General Recommendations for DNA Electrophoresis

This protocol is for the 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 1. 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

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Table 2. 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	0 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. The following guidelines are recommended:

- Only high purity agarose should be used. TopVision™ Agarose was used to prepare the gels.
- Only freshly prepared electrophoresis buffers should be used. The buffers were prepared from 50X TAE Buffer and 10X TBE Buffer.
- 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

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