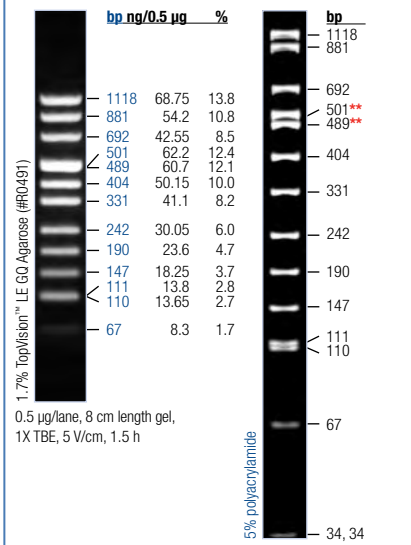
pBR322 DNA/BsuRI (HaeIII) Marker, 5
#SM0271



Range

22 fragments (in bp): 587**, 540**, 502, 458**, 434, 267, 234, 213, 192, 184, 124, 123, 104, 89, 80, 64, 57, 51, *21, 18, 11, 8 (oblique fragments comprise 5.4%)*.

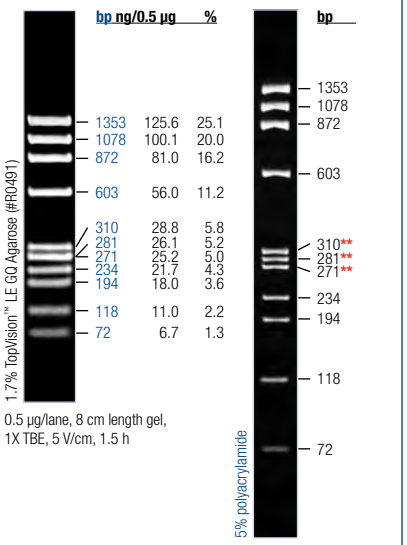
pUC Mix Marker, 8
#SM0301/2/3



Range

17 fragments (in bp): 1118, 881, 692, 501**, 489**, 404, 331, 242, 190, 147, 111, 110, 67, 34, 34, *26, 19 (oblique fragments comprise 0.9%)*.

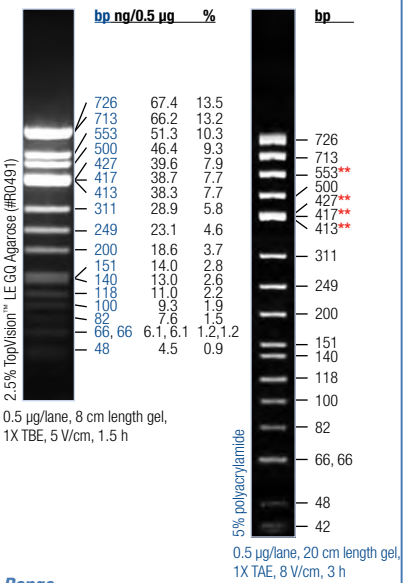
ΦX174 DNA/BsuRI (HaeIII) Marker, 9
#SM0251/2/3



Range

11 fragments (in bp): 1353, 1078, 872, 603, 310**, 281**, 271**, 234, 194, 118, 72.

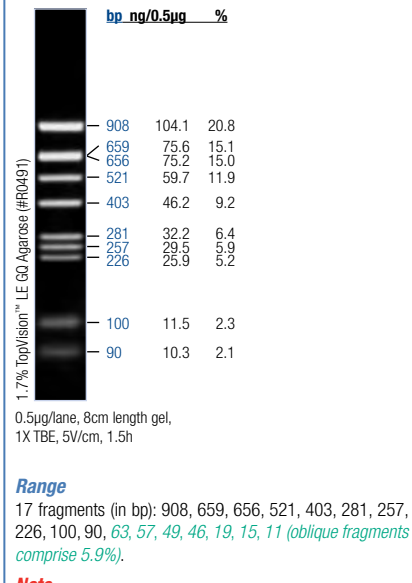
ΦX174 DNA/HinfI Marker, 10
#SM0261



Range

21 fragments (in bp): 726, 713, 553**, 500, 427**, 417**, 413**, 311, 249, 200, 151, 140, 118, 100, 82, 66, 66, 48, 42, *40, 24 (oblique fragments comprise 1.9%)*.

pBR322 DNA/AluI Marker, 20
#SM0121/3



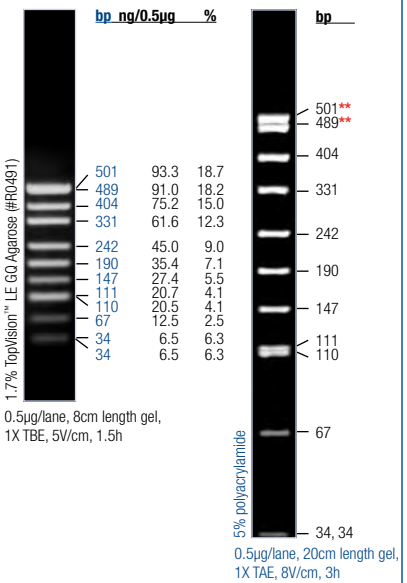
Range

17 fragments (in bp): 908, 659, 656, 521, 403, 281, 257, 226, 100, 90, *63, 57, 49, 46, 19, 15, 11 (oblique fragments comprise 5.9%)*.

Note

Loading the marker in 5% polyacrylamide gels is not recommended due to anomalous migration of 659, 656, 403 and 257 fragments.

pUC19 DNA/MspI (HpaII) Marker, 23
#SM0221/2/3



Range

13 fragments (in bp): 501**, 489**, 404, 331, 242, 190, 147, 111, 110, 67, 34, 34, *26 (it comprises 1.0%)*.

Markers for Genomic DNA Analysis (702-29,946 bp) -20°

DNA Marker I for Genomic DNA Analysis

#SM0341 6 µg
(for 120 applications 50 ng/lane)
Supplied with:
10X DNA Loading Dye 1 ml

DNA Marker II for Genomic DNA Analysis

#SM0351 6 µg
(for 120 applications 50 ng/lane)
Supplied with:
10X DNA Loading Dye 1 ml

Related Products

- TopVision™ Agaroses p.440
- Loading Dyes p.438
- 50X TAE Buffer p.437
- Agarase p.348
- DNA Gel Extraction Kit p.347
- Klenow Fragment, exo⁻ p.253
- DecaLabel™ DNA Labeling Kit p.376
- Biotin DecaLabel™ DNA Labeling Kit p.375
- Biotin-11-dUTP p.483
- dNTP Set p.478
- Biotin Chromogenic Detection Kit p.380
- BCIP-T p.497
- NBT p.497
- T4 Polynucleotide Kinase p.246
- ATP p.481
- SensiBlot™ Plus Nylon Membrane p.380
- 0.5 M EDTA, pH 8.0 p.491
- Water, nuclease-free p.490

50 ng of the marker was run on a 20 cm length 0.7% agarose gel in 1XTAE buffer at 3 V/cm for 18 hours (until bromophenol blue dye reached the bottom of the gel). Blotting was carried out on SensiBlot™ Plus Nylon Membrane (#M1001), and a [α^{32}P] labeled marker was used as a probe for hybridization.

Range

The DNA Marker I – 28 fragments: from 702 to 14321 bp.
The DNA Marker II – 33 fragments: from 702 to 29946 bp.

Description

Genomic DNA Markers I and II are specially designed for genomic DNA analysis by Southern blotting.
Both markers are prepared from lambda DNA and ΦX174 DNA; they do not possess any plasmid sequences. The DNA is digested to completion with the appropriate Fermentas PureExtreme® restriction enzyme(s), purified, dissolved in storage buffer and then mixed.
The markers contain both blunt and sticky ended DNA fragments.
Recommended for random-primed labeling with Klenow Fragment, exo⁻ (#EP0421) and Fermentas DecaLabel™ (#K0621) and Biotin DecaLabel™ (#K0651) DNA Labeling kits (*see* protocol on p.384). Alternatively the markers can be labeled radioactively with T4 Polynucleotide Kinase (#EK0031) (*see* protocol on p.381).
50 ng of radioactively labeled marker is sufficient for 3-5 hybridization reactions.

Features

- Broad range DNA markers recommended for genomic DNA analysis in Southern blotting (*see* protocol on p.388).
- Free of plasmid DNA sequences.
- Sharp bands.
- Bright bands of 3194 bp, 3976 bp and 5220 bp serve as reference fragments.
- The labeled marker probe can be used in a mixture with a test probe for genomic DNA hybridization.
- The markers do not hybridize with genomic DNA from *Homo sapiens*, *Haemophilus influenzae*, *Bacillus subtilis*, *Physalis pruinosa*, *Saccharomyces cerevisiae*.

Concentration

0.2 µg/µl

Storage Buffer

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

10X DNA Loading Dye

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

Quality Control

Tested in appropriate gel electrophoresis applications. The DNA concentration of the complete marker determined spectrophotometrically. The absence of nucleases confirmed by appropriate tests.

Note

The cohesive ends (the 12 nt *cos* site of lambda DNA) of some fragments may anneal and form additional bands. These fragments can be separated by heating at 65°C for 5 min and then cooling on ice for 3 min.

Storage

Store at -20°C.

Protocols and Recommendations

- General Recommendations p.441
- Preparation of Gels for DNA Electrophoresis p.442
- Labeling 5'-termini of DNA by T4 Polynucleotide Kinase p.381
- Radioactive Random-primed DNA Labeling with Klenow Fragment, exo⁻ p.384
- Non-radioactive Random-primed DNA Labeling with Klenow Fragment, exo⁻ p.384
- Southern Blotting p.388
- Troubleshooting Guide p.447

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Ready-to-use | 4° Store at 4°C | RT Store at Room Temperature | -20° Store at -20°C

Reagents for DNA Electrophoresis

Electrophoresis Buffers RT

Buffer	Cat #	Size	1X composition	Application and features	Usage recommendations
50X TAE Buffer (Tris-acetate-EDTA)	**B49**	1 L	40 mM Tris 20 mM acetic acid 1 mM EDTA pH of 50X TAE: 8.4	• Electrophoresis of nucleic acids in agarose and polyacrylamide gels. • Used both as a running buffer and as a gel preparation buffer. • Recommended for resolution of RNA and DNA fragments larger than 1500 b(p), for genomic DNA and for large supercoiled DNA. • Filtered through a 0.22 µm membrane.	• Use fresh 1X TAE both for the gel and for the electrophoresis run. • To prepare 1X TAE buffer, add 20 ml of 50X TAE buffer to 980 ml of deionized water and mix well. ***Note*** TAE buffer has a relatively low buffering capacity, therefore periodic replacement of the buffer during prolonged electrophoresis is recommended.
10X TBE Buffer (Tris-borate-EDTA)	**B52**	1 L	89 mM Tris 89 mM boric acid 2 mM EDTA pH of 10X TBE: 8.3	• Electrophoresis of nucleic acids in agarose and polyacrylamide gels. • Used both as a running buffer and as a gel preparation buffer. • Recommended for electrophoresis of RNA and DNA fragments smaller than 1500 b(p). • Filtered through a 0.22 µm membrane.	• Use fresh 1X TBE both for the gel and for the electrophoresis run. • To prepare 1X TBE buffer, add 100 ml of 10X TBE buffer to 900 ml of deionized water and mix well. ***Note*** Double-stranded linear nucleic acid molecules migrate about 10% slower in TBE buffer than in TAE buffer.

Related Products

- TopVision™ Agaroses p.440
- RiboRuler™ RNA Ladders p.455
- 2X RNA Loading Dye p.456
- PageSilver™ Silver Staining Kit p.398
- Agarase p.348
- DNA Markers/Ladders pp.419-436
- DNA Loading Dyes p.438
- DNA Gel Extraction Kit p.347
- DEPC-treated Water p.490

Quality Control

The absence of endo-, exodeoxyribonucleases and ribonucleases confirmed by appropriate quality tests.

Storage

There are no time limitations for storage of the electrophoresis buffers at room temperature. If the buffer is stored at lower temperatures, a precipitate may form, which is easily dissolved by gentle heating.

Protocols and Recommendations

» General Recommendations p.441
» Non-denaturing and Alkaline Agarose Gel Electrophoresis p.442
» Non-denaturing and denaturing PAGE p.443
» DNA Ladder/Sample Preparation p.445
» Troubleshooting Guide p.447

Bulk quantities and custom formulations available upon request

Loading Dyes

RT

Related Products

- TopVision™ Agaroses p.440
- 50X TAE Buffer p.437
- 10X TBE Buffer p.437
- Agarase p.348
- DNA Markers/Ladders pp.419-436
- RiboRuler™ RNA Ladders p.455
- DNA Gel Extraction Kit p.347

Description

Loading dye solutions are used to prepare DNA markers and samples for loading on agarose or polyacrylamide gels. The optimized solutions contain different dyes (bromophenol blue, xylene cyanol FF or orange G) for visual tracking of DNA migration during electrophoresis. The presence of glycerol ensures that the DNA in the ladder and sample forms a layer at the bottom of the well. The EDTA included in the solutions binds divalent metal ions and inhibits metal-dependent nucleases.

6X DNA Loading Dye, **6X MassRuler™ DNA Loading Dye**, **6X Orange DNA Loading Dye**, **6X TriTrack™ DNA Loading Dye** are all used for conventional DNA electrophoresis (*see* recommendations for use on p.441).

6X DNA Loading Dye & SDS Solution is recommended for agarose gel analysis of DNA samples that contain high amounts of DNA binding proteins. The presence of SDS eliminates DNA-protein interactions and prevents gel-shifts. The high concentration of EDTA protects DNA from degradation by metal-dependant nucleases (*see* recommendations for use on p.441)

2X RNA Loading Dye is mainly used for preparation of RNA samples for electrophoresis, but is also recommended for denaturing DNA electrophoresis since it contains the denaturing agent formamide (*see* recommendations for use on p.441)

For the detailed information about electrophoresis loading dyes, please refer to a table on p.439.

Quality Control

Tested for DNA sample preparation prior to agarose gel electrophoresis. The absence of deoxyribonucleases confirmed by appropriate tests.

2X RNA Loading Dye tested for RNA sample preparation prior to agarose gel electrophoresis. The absence of ribonucleases confirmed by appropriate tests.

Storage

Store at room temperature or at 4°C up to 12 months. For longer periods, store at -20°C.

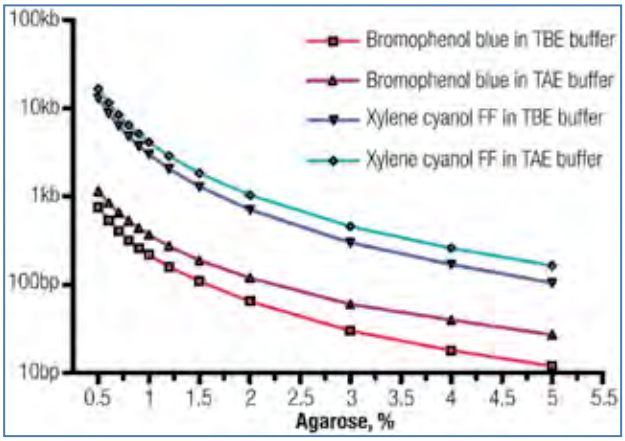

Figure 9.7. Tracking dye migration in agarose gels.

Protocols and Recommendations

Ready-to-use | 4° Store at 4°C | RT Store at Room Temperature | -20° Store at -20°C

Loading dye	Cat #	Size, ml	Composition	Features	Applications	Migration of dyes (1% agarose, TAE or TBE buffers)	Picture of tracking dyes*
6X DNA Loading Dye	**R0611**	5x1	***6X Solution*** • 10 mM Tris-HCl (pH 7.6) • 0.03% bromophenol blue • 0.03% xylene cyanol FF • 60% glycerol • 60 mM EDTA	• Two-color tracking of DNA migration during electrophoresis. • No DNA masking during gel exposure to UV light. • EDTA binds divalent metal ions and inhibits metal dependent nucleases.	• Preparation of DNA ladders, markers and samples for loading on agarose or polyacrylamide gels.	**Xylene cyanol FF:** TAE: 4160 bp TBE: 3030 bp **Bromophenol blue:** TAE: 370 bp TBE: 220 bp	
6X MassRuler™ DNA Loading Dye	**R0621**	5x1	***6X Solution*** • 10 mM Tris-HCl (pH 7.6) • 0.03% bromophenol blue • 60% glycerol • 60 mM EDTA	• One-color tracking of DNA migration during electrophoresis. • No DNA masking during gel exposure to UV light. • EDTA binds divalent metal ions and inhibits metal dependent nucleases.	• Analysis of large DNA molecules. • Preparation of DNA ladders, markers and samples for loading on agarose or polyacrylamide gels.	**Bromophenol blue:** TAE: 370 bp TBE: 220 bp	
6X Orange DNA Loading Dye	**R0631**	5x1	***6X Solution*** • 10 mM Tris-HCl (pH 7.6) • 0.15% orange G • 0.03% xylene cyanol FF • 60% glycerol • 60 mM EDTA	• Two-color tracking of DNA migration during electrophoresis. • No DNA masking during gel exposure to UV light. • EDTA binds divalent metal ions and inhibits metal dependent nucleases.	• Analysis of small DNA molecules. • Preparation of DNA ladders, markers and samples for loading on agarose or polyacrylamide gels.	**Xylene cyanol FF:** TAE: 4160 bp TBE: 3030 bp **Orange G:** TAE/TBE: <50 bp	
6X TriTrack™ DNA Loading Dye	**R1161**	5x1	***6X Solution*** • 10 mM Tris-HCl (pH 7.6) • 0.03% bromophenol blue • 0.03% xylene cyanol FF • 0.15% orange G • 60% glycerol • 60 mM EDTA	• Three-color tracking of DNA migration during electrophoresis. • No DNA masking during gel exposure to UV light. • EDTA binds divalent metal ions and inhibits metal dependent nucleases.	• Preparation of DNA ladders, markers and samples for loading on agarose or polyacrylamide gels.	**Xylene cyanol FF:** TAE: 4160 bp TBE: 3030 bp **Bromophenol blue:** TAE: 370 bp TBE: 220 bp **Orange G:** TAE/TBE: <50 bp	
6X DNA Loading Dye & SDS Solution	**R1151**	5x1	***6X Solution*** • 0.03% bromophenol blue • 0.03% xylene cyanol FF • 60% glycerol • 1% SDS • 100 mM EDTA (pH 7.6, adjusted with Tris)	• 1% SDS eliminates DNA-protein interactions, prevents appearance of additional bands due to annealing of DNA molecules with cohesive ends. • 100 mM EDTA inhibits metal-dependent nucleases.	• Analysis of DNA samples with high amounts of DNA binding proteins. • Kinetic experiments. • DNA agarose gel analysis after DNA restriction digestions, ligation or dephosphorylation reactions.	**Xylene cyanol FF:** TAE: 4160 bp TBE: 3030 bp **Bromophenol blue:** TAE: 370 bp TBE: 220 bp	
2X RNA Loading Dye	**R0641**	1	***2X Solution*** • 95% formamide • 0.025% SDS • 0.025% bromophenol blue • 0.025% xylene cyanol FF • 0.025% ethidium bromide • 0.5 mM EDTA	• Two-color tracking of DNA, RNA migration during electrophoresis. • No DNA, RNA masking during gel exposure to UV light. • Denaturation of DNA, RNA due to the presence of formamide.	• Preparation of DNA ladders, markers and samples for loading on denaturing gels • Preparation of RNA ladders, markers and samples for loading on agarose or polyacrylamide gels.	**Xylene cyanol FF:** TAE: 4160 bp TBE: 3030 bp **Bromophenol blue:** TAE: 370 bp TBE: 220 bp	

* For more detailed information regarding the migration rates of dyes in agarose and polyacrylamide gels *see* Tables 9.4 and 9.5 on p.441.

Bulk quantities and custom formulations available upon request

TopVision™ GQ Agarose

RT

Agarose	Cat #	Size	Characteristics	Features	Applications
TopVision™ LE GQ Agarose (low electroendosmosis)	**R0491**	100 g	• Electroendosmosis EEO 0.05-0.13 • Gel strength (1% gel) ≥1200 g/cm² • Gel strength (1.5% gel) ≥2500 g/cm² • Gelling temperature 36±1.5°C • Melting temperature 88±1.5°C • Moisture <7%	• Optimal concentration between 0.4-5% in all typical buffer systems. • GQ (Genetic Quality) certified, which ensures that nucleic acids recovered from preparative gels can be used for downstream applications (enzymatic reactions etc.). • Low DNA/RNA binding. • Excellent gel transparency. • DNase and RNase free.	• Analytical electrophoresis of nucleic acids. • Preparative electrophoresis. • Blotting assays.
TopVision™ LM GQ Agarose (low melting point)	**R0801**	25 g	• Electroendosmosis EEO <0.12 • Gel strength (1% gel) ≥250 g/cm² • Gelling temperature 24-28°C • Melting temperature <65.5°C • Moisture <7%	• Optimal concentration between 0.7-2% in all typical buffer systems. • GQ (Genetic Quality) certified, which ensures that nucleic acids recovered from preparative gels can be used for various applications (enzymatic reactions etc.). • Low DNA/RNA binding. • Excellent transparency of gels. • DNase and RNase free.	• In-gel enzymatic processing experiments. • Analytical electrophoresis of nucleic acids. • Preparative electrophoresis followed by Agarase (#EO0461) treatment.

Related Products

- 50X TAE Buffer p.437
- 10X TBE Buffer p.437
- Agarase p.348
- DNA Markers/Ladders pp.419-436
- Loading Dyes p.438
- RiboRuler™ RNA Ladders p.455
- DNA Gel Extraction Kit p.347
- 0.5 M EDTA, pH 8.0 p.491
- DEPC-treated Water p.490

Quality Control

The absence of endo-, exodeoxyribonucleases and ribonucleases confirmed by appropriate quality tests.

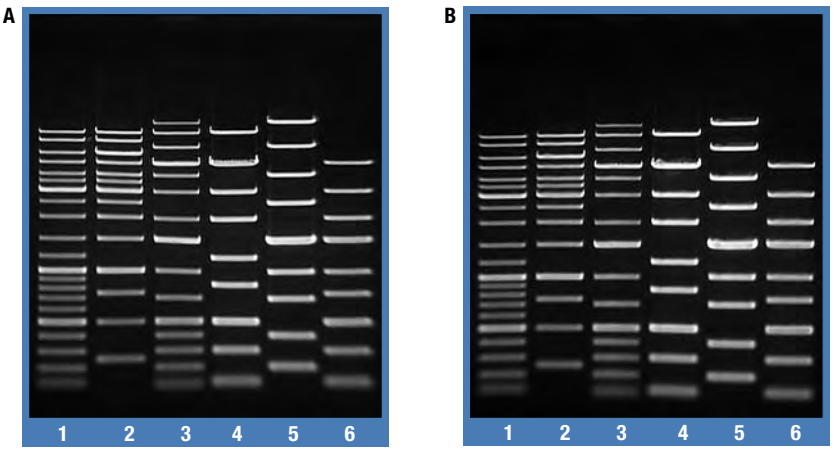

Figure 9.8. Separation of Fermentas DNA Ladders in (A) TopVision™ LE GQ Agarose, (B) TopVision™ LM GQ Agarose.

Electrophoresis conditions: 1% gel, 0.5 µg/lane, 1X TAE, 7V/cm, 40 min.

1 – GeneRuler™ DNA Ladder Mix, ready-to-use (p.422)
2 – GeneRuler™ 1kb DNA Ladder, ready-to-use (p.422)
3 – GeneRuler™ 1kb Plus DNA Ladder, ready-to-use (p.422)
4 – ZipRuler™ Express DNA Ladder 1, ready-to-use (p.431)
5 – ZipRuler™ Express DNA Ladder 2, ready-to-use (p.431)
6 – GeneRuler™ Express DNA Ladder, ready-to-use (p.423)

Protocols and Recommendations

» General Recommendations for DNA Electrophoresis p.441
» General Recommendations for RNA Electrophoresis p.459
» Non-denaturing and Alkaline Agarose Gel Electrophoresis p.442
» Non-denaturing and denaturing PAGE p.443
» DNA Ladder/Sample Preparation p.445
» Troubleshooting Guide p.447

www.fermentas.com

Ready-to-use | 4° Store at 4°C | RT Store at Room Temperature | -20° Store at -20°C

Protocols and Recommendations

General Recommendations for DNA Electrophoresis

- Use the same DNA loading dye (supplied with the DNA ladder/marker) for both the sample DNA and the ladder/marker DNA.
- If possible, always load equal volumes of the sample DNA and the ladder/marker DNA. The sample can be diluted with 1X DNA loading dye.
- Avoid high salt concentrations in the DNA samples as this may cause bands to shift during electrophoresis.
- Following electrophoresis, visualize DNA by staining in 0.5 µg/ml ethidium bromide solution or SYBR® Green I.
- Choose the gel percentage according to the tables below:

Table 9.4. Recommended Agarose Gels for Electrophoretic Separation of DNA Fragments.

Agarose gel, %	Range of effective separation, bp	Approximate positions of tracking dyes, bp*			
		Bromophenol blue		Xylene cyanol FF	
		TBE buffer	TAE buffer	TBE buffer	TAE buffer
0.5	2000-50000	750	1150	13000	16700
0.6	1000-20000	540	850	8820	11600
0.7	800-12000	410	660	6400	8500
0.8	800-10000	320	530	4830	6500
0.9	600-10000	260	440	3770	5140
1.0	400-8000	220	370	3030	4160
1.2	300-7000	160	275	2070	2890
1.5	200-3000	110	190	1300	1840
2.0	100-2000	65	120	710	1040
3.0	25-1000	30	60	300	460
4.0	10-500	18	40	170	260
5.0	10-300	12	27	105	165

Note

* Positions of the tracking dyes can only be estimated approximately because the dye front migrates as wide band. The following guidelines are recommended:

- Only high purity agarose should be used. TopVision™ LE GQ Agarose (#R0491) was used to prepare the gels.
- Only freshly prepared electrophoresis buffers should be used. The buffers were prepared from Fermentas 50X TAE Buffer (#B49) and 10X TBE Buffer (#B52).

Table 9.5. Recommended Polyacrylamide Gels for Electrophoretic Separation of DNA Fragments (1).

Polyacrylamide gel (with BIS at 1:20), % (w/v)	Range of effective separation*	Approximate positions of tracking dyes*	
		Bromophenol blue	Xylene cyanol FF
Denaturing gels			
4.0	100-500 b	50 b	230 b
5.0	70-400 b	35 b	130 b
6.0	40-300 b	26 b	105 b
8.0	30-200 b	19 b	75 b
10.0	20-100 b	12 b	55 b
15.0	10-50 b	10 b	40 b
20.0	5-30 b	8 b	28 b
30.0	1-10 b	6 b	20 b
Non-denaturing gels			
3.5	100-1000 bp	100 bp	460 bp
5.0	80-500 bp	65 bp	260 bp
8.0	60-400 bp	45 bp	160 bp
12.0	50-200 bp	20 bp	70 bp
15.0	25-150 bp	15 bp	60 bp
20.0	5-100 bp	12 bp	45 bp

Note

* Positions of the tracking dyes can only be estimated approximately because the dye front migrates as wide band.

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Reference

1. Sambrook, J., et al., Molecular Cloning. A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 12.89, 5.42, 2001.

- Choose electrophoresis conditions according to the recommendations below:

Size of the DNA	Voltage	Buffer
< 1 kb	5-10 V/cm	TBE
1-5 kb	4-10 V/cm	TAE or TBE
> 5 kb	1-3 V/cm	TAE
Up to 10 kb, fast electrophoresis with **Express DNA ladders**	up to 23 V/cm	TAE

Recommendations for Accurate Gel Quantification

- Always use the same DNA loading dye (supplied with the DNA ladder/marker) for both the sample DNA and the ladder/marker DNA.
- Always compare the sample band with the ladder band of the closest size.
- If possible, adjust the concentration of the sample to approximately equalize it with the amount of DNA in the nearest band.
- dNTPs, oligonucleotides, genomic DNA, RNA, NTPs or buffer components can interfere with spectrophotometrical measurements and lead to inaccurate quantification of sample DNA. In these cases, it is best to rely on gel quantification data.
- For the most accurate quantification, use video-densitometry analysis.

Preparation of Gels for DNA Agarose Gel Electrophoresis

Non-denaturing Agarose Gel Electrophoresis

Note

- Use a flask of at least three times larger volume than that of the solution to avoid boiling over.
- Use the same 1X electrophoresis buffer to prepare the gel and to run electrophoresis.
- Dilute 50X TAE Buffer (#B49) or 10X TBE Buffer (#B52) to a 1X concentration immediately before use.
- Use TBE buffer for analysis of DNA bands smaller than 1500 bp. For larger DNA, use TAE buffer.
- For intensified gel staining, add ethidium bromide to both the gel and the electrophoresis buffer at a final 0.5 µg/ml concentration. Alternatively, stain the gel after electrophoresis (*see* below).
  Wear gloves when handling ethidium bromide.
- For reliable analysis of supercoiled/relaxed plasmid ethidium bromide should not be included in the electrophoresis buffer or gel. The gel should be stained only after electrophoresis is complete.
- Ethidium bromide and exposure to UV light may cause DNA alterations. Therefore, avoid UV exposure and do not stain DNA with ethidium bromide if the purified fragments will be used for cloning experiments (*see* p.341).

❶ Weigh out the required amount of agarose (#R0491 or #R0801) (depending on the gel percentage) into an Erlenmeyer flask.
❷ Add the appropriate volume of either 1X TBE or 1X TAE buffer and swirl to mix.
❸ Weigh the flask with the solution.

For high percentage gels (3-5%): *add an excess amount of distilled water to increase the weight by 10-20%.*

❹ Boil the mixture in a microwave oven (at medium power) until the agarose melts completely; swirl the flask several times while boiling. To prepare the highest quality agarose gels of any percentage, an additional 3-5 min of boiling after completely melting the agarose is recommended. A significant amount of water evaporates during this procedure and therefore restoring of the initial weight (in step 5) is required to obtain the desired percentage gel.
❺ Weigh the flask again and if necessary, add hot distilled water to restore the initial weight.

For high percentage gels (3-5%): *check (by weighing) that the excess 10-20% of water has evaporated and, if needed, continue boiling to remove any excess, or add hot distilled water to restore the initial weight.*
Optional: *for intensified gel staining add ethidium bromide to a final concentration of 0.5 µg/ml. Mix well and heat for 1 min without boiling.*

❻ Cool the solution to 65-70°C. Pour carefully on a clean casting plate with an appropriate comb. If bubbles appear, push them carefully away to the sides with a pipette tip.
❼ Solidify the gel for approximately 30 min before use. Low percentage LM agarose gels need to be solidified at 4°C.
❽ Immerse the gel into the desired electrophoresis buffer. Load the samples onto the gel.
❾ Run electrophoresis at 5-7 V/cm until the bromophenol blue runs approximately two-thirds of the way down the gel.
❿ After electrophoresis the gel can be stained by immersing it into a 0.5 µg/ml ethidium bromide solution for 15-20 min, stained with SYBR® Green I or any other DNA staining technique.

Warning. *Hot agarose solution should be handled very carefully.*

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Alkaline Agarose Gel Electrophoresis

Note

- Double stranded DNA ladders are not recommended for denaturing electrophoresis as they may form an atypical pattern. However, these discrepancies are normally acceptable for analysis of cDNA or other ssDNA in alkaline gels.
- Use a flask of at least three times larger volume than that of the solution to avoid boiling over.
- Wear gloves when handling ethidium bromide.

1. Weigh out the required amount of agarose (depending on the gel percentage) into an Erlenmeyer flask.
2. Add the appropriate volume of the buffer (30 mM NaCl, 2 mM EDTA, pH 7.5) and swirl to mix.
3. Weigh the flask with the solution.

For high percentage gels (3-5%): *add an excess amount of distilled water to increase the weight by 10-20%.*

4. Boil the mixture in a microwave oven (at medium power) until the agarose melts completely; swirl the flask several times while boiling. To prepare the highest quality agarose gels of any percentage, an additional 3-5 min of boiling after completely melting the agarose is recommended. A significant amount of water evaporates during this procedure and therefore restoring of the initial weight (in step 5) is required to obtain the desired percentage gel.
5. Weigh the flask again and if necessary, add hot distilled water to restore the initial weight.

For high percentage gels (3-5%): *check (by weighing) that the excess 10-20% of water has evaporated and, if needed, continue boiling to remove any excess, or add hot distilled water to restore the initial weight.*

6. Cool the solution to 65-70°C. Pour carefully on a clean casting plate with an appropriate comb. If bubbles appear, push them carefully away to the sides with a pipette tip.
7. Solidify the gel for approximately 30 min before use.
8. Immerse the gel for at least one hour into the alkaline electrophoresis buffer (30 mM NaOH, 2 mM EDTA). Dilute 5 volumes of the DNA sample or ladder with one volume of 6X alkaline electrophoresis loading buffer (180 mM NaOH, 6 mM EDTA, 18% Ficoll 400, 0,05% bromcresol green).
9. Heat samples and ladder at 70°C for 5 min, then chill on ice for 3 minutes. Load onto the gel.
10. Run electrophoresis at 3 V/cm in alkaline electrophoresis buffer (30 mM NaOH, 2 mM EDTA) until the dye runs approximately two-thirds of the way down the gel.
    After electrophoresis the gel should be immersed for 30 min in 100-300 ml of 0.5 M Tris-HCl buffer, pH 7.5 and later stained in a 0.5 µg/ml ethidium bromide solution for 30 min. If staining is not enough, the whole procedure can be repeated.

Warning. *Hot agarose solution should be handled very carefully.*

Preparation of Gels for PAGE

Non-denaturing PAGE

Reagents:

- Deionized water
- 10X TBE Buffer (#B52)
- 20% acrylamide/bisacrylamide solution
- TEMED
- 10% (w/v) ammonium persulfate (APS) in water
- 0.5µg/ml ethidium bromide solution
- Denaturants –free loading dye solution for sample and ladder DNA

1. For a nondenaturing 5% polyacrylamide gel solution of 40 ml, mix the following:

10X TBE Buffer (#B52)	4 ml
20% acrylamide/bisacrylamide	10 ml
deionized water	26 ml

Caution: *acrylamide is a neurotoxin; always wear gloves, safety glasses, and a surgical mask when working with acrylamide powder.*

2. Vigorously agitate the solution for 1 min by magnetic stirring to ensure complete mixing.
3. Add 48 µl of TEMED and swirl the flask to ensure that the solution is thoroughly mixed.
4. Immediately add 240 µl of fresh 10% (w/v) APS and mix thoroughly.
5. Pour the acrylamide between the gel plates and insert the comb.
   Clamp the comb in place at the top of the gel to avoid separation of the gel from the plates as the acrylamide polymerizes. Allow the gel to polymerize for 30 min.

Important note: *polymerization begins as soon as APS is added to the mixture, so all subsequent steps must be performed quickly.*

(continued on next page)

6. After polymerization is complete, remove the comb and any bottom spacers from the gel. Wash the gel plates to clean any spilled acrylamide and be sure that the spacers are properly seated and clean. Fill the lower reservoir of the electrophoresis tank with 1X TBE buffer. Initially, place the gel into the lower tank at an angle to avoid the formation of air bubbles between the plates and the gel bottom. Clamp the gel plates to the top of the electrophoresis tank and fill the upper reservoir with 1X TBE so that the wells are covered.
7. Pre-run and warm the gel for at least 30 min at 5 V/cm (constant voltage).
8. Prepare the ladders for electrophoresis as it is recommended in the certificate of the product or in the Table 9.6 on p.445.
   Load the recommended volume of the ladder, premixed with the appropriate electrophoresis loading dye solution. Use the same loading dye for the sample DNA.
9. Run the gel at 5 V/cm, taking care to avoid excessive heating. Run the gel for the time indicated in the certificate of analysis of the ladder.
10. Stain the gel in a 0.5 µg/ml ethidium bromide aqueous solution for about 30 min. Examine the gel under the UV light.

Denaturing Polyacrylamide/Urea Gel Electrophoresis

Note

Double stranded DNA ladders are not recommended for denaturing electrophoresis as they may form an atypical pattern. However these usual discrepancies are normally acceptable for analysis of cDNA or other ssDNA in denaturing PAGE, *see* Fig.9.10 below.

Reagents:

- Deionized water
- 10X TBE Buffer (#B52)
- 20% acrylamide/bisacrylamide solution
- TEMED
- 10% (w/v) ammonium persulfate (APS) in water
- 0.5µg/ml ethidium bromide solution
- UREA
- 2X RNA Loading Dye (#R0641)

Sample preparation for denaturing PAGE:

Note Use the same loading dye solution for the sample and the ladder DNA.

1. Mix the DNA sample with an equal volume of 2X RNA Loading Dye.
2. Heat at 95ºC for 5 min.
3. Chill the sample on ice for 3 min.
4. Keep samples on ice while loading.
5. For a denaturing 10% polyacrylamide gel solution of 40 ml, mix the following:

10X TBE Buffer (#B52)	4 ml
20% acrylamide/bisacrylamide	10 ml
UREA	19.2 g (to 8 M final concentration)
deionized water	to 40 ml

Caution: *acrylamide is a neurotoxin; always wear gloves, safety glasses, and a surgical mask when working with acrylamide powder.*

6. Vigorously agitate the solution by magnetic stirring to ensure complete mixing and solving of UREA powder.
7. Add 40 µl TEMED and swirl the flask to ensure thorough mixing.
8. Immediately add 400 µl of fresh 10% (w/v) APS and mix thoroughly.
9. Pour the acrylamide between the gel plates and insert the comb.
10. Clamp the comb in place at the top of the gel to avoid separation of the gel from the plates as the acrylamide polymerizes. Allow the gel to polymerize for 30 min.

Important note: *polymerization begins as soon as APS is added to the mixture, so all succeeding actions must be performed promptly.*

Run the gel:

1. After polymerization is complete, remove the comb and any bottom spacers from the gel. Fill the lower reservoir of the electrophoresis tank with 1X TBE buffer. Initially, place the gel into the lower tank at an angle to avoid the formation of air bubbles between the plates and the gel bottom. Clamp the gel plates to the top of the electrophoresis tank and fill the upper reservoir with 1X TBE so that the wells are covered.
2. Pre-run and warm the gel for at least 30 min at 5 V/cm (constant voltage).

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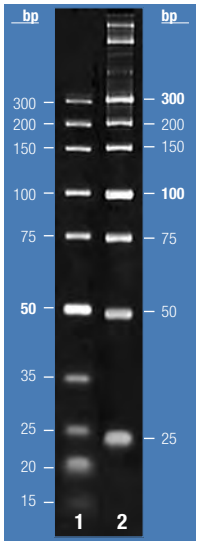

Note

Heat the gel (buffer) during the whole run at 60-70ºC.

❸ Wash the wells with 1X TBE buffer to remove UREA and gel pieces.
❹ Load the samples.
❺ Run the gel at 6 V/cm till the lower dye front reaches the three thirds of the gel.
❻ Soak the gel for about 15 min in 1X TBE to remove the urea prior to staining.
❼ Stain the gel in a 0.5 µg/ml ethidium bromide aqueous solution for about 30 min.
❽ Examine the gel under the UV light.

Figure 9.9. Migration of GeneRuler™ Ultra Low Range (#SM1211/2) and Low Range (#SM1191/2) DNA ladders under denaturing conditions.
Ladders are prepared for electrophoresis using formamide containing 2X RNA Loading Dye (#R0641), as specified in above protocol and loaded on 10% denaturing PAGE with 8 M UREA.

1 – GeneRuler™ Ultra Low Range DNA Ladder (#SM1211)
2 – GeneRuler™ Low Range DNA Ladder (#SM1191)

Note

- GeneRuler™ Ultra Low Range DNA Ladder: 10 bp band is not visible, 15 bp is the first one seen.
- GeneRuler™ Low Range DNA Ladder: upper bands (400, 500, 700 bp) are not separated well under denaturing conditions.

Preparation of DNA Ladders/Markers for Electrophoresis

Table 9.6. Recommendations for loading the conventional formulation (supplied in TE buffer) DNA ladders/markers.

Technical specifications	Conventional formulation (supplied in TE buffer) DNA ladders/markers			
	GeneRuler™ DNA ladders	Conventional Lambda DNA markers	Conventional Phage & Plasmid DNA markers	DNA Markers for Genomic DNA Analysis
Supplied amount/ number of applications	50 µg (100 µl) is sufficient for: – 100 applications on agarose gel – 50 applications on native PAGE	50 µg (100 µl) is sufficient for 100 applications on agarose gel	50 µg (100 µl) is sufficient for: – 100 applications on agarose gel – 50 applications on native PAGE	6 µg (30 µl) is sufficient for 120 applications on agarose gel
Amount used per 1 mm width of a gel lane	0.1 µg (0.2 µl) for agarose gel 0.2 µg (0.4 µl) for PAGE	0.1 µg (0.2 µl) for agarose gel	0.1 µg (0.2 µl) for agarose gel 0.2 µg (0.4 µl) for PAGE	6 ng
Dilution	Not needed	Not needed	Not needed	Mix 1 µl (0.2 µg) of DNA marker with 39 µl of nuclease-free water
Heating	Do not heat	**Heat at 65°C** for 5 min; chill on ice for 3 min before use	Do not heat	**Heat at 65°C** for 5 min; chill on ice for 3 min before use
I. Loading on agarose gel:				
DNA ladder/marker **loading dye** **Water, nuclease-free** (#R0581)	1 µl (0.5 µg) 2 µl 9 µl	1 µl (0.5 µg) 2 µl 9 µl	1 µl (0.5 µg) 2 µl 9 µl	10 µl (50 ng) of diluted marker 1 µl
Mix gently and load on gel				
II. Loading on polyacrylamide gel:				
DNA ladder/marker **loading dye** **Water, nuclease-free** (#R0581)	2 µl (1 µg) 0.5 µl 0.5 µl	Not recommended for PAGE	2 µl (1 µg) 0.5 µl 0.5 µl	Not recommended for PAGE
Mix gently and load on gel				

Table 9.7. Recommendations for loading ready-to-use DNA ladders/markers.

Technical specifications	DNA ladders/markers, ready-to-use						
	GeneRuler™ & O'GeneRuler™ DNA ladders	MassRuler™ DNA ladders	FastRuler™ DNA ladders	O'RangeRuler™ DNA ladders	ZipRuler™ Express DNA ladders	Conventional Lambda DNA markers	Conventional Phage & Plasmid DNA markers
Supplied volume/ number of applications	500 µl for 100 applic.	2x500 µl for 50-200 applic.	2x500 µl for 50-333 applic.	500 µl for 100 applic.	2x500 µl for 100-200 applic.	500 µl for 100 applic.	500 µl for 100 applic.
						Heat at 65°C for 5 min; **chill on ice** for 3 min before use	
Mix gently and load on gel							
Volume per 1 mm width of a gel lane	1-2 µl	variable	variable	1-2 µl	1-2 µl	1-2 µl	1-2 µl

Bulk quantities and custom formulations available upon request

Preparation of DNA Samples for Electrophoresis

Preparation of DNA Samples for Conventional DNA Electrophoresis

6X DNA Loading Dye (#R0611), **6X MassRuler™ DNA Loading Dye** (#R0621), **6X Orange DNA Loading Dye** (#R0631), **6X TriTrack™ DNA Loading Dye** (#R1161) are all used according to below protocol:

1. Add 1 volume of 6X DNA loading dye to 5 volumes of DNA sample.
2. Mix well, spin down and load.

Preparation of DNA Samples from Enzymatic Reaction Mixtures or with Samples Containing High Amounts of DNA Binding Proteins

Use **6X DNA Loading Dye & SDS Solution** (#R1151) to prevent the appearance of additional bands or gel shifts when analyzing:

- probes after DNA restriction digestions, ligation or dephosphorylation reactions,
- DNA samples with high amounts of DNA binding proteins,
- DNA molecules with cohesive ends,
- or to stop an enzymatic reaction during kinetic experiments.

To mix 6X DNA Loading Dye & SDS Solution with sample DNA:

1. Add 1 volume of 6X DNA Loading Dye & SDS Solution to 5 volumes of DNA sample.
2. Mix well.
3. Heat at 65°C for 10 minutes.
4. Chill on ice, spin down and load.

Note

The prepared sample can be stored at -20ºC and reused for electrophoresis after several freeze-thaw cycles.

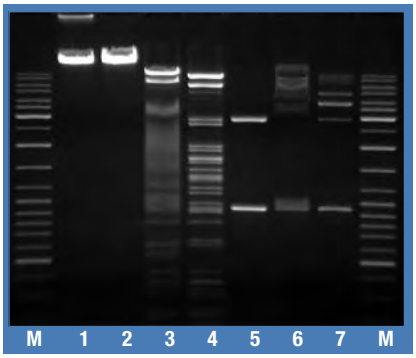

Figure 9.10. The effect of SDS on electrophoresis of DNA samples containing high amounts of DNA binding proteins.

M – GeneRuler™ DNA Ladder Mix (#SM0331)
1 – 0.5 µg λ DNA prepared for loading with 6X DNA Loading Dye (#R0611)
2 – 0.5 µg λ DNA prepared for loading with 6X DNA Loading Dye & SDS Solution (#R1151)
3 – 0.5 µg λ DNA digested with TsoI (#ER1991), probe prepared for loading with 6X DNA Loading Dye
4 – 0.5 µg λ DNA digested with TsoI, probe prepared for loading with 6X DNA Loading Dye & SDS Solution
5 – 0.4 µg of the 2 fragment ligation mixture prior the addition of T4 DNA Ligase (#EL0014)
6 – 0.4 µg of the 2 fragment ligation mixture after the ligation with T4 DNA Ligase, probe prepared for loading with 6X DNA Loading Dye
7 – 0.4 µg of the 2 fragment ligation mixture after the ligation with T4 DNA Ligase, probe prepared for loading with 6X DNA Loading Dye & SDS Solution

Preparation of DNA Samples for Denaturing Polyacrylamide/Urea Gel Electrophoresis

See the protocol on p.444.

Preparation of DNA Samples for Denaturing Alkaline Agarose Gel Electrophoresis

See the protocol on p.443.

Labeling of DNA Ladders/ Markers

For protocols *see* chapter **Molecular Labeling & Detection** on p.381

Troubleshooting Guide for DNA Electrophoresis

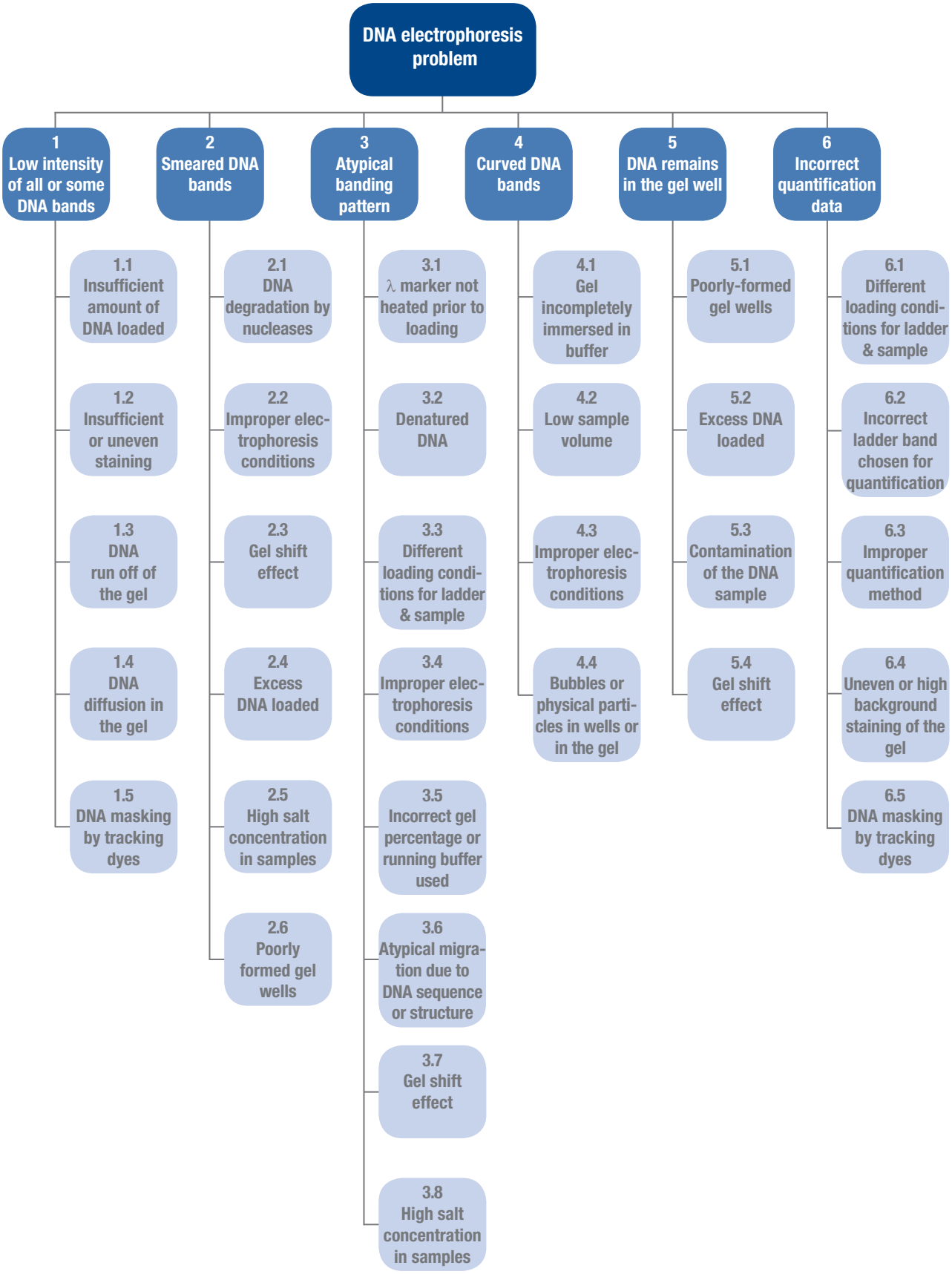

Table 9.8. Troubleshooting Guide for DNA Electrophoresis.

Problem	Possible cause and recommended solution
1. Low intensity of all or some of the DNA bands	**1.1. Insufficient amount of ladder was loaded.** Follow the recommendations for loading described in the certificate of analysis of the DNA ladders/markers (~0.1-0.2 µg per 1 mm gel lane width) or in the Table 9.6 on p.445.
	1.2. Insufficient or uneven staining. Following electrophoresis, visualize DNA by staining in ethidium bromide solution (final concentration 0.5 µg/ml) or SYBR® Green I. Alternatively, if the DNA will not be used for cloning, add ethidium bromide to both the gel and electrophoresis buffer at a final 0.5 µg/ml concentration. After alkaline agarose gel electrophoresis the gel should be immersed for 30 min in 300 ml 0.5 M Tris-HCl buffer, pH 7.5 and only later stained in a 0.5 µg/ml ethidium bromide solution for 30 min. After denaturing polyacrylamide gel electrophoresis with urea, soak the gel for about 15 minutes in 1X TBE to remove the urea prior to staining. Stain the gel in 0.5 µg/ml ethidium bromide in 1X TBE solution for 15 min. Make sure that the gel is immersed completely in the staining solution.
	1.3. DNA run off the gel. Perform electrophoresis until the bromophenol blue dye passes 2/3 (orange G, 4/5) of the gel. Refer to the table on p.441 for migration of tracking dyes in different gels. Make sure that the entire gel is immersed completely in the electrophoresis buffer during the run. Make sure that gel and apparatus are positioned horizontally during the run.
	1.4. DNA diffusion in the gel. Avoid prolonged electrophoresis or excessive staining and destaining procedures as this may cause diffusion of smaller DNA fragments in the gel. Avoid long term storage of the gel before taking a picture, as this may cause diffusion of DNA fragments and low band intensity.
	1.5. DNA masking by electrophoresis tracking dyes. Do not exceed the amount of electrophoresis tracking dyes used for sample/ladder preparation. Use the loading dye solutions supplied with every Fermentas DNA ladder/marker, as these solutions contain equilibrated amount of tracking dyes which will not mask DNA under UV light. Prepare DNA ladders and probes according to recommendations on p.445.
2. Smeared DNA bands	**2.1. DNA degradation by nucleases.** Use fresh electrophoresis buffers, freshly poured gels, nuclease free vials and tips to minimize nuclease contamination of DNA solutions.
	2.2. Improper electrophoresis conditions. Prepare gels according to recommendations on p.442, always use the same electrophoresis buffer for both preparation of the gel and running buffer. Make sure that the whole gel is immersed completely in the electrophoresis buffer during the run. Do not use an excessively high voltage for electrophoresis. Run the gels at 5-8 V/cm. To increase the band sharpness, use a lower voltage for several minutes at the beginning of electrophoresis. For fast electrophoresis under high voltage (up to 23 V/cm) use GeneRuler™ or O'GeneRuler™ Express DNA ladders (#SM1551/2/3 or #SM1563, p.423). An excessively low voltage during the entire run may result in diffusion of bands during electrophoresis. Excessively high voltage may result in gel heating and DNA denaturation. To calculate the optimal electrophoresis conditions (voltage) and to use the recommended V/cm value (usually 5-8 V/cm, depending on the ladder) one has to: – measure the distance between electrodes (cathode and anode) – X, cm. – and multiply that X, cm value by the recommended voltage (Y, V/cm) – the result (X, cm x recommended Y, V/cm) is Z – recommended voltage to be applied.

(continued on next page)

Table 9.8. Troubleshooting Guide for DNA Electrophoresis.

Problem	Possible cause and recommended solution
	2.3. Gel shift effect. DNA binding proteins, such as ligases, phosphatases or restriction enzymes may alter DNA migration on gels and cause the DNA to remain in the gel well or gel shifting. Lambda DNA or other DNA with long complementary overhangs may anneal and migrate atypically (*see* p.432). To correct for the above mentioned effects, use 6X DNA Loading Dye & SDS Solution (#R1151, p.441) which is supplemented with 1% SDS to eliminate DNA-protein interactions and to prevent annealing of DNA molecules via long cohesive ends. Always heat these samples with SDS at 65°C for 10 min, chill on ice, spin down and load.
	2.4. Excess DNA loaded. Follow the recommendations for loading described in the certificate of analysis of the DNA ladders/markers (~0.1-0.2 µg per 1 mm gel lane width) or in the Table 9.6 on p.445. If possible apply same requirements for DNA quantities for the samples as well.
	2.5. High salt concentration in the sample. Samples containing high concentrations of salts may result in smeared or shifted band patterns. Ethanol precipitation and washing the pellet with ice cold 75% ethanol or spin column purification prior to resuspending the sample in water or TE buffer helps eliminate saltspresent in the sample.
	2.6. Poorly formed (slanted) gel wells. When inserting the comb into the gel, make sure that it is vertical to the gel surface and stable during gel casting and its solidification.
3. Atypical banding pattern	**3.1. Lambda DNA marker was not heated prior to loading.** All DNA markers generated from Lambda DNA, as well as lambda DNA digestion products should be heated at 65°C for 5 min and chilled on ice before loading on the gel in order to completely denature the cohesive ends (the 12 nt cos site of lambda DNA) that may anneal and form additional bands. *See* p.445 for preparation of lambda markers for electrophoresis.
	3.2. Denatured DNA. Excessively high voltage may result in gel heating and DNA denaturation. To calculate the optimal electrophoresis conditions (voltage) and to use the recommended V/cm value (often 5-8 V/cm, depending on the ladder) one has to: – measure the distance between electrodes (cathode and anode) – X, cm. – and multiply that X, cm value by the recommended voltage (Y, V/cm) – the result (X, cm x recommended Y, V/cm) is Z – recommended voltage to be applied. For non-denaturing electrophoresis use the loading dye solutions supplied with every Fermentas DNA ladder/marker, as these solutions do not contain denaturing agents. Prepare DNA ladders and probes according to recommendations on p.445. Do not heat them before loading. Heating is required only for lambda DNA markers.
	3.3. Different loading conditions for the sample and the ladder DNA. Always use the same loading dye solution (supplied with the DNA ladder/marker) for both the sample DNA and the ladder/marker DNA. If possible always load equal or very similar volumes of the sample DNA and the ladder/marker DNA. The sample can be diluted with 1X loading dye.
	3.4. Improper electrophoresis conditions. Excessive electrophoresis run times or voltage may result in migration of small DNA fragments off of the gel. Very short or slow electrophoresis may result in incompletely resolved bands. Run gels at 5-8 V/cm until the bromophenol blue passes 2/3 (orange G, 4/5) of the gel. Refer to the Table 9.4 on p.441 for migration of tracking dyes in different gels. For fast electrophoresis under high voltage (up to 23 V/cm) use GeneRuler™ or O'GeneRuler™ Express DNA ladders (#SM1551/2/3 or #SM1563, p.423).

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Table 9.8. Troubleshooting Guide for DNA Electrophoresis.

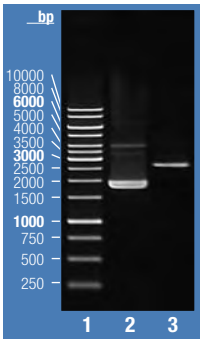
Problem	Possible cause and recommended solution
3. Atypical banding pattern	**3.5. Incorrect gel percentage or running buffer used.** TAE buffer is recommended for analysis of DNA fragments larger than 1500 bp and for supercoiled DNA. TBE buffer is used for DNA fragments smaller tha 1500 bp and for denaturing polyacrylamide gel electrophoresis. Large DNA fragments will not separate well in TBE buffer. The correct gel percentage is important for optimal separation of the ladder DNA; prepare gels according to recommendations on p.442. When preparing agarose gels always adjust the volume of water to accommodate for evaporation during boiling. Otherwise, the gel percentage will be too high and result in bad separation of larger DNA bands. Refer to the Table 9.4 on p.441 for the range of effective separation of DNA in different gels. Ethidium bromide interferes with separation of large DNA fragments. Do not include ethidium bromide in the gel and run buffer when large DNA (more than 20 kb) or supercoiled DNA is analyzed. Stain the gel following electrophoresis in a 0.5 µg/ml ethidium bromide solution for 30 min.
	3.6. Atypical migration due to different DNA sequence or structure. During high resolution electrophoresis DNA fragments of equal size can migrate differently due to differences in DNA sequences. AT rich DNA may migrate slower than an equivalent size GC rich DNA fragment. The sequences of Fermentas DNA ladders are chosen to allow for highly accurate DNA migration according to size, however, due to differencies in nucleotide sequence or the overall DNA structure, sample migration can sometimes slightly differ from ladder band migration. DNA structures such as nicked, supercoiled or dimeric molecules will always show different mobility on gels compared to an equivalent DNA size standard. *See* the picture below for migration of plasmid DNA forms:  **1** GeneRuler™ 1 kb DNA Ladder (#SM0311) **2** Undigested plasmid pUC19 2,7 kb DNA, forms: – upper band (~4 kb) – dimeric plasmid – below, less visible (~3.5 kb) – nicked plasmid – lowest band (~1.9 kb) – supercoiled plasmid. **3** Linearized plasmid pUC19 (2,7 kb) – migrates according to its size High level DNA modifications such as methylation, labeling with biotin or large fluorescent molecules also result in slower migration compared to un-modified DNA of the same size.
	3.7. Gel shift effect. The presence of DNA binding proteins in the sample, such as ligases, phosphatases or restriction enzymes may alter DNA migration in the gel or cause the DNA to remain in the gel wells. Lambda DNA or other DNA with long complementary overhangs may anneal resulting in an atypical migration pattern. To eliminate these effects, use 6X DNA Loading Dye & SDS Solution (#R1151) which is supplemented with 1% SDS to eliminate DNA-protein interactions and to prevent annealing of DNA molecules via long cohesive ends. Always heat these samples with SDS at 65°C for 10 min, chill on ice, spin down and load. High salt concentration in the sample may also cause gel shift effects, *see* 3.8.
	3.8. High salt concentration in the sample. Samples with a high salt concentration may give smeared or shifted band patterns. Ethanol precipitation and washing the pellet with ice cold 75% ethanol or spin column purification prior resuspending DNA in water or TE buffer, helps eliminate salt from the sample.

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Table 9.8. Troubleshooting Guide for DNA Electrophoresis.

Problem	Possible cause and recommended solution
4. Curved DNA bands	**4.1. Gel incompletely immersed in electrophoresis buffer.** Electrophoresis buffer should completely cover the entire gel during sample loading and run.
	4.2. Low sample volume. The sample or the ladder volume should be large enough to fill 1/3 of the total capacity of the well. Large wells should not be used with small sample volumes. If needed the sample volume can be adjusted with 1X loading dye.
	4.3. Improper electrophoresis conditions. Do not use an excessively high voltage for electrophoresis. Run the gels at 5-8 V/cm. To minimize band curving, use a lower voltage for several minutes at the beginning of electrophoresis. For fast electrophoresis under high voltage (up to 23 V/cm) use GeneRuler™ or O'GeneRuler™ Express DNA ladders (#SM1551/2/3 or #SM1563, p.423). To calculate the optimal electrophoresis conditions (voltage) and to use the recommended V/cm value (which is in many cases 5-8 V/cm, depending on the ladder) one has to: – measure the distance between electrodes (cathode and anode) – X, cm. – and multiply the X value by the recommended voltage (Y, V/cm) – the result (X x Y) is the recommended voltage to be applied.
	4.4. Bubbles or physical particles in the gel wells or in the gel. Use pure water, clean flasks and clean equipment for preparation of gels. Pour the gel slowly avoiding formation of bubbles. Bubbles can be removed with a pipette tip.
5. DNA remains in the gel	**5.1. Poorly formed gel wells.** Remove the gel comb only after complete polymerization of the gel. Pour the buffer onto the gel immediately. Rinse the wells with electrophoresis buffer to remove urea from denaturing polyacrylamide gels prior to loading the sample.
	5.2. Excess DNA loaded. Follow the recommendations for loading described in the certificate of analysis of the DNA ladders/markers (~0.1-0.2 µg per 1mm gel lane width) or in the Table 9.6 on p.445. If possible load the same quantity of the sample.
	5.3. Contamination of the DNA sample. Make sure that your sample DNA solution does not contain any precipitate.
	5.4. Gel shift effect. The presence of DNA binding proteins in the sample, such as ligases, phosphatases or restriction enzymes may alter DNA migration in the gel and cause the DNA to remain in the gel wells. Lambda DNA or other DNA with long complementary overhangs may anneal resulting in an atypical band migration pattern. To eliminate these effects, use 6X DNA Loading Dye & SDS Solution which is supplemented with 1% SDS to eliminate DNA-protein interactions and to prevent annealing of DNA molecules via long cohesive ends. Always heat these samples with SDS at 65°C for 10 min, chill on ice, spin down and load.
6. Incorrect quantification data	**6.1. Different loading conditions for the sample and the ladder DNA.** Always use the same loading dye solution (supplied with the DNA ladder/marker) for both the sample DNA and the ladder/marker DNA. If necessary, adjust the concentration of the sample to approximately equalize it with the amount of DNA in the nearest band. If possible always load equal or very similar volumes of the sample DNA and the ladder/marker DNA. The sample can be diluted with 1X loading dye solution.
	6.2. Incorrect ladder band chosen for quantification of the sample. Always compare the sample band with a similar sized ladder band.
	6.3. Improper quantification method used. If possible, quantify by video-densitometry while subtracting the gel background as this method is more precise than a visual comparison of the bands.

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Table 9.8. Troubleshooting Guide for DNA Electrophoresis.

Problem	Possible cause and recommended solution
6. Incorrect quantification data	**6.4. Uneven staining of the gel and high background staining can also interfere with gel quantification results.** Make sure that the gel is immersed completely in the staining solution. Following electrophoresis, visualize DNA by staining in ethidium bromide solution (final concentration 0.5 µg/ml) or SYBR® Green I. Do not exceed the recommended concentration of the dye for staining. Avoid prolonged staining for more than 30 min as this may result in high background. If the gel is to be stained during the run, ensure that the ethidium bromide is included in both the gel and running buffer, otherwise the staining will be uneven. After alkaline agarose gel electrophoresis the gel should be immersed for 30 min in 300 ml of 0.5 M Tris-HCl buffer, pH 7.5 and only later stained in a 0.5 µg/ml ethidium bromide solution for 30 min. After denaturing polyacrylamide gel electrophoresis with urea, soak the gel for about 15min in 1X TBE to remove the urea prior to staining. Stain the gel in 0.5µg/ml ethidium bromide in 1X TBE solution for 15 min.
	6.5. DNA masking by electrophoresis tracking dyes. Do not exceed the recommended amount of electrophoresis tracking dyes used for sample/ladder preparation. Use the loading dye solutions supplied with every Fermentas DNA ladder/marker, as these solutions contain equilibrated amount of tracking dyes which will not mask DNA under UV light. Prepare DNA ladders and probes according to the recommendations on p.445.