

Characterization of the alfalfa (*Medicago sativa*) genome by DNA reassociation

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Source and description of DNA

Total leaf DNA from diploid ($2n = 2x = 16$) alfalfa, *Medicago sativa* HG-2 [1] was purified by CsCl banding [2] and treatment with RNase A and proteinase K. The G+C content was calculated to be 36.3% from $T_m = 84.2^\circ\text{C}$ [3]. Control DNA for reassociation analysis was *Escherichia coli* (K-12) DNA. Plasmid probes for alfalfa unique copy chloroplast DNA measurements were: p 18.0 (18 kb ribosomal DNA) and p 7.0 (7 kb psbA gene fragment) from Dr J. D. Palmer [4].

Reassociation analysis

Denatured, sonicated DNA, average size 600–900 nucleotides, was reannealed in 0.12 M neutral sodium phosphate (PB) buffer at 60°C . The degree of reassociation was measured spectrophotometrically by following the decrease in hyperchromicity at 260 nm with DNA concentrations of 35–1245 $\mu\text{g/ml}$. Reassociation of the different DNA classes showed 4–5% mismatch as determined by ΔT_m at the end of reassociation. Data were corrected for PB absorbance, digitized and analyzed by the pro-

Table 1. Kinetic analysis of alfalfa DNA reassociation

Reassociation DNA	Component	Fraction reassociated ^a	K kinetic constant	Cot 1/2 ^b	Determined complexity ^c bp	Average copy number ^d
Alfalfa DNA	Highly rep.	0.22	14.0	0.071	1.2×10^3	18182
	Mid rep.	0.42	0.042	23.8	7.6×10^6	55
	Unique	0.36	0.00077	1299	3.6×10^8	1
<i>E. coli</i> DNA	Unique	0.99	0.17	5.88	4.5×10^6	1

Highly repetitive DNA includes 5% foldback DNA.

^a Measured as decrease in hyperchromicity at 260 nm as shown in Fig. 1. Reflects 8% unreassociated total DNA.

^b K is expressed in $\text{M}^{-1} \text{s}^{-1}$ and represents a second-order rate constant. $1/K = \text{Cot } 1/2$.

^c Complexity of DNA = $4.5 \times 10^6 \text{ bp} \times \frac{\text{Cot } 1/2 \text{ (alfalfa DNA)}}{\text{Cot } 1/2 \text{ (E. coli DNA)}} \times \text{fraction of genome}$.

^d Average copy number = K component/K single component [8].

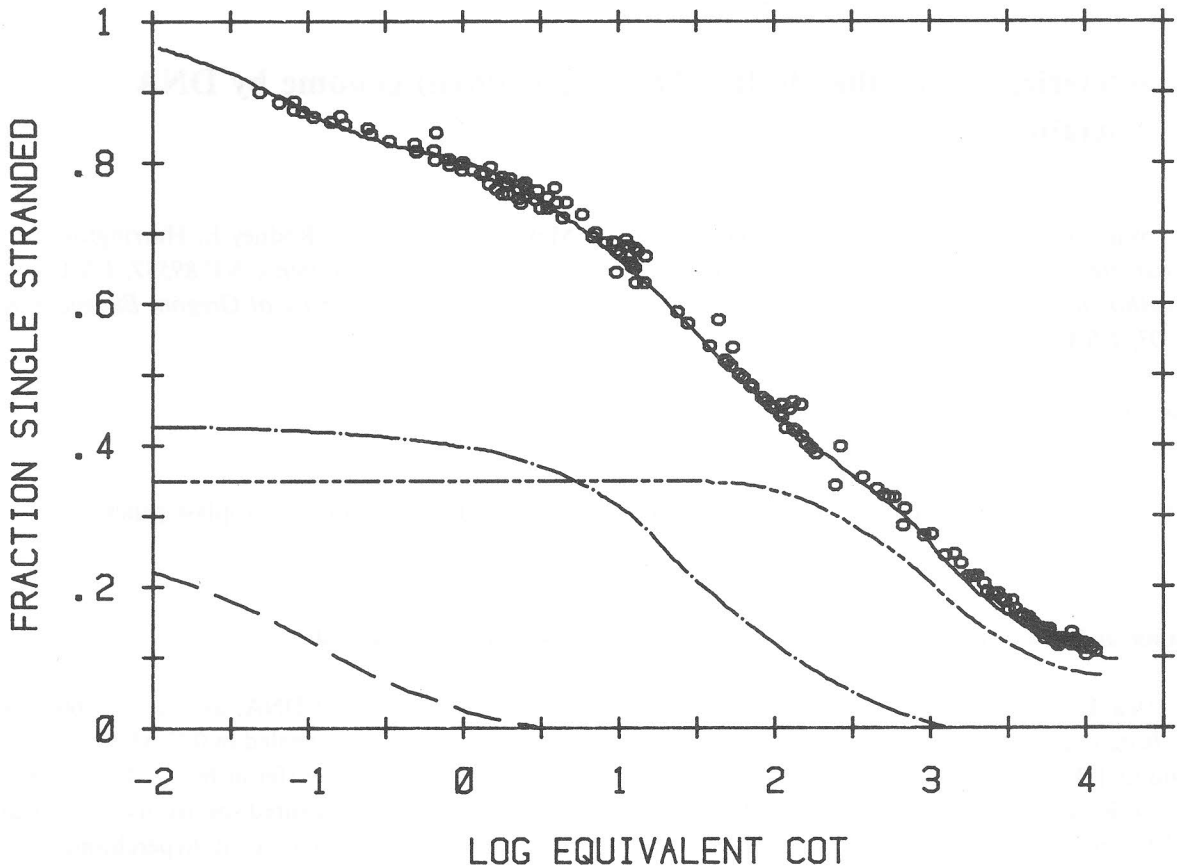


Fig. 1. Reassociation kinetics of unlabeled alfalfa DNA. Data are plotted as fraction single-stranded DNA measured by decrease in hyperchromicity as a function of log equivalent Cot. The curve drawn through the points represents the least squares best fit of three components assuming second-order reassociation kinetics. Overall curve fit RMS = 0.0121. The dashed lines represent the resolved reassociation curves of pure components of highly repetitive, mid repetitive and slowly reassociating DNA as predicted from the fitted data. Kinetic parameters of the resolved curves are listed in Table 1.

gram FLING [5] for best fit to a Cot curve composed of 3 components (RMS=0.0121) as shown in Fig. 1. DNA complexity calculations were done by comparison with *E. coli* hybridization kinetic parameters as unique DNA [6] at identical conditions and are shown in Table 1.

Genome composition

Calculations show the alfalfa $1n = 1x$ haploid genome is 1.0×10^9 bp or 1.1 pg DNA, and consists of 22% highly repetitive plus foldback sequences; 42% mid repetitive sequences and 36% unique sequences. The complexity of unique sequences is 3.6×10^8 bp.

Other comments

Chloroplast DNA comprises 3% of total leaf DNA as measured by quantitative slot blots [7] with two alfalfa chloroplast DNA probes, equivalent to about 500 chloroplast genome copies per cell.

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