

# Genes Leave Their Characteristic Marks on the Melting Profile of Plasmid DNAs

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High resolution melting profiles of four linearised plasmids (pUC9, pGV403, pHP2, and pBR322) were recorded by means of UV absorbance vs. temperature scanning. The set of transitions obtained for each plasmid are compared to each another and to the transitions obtained for their excised particular antibiotic resistance gene. It can be shown that each gene leaves a characteristic mark on the melting profile of its parental plasmid.

## Introduction

Strand separation in double stranded DNA may be caused by a variety of physical and chemical factors such as temperature or pH, and the melting temperature,  $T_m$  (defined as the temperature at which 50% of the total absorbance change has occurred) is a function of the base composition of DNAs. Experimentally this process can be monitored by spectroscopic methods such as high-resolution thermal denaturation (Yabuki *et al.*, 1975; Gotoh *et al.*, 1976). Recording changes in UV absorbance while increasing the temperature has become one of the most popular experimental techniques.

The UV absorbance of purine and pyrimidine bases is known to be caused by a  $\pi-\pi^*$  electronic transition. The absorbance intensity increases by about 30% due to abolishing the degeneracy of transition moments when the bases are stacked in their double helical conformation. A thermal profile, recorded as a change in absorbance versus temperature is characterised by its  $T_m$  (Doty *et al.*, 1959; De Ley, 1969). As a result of many studies of the melting profiles of various DNA sequences a linear relationship between the melting temperature and the GC content has been established. Thus, the determination of the melting temperature has become a valuable tool for determining

the gross GC content of a particular DNA sequence (Yabuki *et al.*, 1975; Wada *et al.*, 1977; Akiyama *et al.*, 1977).

The discovery that the melting of DNA sequences does not occur in a single transition but in discrete steps (Yabuki *et al.*, 1975; Akiyama *et al.*, 1977; Wada *et al.*, 1976) was a great step forward towards a thermodynamic description of gene sequences and this effect was convincingly corroborated in recent years illustrated by a number of reports and fine structure-stability maps of DNA sequences have been published (Yabuki *et al.*, 1975; Gotoh *et al.*, 1976; Wada *et al.*, 1976; Reiss *et al.*, 1974; Vizard and Ansevin, 1976; Lyubchenko *et al.*, 1976). The high resolution study of the genome of fd phage (Tachibana *et al.*, 1978) suggested that there is a correlation between genes and the specific unit within the DNA sequence which is termed a cooperative melting unit. The cooperative melting unit is defined as a stretch of base pairs within a specific DNA which gives rise to one or more thermal transitions observed as peaks in the first derivative of the absorbance versus temperature plot. This was confirmed by other groups (Freire *et al.*, 1978; Freire *et al.*, 1978<sup>a</sup>; Freire *et al.*, 1978<sup>b</sup>; Vollenveider *et al.*, 1979). There is, however, a shortcoming in the whole genome approach in so far as a single transition peak can contain contribution from melting regions of different sequences within the genome.

Here we would like to present a high resolution thermal denaturation analysis of simple prokaryotic genes, located on convenient plasmids.

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Materials and Methods

Materials

Plasmids pUC9 (Vieira *et al.*,1982), pGV403 (Amersham Catalog, 1986), pHP2, and pBR322 (Sutcliffe, 1978) were prepared using the well documented method of Clewell and Helinski ( Clewell and Helinski, 1969).

Enzymatic digestion and purification

Plasmid DNA was incubated with restriction enzymes at a ration of 1µg of DNA per 1 unit of enzyme with required buffer at 37 °C for fifteen hours. The completeness of digestion was checked by agarose gel electrophoresis. All restriction enzymes and molecular weight marker were obtained from Boehringer-Mannheim, Germany. Genes were purified on a HPLC NUCLEOGEN 400-DEAE column, (U.K.) with elution buffer A: 20 mM phosphate buffer, 6 M urea, pH 6.7; buffer B: buffer A plus 1.2 M KCl. The gradient applied varied from 840 mM to 1200 mM KCl over 90 min at flow rate of 2 ml/min.

UV melting experiment

DNA samples from the stock solution were diluted with the following buffer: 50 mM NaCl, 1 mM tris(hydroxymethyl)aminomethane (Tris)-HCl, 0.5 EDTA, pH 7.6 to an absorbance of approximately 0.3 (260 nm) in a quartz cuvette (d=5 mm). The thermal denaturation process was monitored at 260 nm with a controlled temperature increase of 1 °C per minute with the accuracy of 0.1 °C. Melting curves were continuously recorded using Uni-

cam SP1800 spectrophotometer equipped with a custom-made heating block.

Results and Discussion

The chloramphenicol-resistance gene isolated from a pGV403 plasmid by restriction enzyme excision was thermally denatured and the progress of the denaturation was monitored as described in Materials and Methods. The melting profile of the excised gene was compared with the melting profile of the parental vector. The results are presented in Table I. Each peak corresponds to a certain DNA sequence within the gene or genome of the size of a cooperative melting unit.

The ampicillin-resistance gene isolated from pUC9 plasmid as well as the parental pUC9 plasmid were subjected to the high resolution thermal denaturation procedure and the results are presented in Table I. The tetracycline-resistance gene and the pBR322 plasmid were subjected to the same procedure as the pGV403 and pUC9 plasmids and their genes and the results are compiled in Table I. The actual melting profile and the first derivative ( $dA_{260}/dT$ ) for the pBR322 plasmid and its tetracycline-resistance gene are displayed in Fig. 1A-B. The superposition of these two figures shows clearly that the thermal transitions observed for the tetracycline-resistance gene corresponds to a set of peaks within the thermal denaturation profile of the parental plasmid.

The chloramphenicol-resistance gene melts in two separate steps ( $T_m$ s equal to 77.5 °C and 82.0 °C of Table I). The two steps correspond to the first and fourth transition obtained within the

Table I. List of the thermal transitions of the pGV403, pUC9, and pBR322 plasmids and their respective genes chloramphenicol-resistance gene, ampicillin-resistance gene, and tetracycline-resistance gene.

Number of transition	$T_m$ [°C] of pGV403 plasmid	$T_m$ [°C] of chloramphenicol-resistance gene	$T_m$ [°C] of pUC9 plasmid	$T_m$ [°C] of ampicillin-resistance gene	$T_m$ [°C] of pBR322 plasmid	$T_m$ [°C] of tetracycline-resistance gene
1	77.5	82	76.6	82.4	73.4	84.5
2	79.1		78.4		77	
3	80.4		80.8		78	
4	82		82.4		80.4	
5	84.6	87.2	83.8	89.3	82.2	
6	86.6		85.8		84.4	
7	88.3		87.4		85.8	
8			89		87.2	
9					88.2	

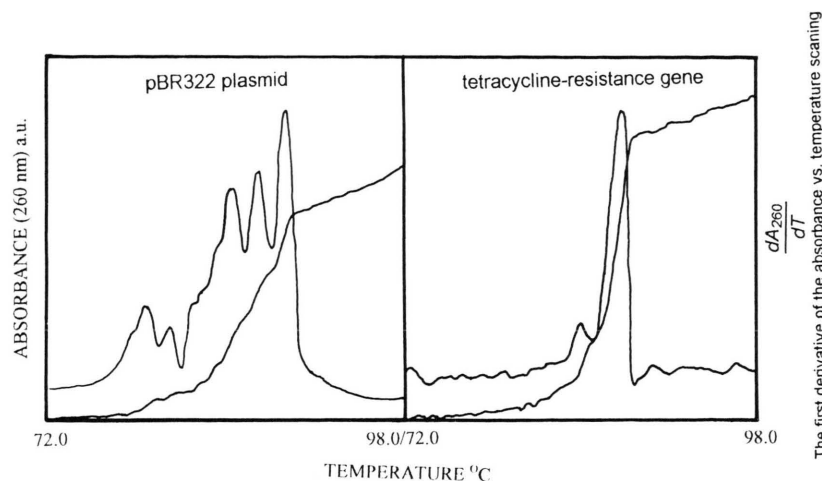


Fig. 1. The complete thermal denaturation profile and its first derivative. A) pBR322 plasmid, B) tetracycline-resistance gene. The heating rate was 1 °C per minute, the buffer: 50 mM NaCl, 0.5 mM EDTA, 1 mM Tris, pH 7.6; a. u., arbitrary units.

pGV403 plasmid melting profile. The ampicillin-resistance gene consists of four separate melting units with  $T_{ms}$  equal to 78.6 °C, 82.4 °C, 85.0 °C, and 89.3 °C. The transitions correspond to the second, fourth, sixth, and eighth step of the melting profile of the parental pUC9 plasmid. The tetracycline-resistance gene melts in two steps ( $T_{ms}$  of 84.5 °C and 87.2 °C) which correspond to the sixth and eighth transition within the melting profile observed for the parental pBR322 plasmid.

Comparing the transitions observed for the pBR322 plasmid and for the ampicillin-resistance gene shows that a set of transitions observed for the ampicillin-resistance gene corresponds to a set of thermal transitions in the pBR322 melting profile.

The transitions 3, 5, 7, and 9 in Table I of pBR322 plasmid correspond to steps 2, 4, 6, and 8 observed for pUC9 plasmid. This correspondence indicates clearly that the sequence of the plasmid pBR322 harbours another gene ( $T_{ms}$  78.6 °C, 82.4 °C, 85.0 °C, and 89.3 °C). Besides the tetracycline-resistance gene there is the ampicillin-resistance gene. There are slight discrepancies for the  $T_{ms}$  of the four subtransitions originating from the melting of the ampicillin-resistance gene. These discrepancies may be due to “cross talk” during the melting of the tetracycline-resistance gene with the ampicillin-resistance gene, or more trivial,

due to slight inaccuracies of the temperature reading device. The “cross talk” is understood here as a simultaneous melting of stretches of DNA enclosed within the two genes present in pBR322 plasmid.

The chloramphenicol-resistance gene melting units ( $T_{ms}$  75.7 °C and 82.0 °C) can be extracted from the comparison of the high resolution profiles of the plasmids pGV403 and pHP2, the latter containing the sequence of the resistance gene as an insert.

Taken together we have conclusively demonstrated that genes can be characterised by their high resolution melting profile, which can be separated out of the complete melting profile of the parental plasmid which harbours the specific gene. The recording and analysis of the melting profile can serve as an analytical tool to characterize a certain gene within its genome.

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