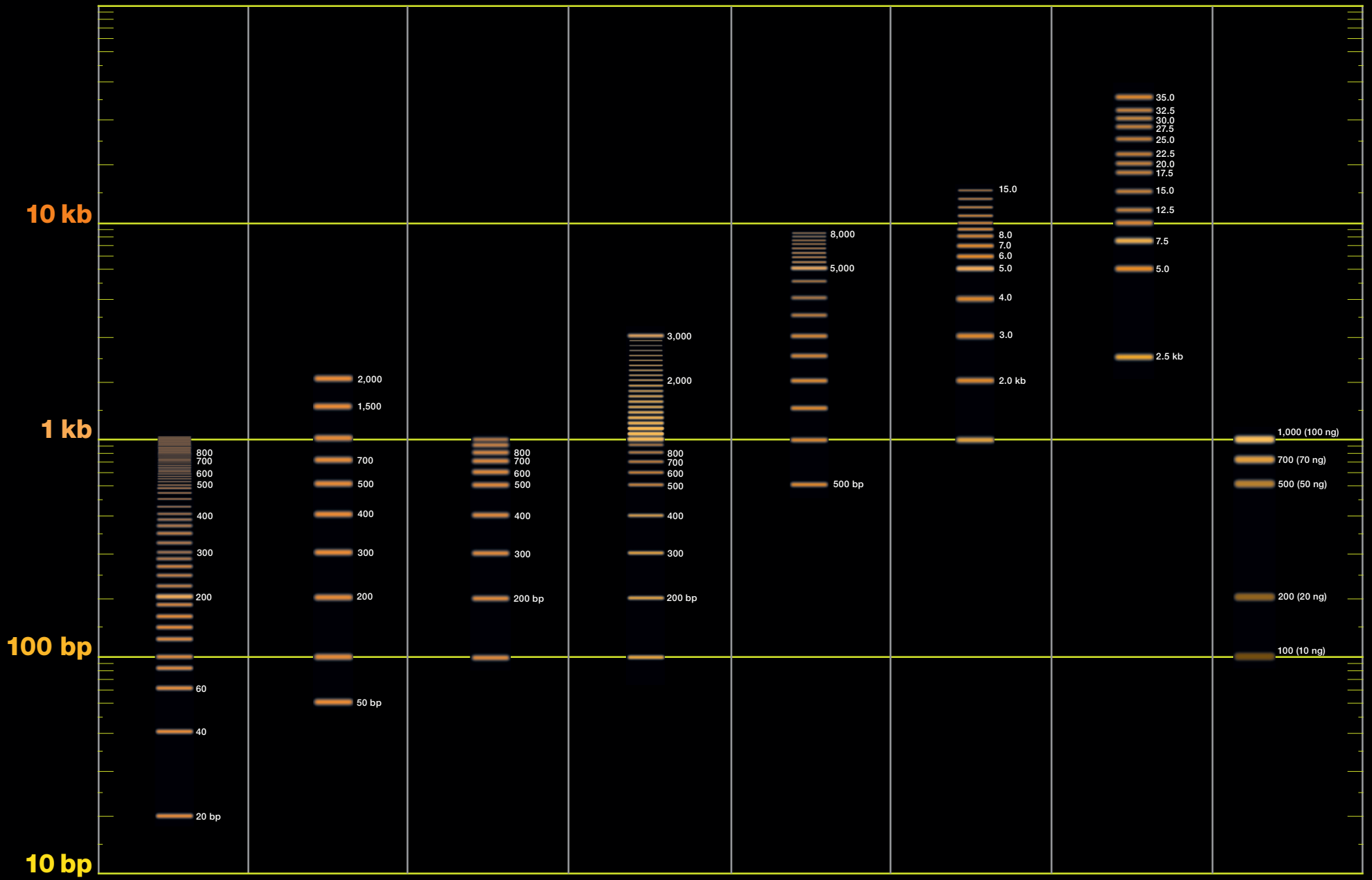
User Guide to Nucleic Acid Standards



	20 bp Molecular Ruler	AmpliSize® Molecular Ruler	100 bp Molecular Ruler	100 bp PCR Molecular Ruler	500 bp Molecular Ruler	1 kb Molecular Ruler	2.5 kb Molecular Ruler	Precision Molecular Mass Ruler
EZ Load™	170-8351		170-8352	170-8353	170-8354	170-8355		170-8356
Standard	170-8201	170-8200	170-8202	170-8206	170-8203	170-8204	170-8205	170-8207

Relative Sample Migration Rates*

Cell Type	Voltage	Bromophenol Blue Migration Rate
Sub-Cell® Model 96	200 V	~6.2 cm/hr
Sub-Cell Model 192	200 V	~5.2 cm/hr
Sub-Cell GT cell, 15 x 15 cm gel	75 V	~3.0 cm/hr
Wide Mini-Sub® cell GT, 15 x 10 cm gel	75 V	~4.5 cm/hr
Mini-Sub cell GT cell, 7 x 10 cm gel	75 V	4.5 cm/hr

* These sample migration rates were determined based on a 0.5 cm thick 1.0 % agarose gel using Bio-Rad's Certified™ Molecular Biology Agarose in 1x TAE electrophoresis buffer (diluted from Bio-Rad's Premixed 50x TAE Buffer). Migration rates will vary depending on the voltage, current, and type of agarose or buffer used.

Properties of Nucleic Acids

Common Conversions of Nucleic Acids

Weight conversions

1 µg = 10^{-6} g
1 ng = 10^{-9} g
1 pg = 10^{-12} g
1 fg = 10^{-15} g

Spectrophotometric conversions

1 A_{260} unit of double-stranded DNA = 50 µg/ml
1 A_{260} unit of single-stranded DNA = 33 µg/ml
1 A_{260} unit of single-stranded RNA = 40 µg/ml

Molar conversions

1 µg of 1,000 bp DNA = 1.52 pmole (3.03 pmoles of ends)
1 µg of pBR322 DNA = 0.36 pmole
1 pmole of 1,000 bp DNA = 0.66 µg
1 pmole of pBR322 DNA = 2.8 µg

Protein conversions

1 kb of DNA = 333 amino acids of coding capacity = 3.7 x 10^4 MW
10,000 MW protein = 270 bp DNA
30,000 MW protein = 810 bp DNA
50,000 MW protein = 1.35 kb DNA
100,000 MW protein = 2.7 kb DNA

DNA Size Migrations With Sample Loading Dyes

Agarose Concentration (%)	Xylene Cyanole	Bromophenol Blue
0.5–1.5	4–5 kb	400–500 bp
2.0–3.0*	750 bp	100 bp
4.0–5.0*	125 bp	25 bp

* Sieving agarose such as AmpliSize agarose.

Commonly Used Electrophoresis Buffers

Working Buffer	Concentrated Stock Solution	Solution (per liter)
Tris-acetate (TAE)	1x: 0.04 M Tris-acetate, 0.001 M EDTA	50x: 242 g Tris base, 57.1 ml glacial acetic acid, 100 ml 0.5 M EDTA (pH 8.0)
Tris-phosphate (TPE)	1x: 0.09 M Tris-phosphate, 0.002 M EDTA	10x: 108 g Tris base, 15.5 ml 85% phosphoric acid (1.679 g/ml), 40 ml 0.5 M EDTA (pH 8.0)
Tris-borate (TBE)	0.5x: 0.045 M Tris-borate, 0.001 M EDTA	5x: 54 g Tris base, 27.5 ml boric acid, 20 ml 0.5 M EDTA (pH 8.0)
Alkaline	1x: 50 mN NaOH, 1 mM EDTA	1x: 5 ml 10 N NaOH, 2 ml 0.5 M EDTA (pH 8.0)

Gel-Loading Buffers

Buffer Type	6x Buffer	Storage Temperature
I	0.25% Bromophenol Blue, 0.25% Xylene Cyanole FF, 40% (w/v) sucrose in water	4°C
II	0.25% Bromophenol Blue, 0.25% Xylene Cyanole FF, 15% Ficoll (Type 400) in water	Room temperature
III	0.25% Bromophenol Blue, 25% Xylene Cyanole FF, 30% glycerol in water	4°C
IV	0.25% Bromophenol Blue, 40% (w/v) sucrose in water	4°C
V	Alkaline loading buffer: 300 mN NaOH, 18% Ficoll (Type 400) in water, 0.15% Bromocresol Green, 0.25% Xylene Cyanole FF	4°C

Effective Range of Separation of DNA in Polyacrylamide Gels

Acrylamide (% [w/v])*	Effective Range of Separation (bp)	Xylene Cyanole FF**	Bromophenol Blue**
3.5	1,000–2,000	460	100
5	80–500	260	65
8	60–400	160	45
12	40–200	70	20
15	25–150	60	15
20	6–100	45	12

* N,N'-methylenebisacrylamide is included at 1/30th the concentration of acrylamide.
** The numbers given are the approximate sizes (in nucleotide pairs) of fragments of double-stranded DNA with which the dye comigrates.

Range of Separation in Agarose Gels

Agarose (% [w/v])	Effective Range of Separation of Linear DNA Molecules (kb)
0.3	5–60
0.6	1–20
0.7	0.8–10
0.9	0.5–7
1.2	0.4–6
1.5	0.2–3
2.0	0.1–2

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