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DNA electrophoresis in agarose gels: A simple relation describing the length dependence of mobility

Electrophoretic mobilities of DNA molecules ranging in length from 100 to 10 000 base pairs (bp) were measured in gels of eleven concentrations of agarose from 0.5 to 1.5%. Excellent fits of the dependence of mobility on DNA length were obtained with the relationship $\frac{1}{\mu(L)} = \frac{1}{\mu_l} - \left(\frac{1}{\mu_l} - \frac{1}{\mu_s}\right) e^{-L/\gamma}$ showing an $e^{-L/\gamma}$ crossover, where L is the length of a DNA fragment and γ is a crossover length ranging from 8000 to 12 000 bp. The other parameters in the fit are μ_s the mobility of short DNA with unit charge in the limit as length is extrapolated to zero, and μ_l, the mobility of long DNA as length is extrapolated to infinity. This exponential relationship should be a useful interpolation function for determining DNA lengths over a wide range. The simplicity of this relationship may be of more fundamental significance and suggests that some common feature dominates the electrophoresis of double stranded DNA fragments in agarose gels, regardless of length.

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Gene mapping, sequencing and other aspects of genomics depend on accurate determinations of double-stranded DNA fragment lengths. DNA separations according to length are commonly accomplished by electrophoresis in agarose gels. Length determination depends on observations, first made over 30 years ago [1–4], that the electrophoretic mobility of DNA in agarose gels decreases to some non-zero limit with increasing chain contour length, regardless of base sequence. Despite widespread use and extensive theoretical development, important questions remain about the mechanism(s) by which the gel matrix sorts DNA fragments according to size (*e.g.* see the review in [5] and references therein). In order to reexamine these issues we ran a series of electrophoretic separations at varying agarose concentrations and tested several methods for fitting the resulting data. This led to the conclusion that none of the standard techniques for fitting were satisfactory for DNA molecules ranging from 100 to 10 000 bp. A simple phenomenological model was developed that was found to fit our data exceptionally well over the entire range of DNA sizes.

The electrophoretic mobilities, $\mu(L)$ of fragments of "100 bp ladder" and "1000 bp ladder" standards were determined for gels of eleven agarose concentrations of T = 0.5, 0.6, 0.7, . . ., 1.5% (Seakem LE, BMA Bioproducts, Rockland, ME, USA) containing TBE buffer (0.89 M Tris, 0.89 M boric acid, 1.0 mM Na_2EDTA, pH 8.3; reagents from Sigma-Aldrich, St. Louis, MO, USA). Gel concentrations are in %T = (grams agarose/100 mL buffer) × 100. TBE buffer was used in the electrode trays (Bethesda Research Laboratories Horizontal Gel Electrophoresis System). Electrophoresis was performed at an ambient temperature of 22°C ± 1°C. The applied voltage was controlled to produce a constant field of 2.78 V/cm to reduce heating and distortions of DNA conformation induced by strong fields [6, 7]. Gels were 25 cm long with an effective thickness of 4 mm. The gel holder was divided so samples could be run simultaneously in three different concentration gels. All experiments were performed with the same equipment, using a single stock solution of TBE buffer for all gels and tray buffers. Gels were post-stained with ethidium bromide (0.5 mg/L) and then photographed using Polaroid Type 667 film. Distances from the well bottom to band midpoints, measured directly from photographs, were used to calculate mobilities. Precision in mobilities varied from ±0.6 to 1.6% (shortest to longest fragments), on 0.5% gels, and from ±0.8 to 6% on 1.5% gels. The data were fit to a relationship that can be represented in most general form by

$$\mu(L) = \frac{1}{\beta + \alpha(1 - e^{-L/\gamma})} \quad (1)$$

where α, β, and γ are adjustable parameters. Excellent fits were obtained for all gel concentrations using an algorithm generated in MathCad (MathSoft, Cambridge, MA, USA) (Fig. 1a). Residuals in $\mu(L)$ were small (<2%) and generally randomly distributed for $T \leq 1.0\%$ (Fig. 1b). Some nonrandom trends were noticeable for the highest

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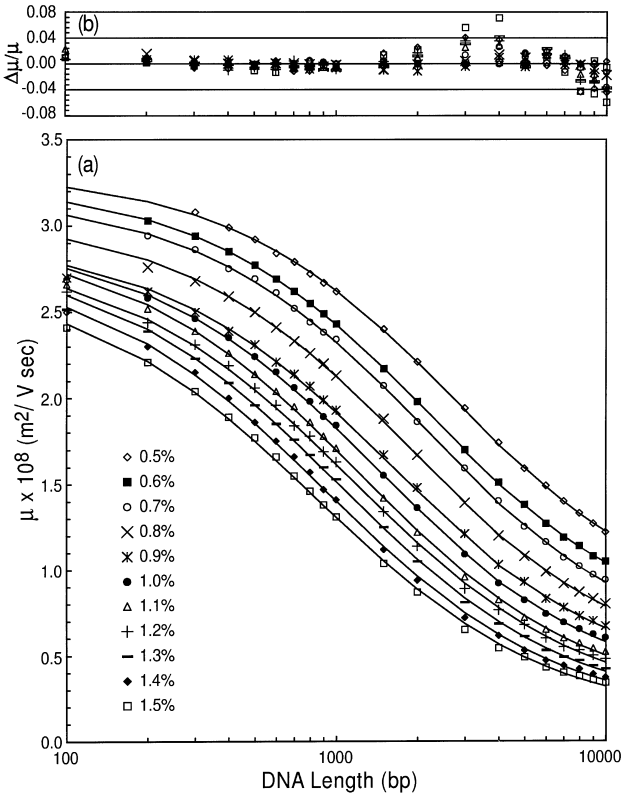

Figure 1. (a) Dependence of DNA mobility on length and agarose gel concentration. DNA samples were a mixture of $N \times 100$ bp and $N \times 1000$ bp "ladder" standards (N = 1 . . . 10) from Promega. Lines are nonlinear least squares fits to Eq. (1) generated by a MathCad (MathSoft) program. Residuals of the fits are shown in frame (b), and were consistent in magnitude with experimental precision.

gel concentrations, but residuals remained less than 2% for $L \leq 2000$ bp, and less than 4% for longer fragments in all gels except for T = 1.5%. The residuals in the fits were also within the precision of the data for all gel concentrations. Chi-squared (χ^2) for gels with $T \leq 1.1\%$ were ≥ 0.9996; values for higher T drifted downward, reflecting the higher residuals for long DNAs, but χ^2 remained ≥ 0.999 for all fits. Data from several other sources were also fit well by Eq. (1), as is illustrated in Fig. 2 for some sample cases. A more comprehensive analysis of literature data is in progress.

Equation (1) expresses an exponential crossover from the mobilities of short DNA fragments to the mobilities of long fragments. An intuitively more appealing representation of this exponential crossover is obtained by substituting the limiting mobilities into Eq. (1). The limiting mobility, μ_s describes the mobility of a particle with charge q in the limit of zero length, *i.e.*,

$$\lim_{L \to 0} \mu(L) \equiv \mu_s \tag{2}$$

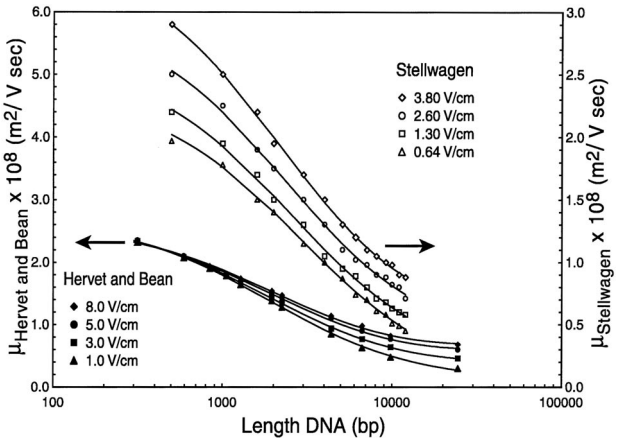

Figure 2. Fits of literature data to Eq. (1) taken from Hervet and Bean [9] for T = 1.0% (left scale), and from Stellwagen [6] for T = 0.6% (right scale) at indicated electric fields.

Note that μ_s is not equivalent to the free solution mobility of long DNA often quoted in other studies and represented by μ_0. In the limit of $L \gg \gamma$ the exponential term is negligible and the limiting mobility μ_l is

$$\lim_{L \to \infty} \mu(L) \equiv \mu_l \tag{3}$$

Inverting Eq. (1) and substituting from Eqs.(2) and (3) yields

$$\frac{1}{\mu(L)} = \frac{1}{\mu_l} - \left(\frac{1}{\mu_l} - \frac{1}{\mu_s}\right) e^{-L/\gamma} \tag{4}$$

or, in particularly simple form,

$$\frac{\dfrac{1}{\mu_l} - \dfrac{1}{\mu(L)}}{\dfrac{1}{\mu_l} - \dfrac{1}{\mu_s}} = e^{-L/\gamma} \tag{5}$$

The reciprocal mobility expresses the resistance that the gel matrix offers to passage of solutes; thus, it is the decrease in gel resistance with decreasing DNA length from the long chain limit that obeys a simple exponential. The parameter γ, which has dimensions of length, determines the rate of decrease.

The critical importance of the gel matrix for DNA separations by electrophoresis is well established. Stellwagen and co-workers [10] have shown that the mobility of DNA in free solution is independent of length for $L \geq 400$ bp, and decreases with decreasing length for $L \leq 400$ bp. This behavior contrasts sharply to the general decrease in mobility with increasing length when electrophoresis occurs in gels. Mobilities of DNA in agarose gels also become independent of length, but this limit occurs only for large $L \geq 20$ kbp or longer, depending on gel concentration [5, 11–13]. The challenge has been to describe

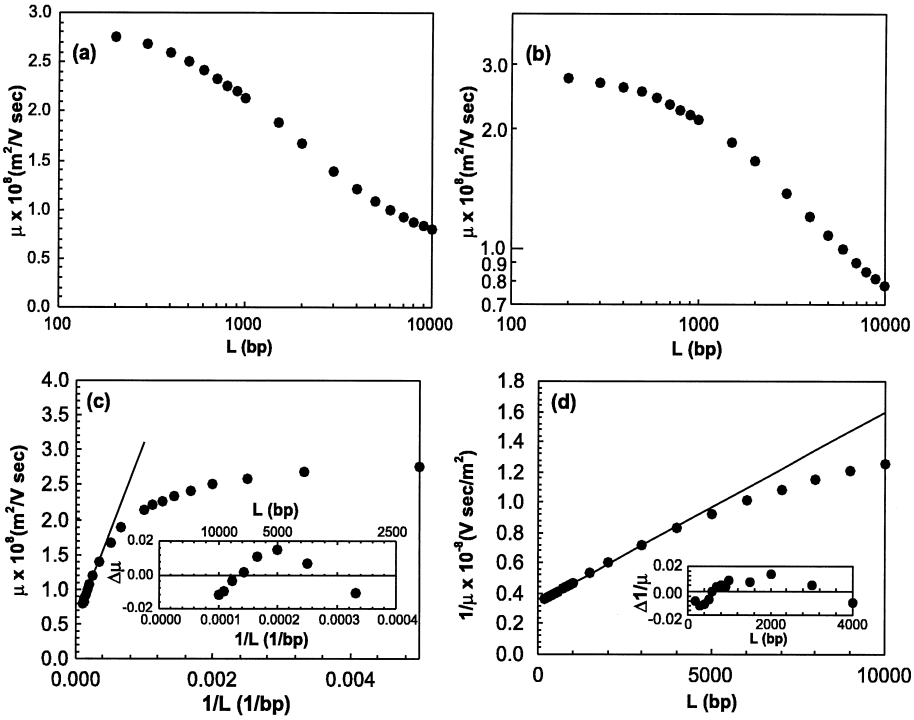

Figure 3. Representative dependencies of DNA mobilities on length plotted in different ways. Electrophoresis was performed on 0.8% Seakem LE agarose gel. (a) μ *vs*. log(*L*), (b) log(μ) *vs*. log (*L*), (c) μ *vs*. 1/*L*, (d) 1/ μ *vs*. *L*. A straight line was fit from 3000 bp to 10 000 bp for μ *vs*. 1/*L* and from 100 bp to 4000 bp for 1/ μ *vs*. *L*. For both graphs, the residuals (which are shown in the lower right panels) show trends indicating that deviations from the straight line were not random.

how DNA molecules (and by inference other semirigid chains) moving in a field interact with the gel matrix, and how these interactions change as the chain length increases over several orders of magnitude.

The usual practice in plotting calibration curves for determining DNA lengths, is to graph electrophoretic mobility *vs*. logarithm of length for a series of standards (*e.g*. see [2] and [14]). This practice has little theoretical basis, and plots of μ *vs*. $\log_{10}$ (*L*) are seldom linear for more than a decade in *L* (Fig. 3a). Plots of $\log_{10}$ (μ) *vs*. $\log_{10}$ (*L*) are of more fundamental interest and are approximately sigmoidal (Fig. 3b), showing that mobility does not scale as a simple power of length ($\mu \neq \alpha L^{\beta}$) over any significant range of *L*. Migration by biased reptation is proposed to dominate when the effective DNA radius significantly exceeds the mean pore size. As originally described by Lumpkin and Zimm [22, 23], and Slater and Noolandi [24], the entire DNA chain is envisioned to migrate within a tube defined by the path through the gel of the first chain segment. The migration of the chain center of mass is biased in the field direction, but the random distribution of chain configurations is considered to be unperturbed during reptation. For this idealized model the ratio of the gel mobility of the reptating chain, μ, to the free solution mobility, μ_0, is predicted to depend inversely on length, *i.e*.,

$$\frac{\mu}{\mu_0} = \frac{\alpha}{L} \tag{6}$$

It is well known that very long DNAs migrate through gels by an inchworm-like motion in which the DNA coil is alternatively stretched and relaxed. Stretching during reptation is predicted to decrease or cause total loss, at sufficiently long lengths, of the dependence of mobility on length [5, 11]. Southern's work [3, 25] sometimes has been cited as evidence for DNA reptation; that is, Southern is said to have shown that $\mu = \alpha/L$. Southern actually showed that plots of reciprocal mobility *vs*. length were linear over a wider range than traditional semi-log plots (μ *vs*. log(*L*)). Plots of $1/\mu$ *vs*. *L* eventually deviate from linearity, but Southern also proposed an empirical relationship that extended the linear range, namely

$$L = \frac{\alpha}{[\mu(L) - \mu_0]} + \beta' \tag{7}$$

which can be rewritten as

$$\mu(L) - \mu_0 = \frac{\alpha}{L - \beta'} \tag{8}$$

Plots of $\mu(L)$ *vs*. 1/*L* will not be linear unless $\beta' = 0$. In fact, plots of $\mu(L)$ *vs*. 1/*L* for the data collected in our study were seldom linear for a full decade (*e.g*. see the quasilinear region for the *L* = 10 000 to 3000 bp range in length in Fig. 3c). By contrast, plots of $1/\mu(L)$ *vs*. *L* were relatively linear over the *L* = 100 to 4000 bp range (Fig. 3d). In both cases, the nonrandom trends make the fits unacceptable, but Southern's approach yields a quasi-straight line for a 40-fold range in length compared to a 3-fold range for $\mu(L)$ *vs*. 1/*L*.

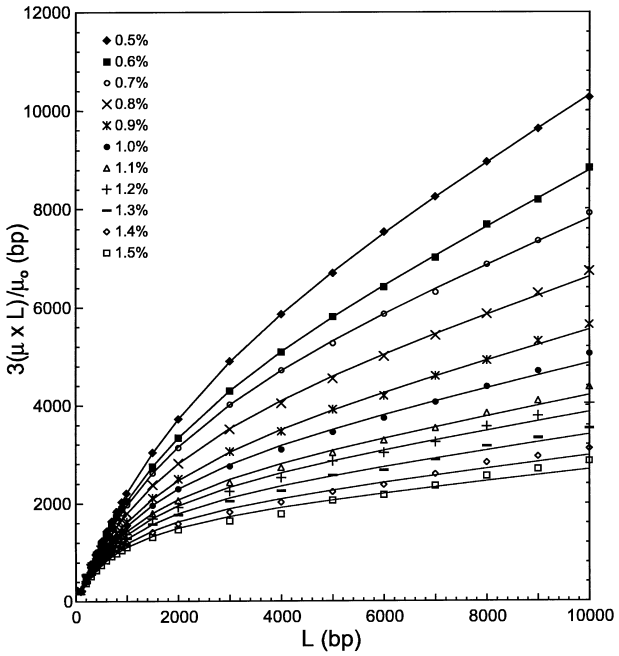

Figure 4. A reptation plot (3 $\mu L/\mu_0$ *vs*. *L*) for agarose gel concentrations *T* = 0.5% to 1.5% for a constant field *E* = 2.78 V/cm.

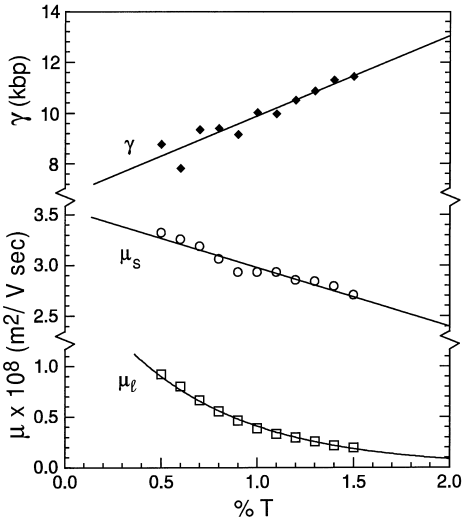

Figure 5. Dependencies of the limiting mobilities, μ_s and μ_L, and the crossover length γ on the gel concentration, *T* (refer to Eq. (6). The following equations, represented by solid lines, describe the dependencies: μ_s, = –0.58 *T* + 3.56, $\mu_l = 1.99e^{-1.59\,T}$, and γ = 2.78 *T* + 7.49, determined using least-squares fitting methods.

Southern's approach is a limiting form of the relations presented here, as is seen by expanding the $(1 - e^{-L/\gamma})$ term in Eq. (1):

$$\frac{1}{\mu(L)} = \beta + \alpha\left(\frac{L}{\gamma} - \frac{L^2}{2!\gamma^2} + \frac{L^3}{3!\gamma^3} - \cdots\right) \tag{9}$$

Thus, Southern's results support the exponential dependence of mobility on length suggested here more strongly than they support the $\mu(L) \propto 1/L$ dependence predicted by the classic reptation model (Eq. 6).

Slater *et al*. [26] have shown that plots of $3\mu L/\mu_0$ *vs*. *L* are illuminating for electrophoresis of single-stranded DNA in polyacrylamide gels. For data taken with relatively high fields and low gel concentrations these plots increased monotonically, with slopes that decrease smoothly. By contrast, plots for data taken with low fields and high gel concentrations exhibited dips, the slopes became non-monotonic. This slope inversion is considered a signature of the entropic trapping regime. Entropic trapping is envisioned to occur when a random coil polymer expands entropically into a gel vacancy, then must assume a smaller, low probability configuration to continue its forced migration through the gel. Analogous plots of our data are consistent with the high field, relatively low gel concentration regime (Fig. 4). Slight deviations of the fits from the data are most pronounced for the higher concentrations, but neither the point-to-point slopes of the data nor its derivatives exhibit significant nonmonotonic behavior.

Barkema *et al*. [27] have shown that some data collapse to a single curve when plotted as $T^{5/2}v\,L^2$ *vs*. *TLE* where *v* is the drift velocity. They proposed a two parameter fit of the collapsed data in the form

$$\frac{T^{5/2}vL^2}{\alpha} = \left[\left(\frac{TLE}{\beta}\right)^2 + \left(\frac{TLE}{\beta}\right)^4\right]^{1/2} \tag{10}$$

The collapsed data they analyzed fit with $\beta = 27 \pm 5$ and $\alpha/\beta = 1.0 \pm 0.1$ at 20°C. We analyzed our data in this way and were unable to obtain reasonable fits even when the α- and β-parameters were allowed to vary freely.

The parameters resulting from fits of our data to Eq. (1) showed clear trends with gel concentration (Fig. 5). The mobility limit for short DNA, μ_s, decreased linearly by about 24% with increasing concentration from *T* = 0.5% to 1.5%. The linear decrease of μ_s with gel concentration observed is consistent with the expectation that the mobility of a point particle through a collection of obstacles will be proportional to the number density of obstacles (at densities well below the percolation limit). Likewise the values of γ changed modestly and linearly with gel concentration, increasing from 8 kbp at *T* = 0.5% to 11.8 kbp at *T* = 1.5%. By contrast, the mobility limit for long DNA, μ_l, decreased with increasing gel concentration, and the ratio of μ_s/μ_l rose from 3 for *T* = 0.5%, to 12 for gels of *T* = 1.5%. In the figure captions, equations are shown representing best fits to allow the reader to extract values for the three parameters.

In summary, relations commonly proposed to describe the dependence of DNA electrophoretic mobility on length are valid only over limited size ranges, generally a factor of 10 or less. These size ranges can be related to models for transport of a particular molecule type through a gel network. Ogston sieving and reptation are two of the best known models and are used to describe electrophoresis of rigid molecules and of unperturbed, worm-like chains, respectively. We have found that a remarkably simple relationship with only a single, exponential term in L describes the electrophoretic mobilities of DNA molecules as small as 100 bp and as large as 10 000 bp or more. To our knowledge this relationship has not been reported previously. The 100 bp molecules are substantially shorter than the DNA persistence length and behave approximately as rods with a modest axial ratio ($\approx$ 14), while 10 kbp molecules are about 70 persistence lengths and are well described as random coils. The exponential relationship described here should be a useful interpolation function for determining DNA lengths over a wide range. The simplicity of this relationship may be of more fundamental significance and suggests that some common feature dominates the electrophoresis of double-stranded DNA fragments in agarose gels, regardless of length.

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