A rapid method for determining the G+C content of bacterial chromosomes by monitoring fluorescence intensity during DNA denaturation in a capillary tube

Hua-Xi Xu, Yoshiaki Kawamura, Na Li, Licheng Zhao, Tie-Min Li, Zhi-Yu Li, Shinei Shu and Takayuki Ezaki

Author for correspondence: Takayuki Ezaki. Tel: +81 58 267 2240. Fax: +81 58 267 0156.
e-mail: tezaki@cc.gifu-u.ac.jp

Department of Microbiology, Gifu University, School of Medicine, 40 Tsukasa-machi, 500-8705, Gifu, Japan

A simple and rapid method to determine the G+C content of bacterial chromosomal DNA was developed. It involves determination of T_m by a Light Cycler and calculation of the G+C content by an empirical formula relating T_m to G+C content. Instead of a conventional thermal denaturation method, which monitors the increase of absorbance at 260 nm, thermal denaturation was monitored by the decrease of fluorescence intensity in the presence of SYBR Green 1. In this method, the apparent T_m of DNA was influenced by the concentration of SYBR Green 1, DNA and salt. In addition, when the G+C content was calculated from a linear equation [(mol % G+C_x = mol % G+C_r + 1.99($T_{m,x} - T_{m,r}$), where x is the unknown organism and r is the reference organism], an error value was introduced among strains with extremely low or high G+C content. Based upon five standards (G+C contents in the range 33–66 mol %), a suitable equation was formulated for the capillary method: mol % G+C_x = mol % G+C_r + 1.4652($T_{m,x} - T_{m,r}$) + 0.0063($T_{m,x}^2 - T_{m,r}^2$). To determine the T_m of organisms within this range of G+C contents, *Escherichia coli* ATCC 11775^T was used as a DNA standard and fixed concentrations of SYBR Green 1, sodium citrate and DNA were used. The data from 37 bacterial strains indicated that this equation behaved well. Because it is rapid and simple, it may prove useful for bacterial identification.

Keywords: T_m , thermal denaturation, G + C content, taxonomy

INTRODUCTION

The G + C content of bacterial chromosomal DNA ranges from 25 to 80 mol %. Several different methods to determine this value have been reported (Marmur & Doty, 1962; Schildkraut *et al.*, 1962; Owen *et al.*, 1969; De Ley, 1970; Ko *et al.*, 1977; Mesbah *et al.*, 1989). The thermal denaturation temperature (T_m) method is one of the most common techniques for determining the G + C content of bacterial DNA (Marmur & Doty, 1962). This method monitors the increase of absorbance at 260 nm during DNA denaturation. Instead of monitoring the absorbance, we explored an alternative procedure that measures the decrease in the fluorescence of SYBR Green 1, which selectively binds double-stranded DNA.

The T_m of DNA is influenced by ionic strength of the DNA solution, and thus the value is difficult to

reproduce from one laboratory to another. To minimize experimental errors, a reference DNA is used as a standard, and the G + C content is calculated as reported by Mandel *et al.* (1970): mol % G + C_x = mol % G + C_r + 1.99($T_{m,x} - T_{m,r}$) (where x is the unknown organism and r is the reference organism). This formula is useful when *Escherichia coli* strain B is used as a standard, for which the G + C content is calculated as 50–51 mol %. However, when the formula was applied to DNA from an organism which had an extremely high or low G + C content, the calculated G + C content differed from the value determined by HPLC (Ezaki *et al.*, 1990).

To solve this problem, we selected reference organisms with low and high G + C contents, and measured their T_m . We then determined a new suitable formula for this capillary method.

Table 1. T_m and G + C content of various strains measured by different methods

The standard G + C content (mol%) of *Escherichia coli* ATCC 11775^T (50.5%) was used in Formulas A and B. Formula A is for conventional T_m method (Mandell *et al.*, 1970). Formula B was created for the capillary method and is described in this paper. Each value represents the mean of three experiments. ND, No data.

Species and strain	T_m		G + C content (mol%)		
	In $0.1 \times$ SSC (capillary tube method)	Published value (reference)	Value calculated by		Published value (reference)
			Formula A	Formula B	
<i>Staphylococcus aureus</i> ATCC 12600 ^T	78.5 $\pm$ 0.8	83.5 (Marmur & Doty, 1962)	36.9	33.4	34.7 (Gause <i>et al.</i> , 1964), 33.5*
<i>Acinetobacter baumannii</i> ATCC 19606 ^T	81.4 $\pm$ 0.5		42.5	40.4	38–47† (Krieg & Holt, 1984)
<i>Acinetobacter lwoffii</i> ATCC 15309 ^T	83.4 $\pm$ 0.3		46.7	45.7	38–47† (Krieg & Holt, 1984)
<i>Empedobacter brevis</i> JCM 1276 ^T	79.0 $\pm$ 0.5		37.9	34.7	31.8–33 (Krieg & Holt, 1984)
<i>Myroides odoratus</i> ATCC 4651 ^T	79.9 $\pm$ 1.0		39.7	36.9	31.4–36.1† (Krieg & Holt, 1984)
<i>Sphingobacterium spiritivorum</i> NCTC 11343 ^T	82.1 $\pm$ 0.3		44.0	42.2	41–41.8 (Krieg & Holt, 1984)
<i>Acinetobacter calcoaceticus</i> ATCC 23055 ^T	81.5 $\pm$ 0.7		42.9	40.9	38–47† (Krieg & Holt, 1984)
<i>Haemophilus influenzae</i> ATCC 9006 ^T	80.3 $\pm$ 0.2	85.6 (Marmur & Doty, 1962)	40.5	37.8	38‡, 39* (Fleischmann <i>et al.</i> , 1995)
<i>Legionella pneumophila</i> ATCC 33152 ^T	81.2 $\pm$ 0.4		42.2	40.0	39 (Krieg & Holt, 1984)
<i>Fluoribacter bozemanæ</i> ATCC 33217 ^T	81.5 $\pm$ 0.2		42.8	40.8	43 (Krieg & Holt, 1984)
<i>Fluoribacter dumoffii</i> ATCC 33297 ^T	81.7 $\pm$ 0.0		43.3	41.4	ND
<i>Legionella dumoffii</i> ATCC 33279 ^T	81.8 $\pm$ 0.2		43.4	41.5	ND
<i>Escherichia coli</i> ATCC 11775 ^T	85.4 $\pm$ 0.5	90.5 (De Ley, 1970)	50.5	50.5	50–51 (Buchanan & Gibbons, 1974), 50.5*‡
<i>Legionella jordanis</i> ATCC 33623 ^T	83.0 $\pm$ 0.2		45.8	44.5	ND
<i>Legionella micdadei</i> ATCC 33218 ^T	83.1 $\pm$ 0.8		46.0	44.7	ND
<i>Legionella feeleyi</i> ATCC 35072 ^T	83.3 $\pm$ 1.6		46.4	45.3	ND
<i>Legionella spiritensis</i> ATCC 35249 ^T	84.6 $\pm$ 0.8		48.9	48.5	ND
<i>Legionella erythra</i> ATCC 35303 ^T	85.0 $\pm$ 0.7		49.7	49.5	ND
<i>Legionella rubrilucens</i> ATCC 35304 ^T	85.4 $\pm$ 0.4		50.7	50.7	52.6 (Takayama <i>et al.</i> , 1965)
<i>Salmonella typhi</i> ATCC 19430 ^T	87.8 $\pm$ 0.6		55.3	56.7	50–53 (Krieg & Holt, 1984)
<i>Pseudomonas aeruginosa</i> ATCC 10145 ^T	91.8 $\pm$ 0.5	97.0–97.7 (De Ley <i>et al.</i> , 1966)	63.4	67.3	67.2 (Krieg & Holt, 1984), 66.4*
<i>Ralstonia pickettii</i> ATCC 27511 ^T	92.0 $\pm$ 0.5		63.7	67.6	64.0 (Krieg & Holt, 1984)

Table 1 (cont.)

Species and strain	T_m		G + C content (mol %)		
	In $0.1 \times$ SSC (capillary tube method)	Published value (reference)	Value calculated by		Published value (reference)
			Formula A	Formula B	
<i>Burkholderia cepacia</i> ATCC 25416 ^T	93.2 $\pm$ 0.1		66.0	70.7	67.4 (Krieg & Holt, 1984)
<i>Brevundimonas diminuta</i> ATCC 11568 ^T	92.6 $\pm$ 0.4	96.9 (De Ley <i>et al.</i> , 1966)	65.0	69.3	66.3–67.3 (Krieg & Holt, 1984)
<i>Pseudomonas fluorescens</i> NCIB 9046 ^T	90.6 $\pm$ 0.1	95.3 (De Ley <i>et al.</i> , 1966)	60.9	63.9	60.5–61.3 (Krieg & Holt, 1984)
<i>Pseudomonas putida</i> ATCC 12633 ^T	90.8 $\pm$ 0.6	96.0 (Colwell & Mandel, 1964)	61.4	64.6	63.7 (Lee & Boezi, 1966)
<i>Alcaligenes faecalis</i> ATCC 8750 ^T	89.7 $\pm$ 0.5		59.2	61.7	56–59 (Krieg & Holt, 1984)
<i>Mycobacterium tuberculosis</i> ATCC 27294 ^T	91.1 $\pm$ 0.4		61.9	65.2	65.6 (Cole <i>et al.</i> , 1998)‡, 65.5*
<i>Mycobacterium kansasii</i> ATCC 12478 ^T	90.8 $\pm$ 0.3		61.3	64.4	62–70† (Sneath <i>et al.</i> , 1986)
<i>Mycobacterium marinum</i> NCTC 2275 ^T	90.6 $\pm$ 0.3		61.0	64.1	62–70† (Sneath <i>et al.</i> , 1986)
<i>Mycobacterium simiae</i> ATCC 25275 ^T	91.4 $\pm$ 0.1		62.6	66.2	62–70† (Sneath <i>et al.</i> , 1986)
<i>Mycobacterium scrofulaceum</i> ATCC 19981 ^T	92.7 $\pm$ 0.4		65.1	69.5	62–70† (Sneath <i>et al.</i> , 1986)
<i>Mycobacterium gordonae</i> ATCC 14470 ^T	91.6 $\pm$ 0.3		62.5	66.0	62–70† (Sneath <i>et al.</i> , 1986)
<i>Mycobacterium avium</i> ATCC 25291 ^T	91.7 $\pm$ 0.6		63.2	67.0	62–70† (Sneath <i>et al.</i> , 1986)
<i>Mycobacterium intracellulare</i> ATCC 13950 ^T	91.3 $\pm$ 0.6		62.2	65.7	62–70† (Sneath <i>et al.</i> , 1986)
<i>Mycobacterium fortuitum</i> ATCC 6841 ^T	90.2 $\pm$ 0.5		60.1	62.8	62–70† (Sneath <i>et al.</i> , 1986)
<i>Mycobacterium chelonae</i> NCTC 946 ^T	90.0 $\pm$ 0.5		59.7	62.4	62–70† (Sneath <i>et al.</i> , 1986)

* Data from HPLC method (this work).

† Data from different species.

‡ Data from sequencing, the strains are *Escherichia coli* K-12 (Blattner *et al.*, 1997); *Mycobacterium tuberculosis* H37Rv (Cole *et al.*, 1998) and *Haemophilus influenzae* Rd (Fleischmann *et al.*, 1995).

METHODS

Bacterial strains, culture and DNA extraction. Five type strains were used to calculate a new formula for G+C content. *Pseudomonas aeruginosa* ATCC 10145^T, *Staphylococcus aureus* ATCC 12600^T, *Haemophilus influenzae* NCTC 8143^T, *Escherichia coli* ATCC 11775^T and *Mycobacterium tuberculosis* ATCC 27294^T. Other type strains of the genera of *Mycobacterium*, *Legionella*, *Pseudomonas*, *Empedobacter*, *Myroides*, *Fluoribacter* and *Acinetobacter* are listed in Table 1. Suitable media and growth conditions were chosen for the particular organisms. BCYE α medium (Washington, 1995) for legionellae, Middlebrook 7H9 broth (Frederick & Metchock, 1995) for mycobacteria, chocolate agar (Campos, 1995) for *H. influenzae*, and brain-heart infusion agar for the remainder of the species shown in Table

1. DNA was extracted and purified using the method described previously (Ezaki *et al.*, 1988a). Briefly, about 2 g (wet weight) bacterial cells was suspended in 9 ml 5 mM EDTA (pH 8.0). Gram-positive cells only were treated with lysozyme (final 1 mg ml⁻¹) and lysed by adding 1 ml 20% SDS. The suspension was incubated at 55 °C for 30 min. Following the addition of 10 ml phenol/chloroform/isoamyl alcohol (25:24:1, by vol.), the mixture was vigorously shaken for 20 min. It was then centrifuged at 15000 g for 10 min and the supernatant was transferred into a beaker. After adding 2 vols ethanol, the precipitated DNA was collected with a glass rod by gently stirring. The spooled DNA was dissolved in 5 ml saline/EDTA and 100 μ l RNase A (10 mg ml⁻¹ in 0.15 M NaCl) was added. After an incubation of the mixture at 37 °C for 30 min, an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1, by

vol.) was added, the suspension was shaken and centrifuged to remove the precipitated RNase A. The DNA was then precipitated, dried and resuspended in sterile distilled water. All DNA samples were stored at -20°C until use. The quantity of each DNA preparation were measured by the absorbance at 260 nm with a Pharmacia LKB Ultrospec Plus spectrophotometer. The DNA used for these experiments possessed an absorbance ratio at A_{260}/A_{280} higher than 1.8.

Determination of T_m . Only the final procedure is described. Fifty nanograms of DNA in 10 μl sterile distilled water was mixed thoroughly in 80 μl of $0.1\times$ SSC solution ($1\times$ SSC is 0.15 M NaCl , $0.015\text{ M trisodium citrate}$, pH 7.0) (Ezaki *et al.*, 1988a, b). The mixture was transferred to a Slide-A-Lyzer cassette (10000 MW membrane; Pierce) and dialysed in $1\text{ l } > 0.1\times$ SSC for at least 4 h. One microlitre of a 4000-fold-diluted SYBR Green 1 solution was added to 9 μl of the dialysed DNA solution. SYBR Green 1 was supplied as a 10000-fold-concentrated stock solution by Molecular Probes and stored at -20°C . Prior to use, the stock solution was diluted 4000-fold with distilled water. The entire 10 μl mixture was placed in a capillary tube and the tube was heated using a Light Cycler (Idaho Technology), which was integrated with a 24-sample thermal cycler (Ririe *et al.*, 1997). The oven temperature was maintained at 70°C for 15 s and then was increased to 98°C at $0.1^{\circ}\text{C s}^{-1}$. Fluorescence intensity was monitored sequentially. The fluorescence data for melting curves were acquired most easily on a plot of the derivative of fluorescence with respect to temperature (dF/dT), and the data were also converted into melting peaks by the Light Cycler. The centre of the melting peak was the T_m . Each value was obtained automatically at the end of the run.

Calculation of the G + C content. T_m values measured by the Light Cycler were used for calculation of G + C content. The G + C content was also determined by the HPLC method using DNA digested with P1 nuclease (Tamaoka, 1986; Mesbah *et al.*, 1989; Ezaki *et al.*, 1990). Briefly, purified DNA (5–10 μg) dissolved in 10 μl distilled water was denatured by boiling for 10 min. Ten microlitres of P1 nuclease (2 U ml^{-1} ; Takara Shuzo) was added and the mixture was incubated at 50°C for 1 h. Ten microlitres of alkaline phosphatase (2.4 U ml^{-1}) was then added and the mixture was incubated at 37°C for 30 min. Aliquots (5–10 μl) of the sample were analysed by HPLC. Standard deviations and regression analysis was carried out using StatView software version 5.0 (Abacus Concepts).

RESULTS

Optimization of the procedure

As the T_m value of DNA varies with the concentration of SSC, the optimal experimental conditions for the capillary tube method were determined (Table 2). When the SSC concentration was $1\times$ or higher, the T_m of *P. aeruginosa* ATCC 10145^T, which has a high G + C content, could not be measured because of the poor shape of the melting curve. When the SSC concentration was between $0.1\times$ and $0.5\times$, the T_m values of high and low G + C content organisms were successfully determined. Thus, we selected $0.1\times$ SSC as a standard concentration for subsequent experiments.

Table 2. Comparison of SSC concentration and T_m value when T_m determined by the capillary method

NP, Not possible to determine. The DNA was fixed at 50 ng and CYBR Green 1 was at 4000-fold dilution. T_m values are means of three experiments.

SSC concn	T_m		
	<i>S. aureus</i> ATCC 12600 ^T	<i>E. coli</i> ATCC 11775 ^T	<i>P. aeruginosa</i> ATCC 10145 ^T
$0.1\times$	78.51 ± 0.77	85.35 ± 0.52	91.84 ± 0.49
$0.5\times$	80.23 ± 0.65	86.10 ± 0.45	93.30 ± 0.25
$1.0\times$	83.50 ± 0.63	89.99 ± 0.31	NP
$2.0\times$	84.60 ± 0.52	90.10 ± 0.25	NP

Fig. 1. T_m of three reference organisms in $0.1\times$ SSC. The DNA concentration of each reference strain was adjusted to $50\text{ }\mu\text{g ml}^{-1}$. Two different SYBR Green 1 concentrations (4000-fold and 8000-fold dilution) were used. $\square$, *P. aeruginosa* ATCC 10145^T, 4000-fold dilution; $\blacksquare$, *P. aeruginosa* ATCC 10145^T, 8000-fold dilution; $\circ$, *Staphylococcus aureus* ATCC 12600^T, 4000-fold; $\bullet$, *Staphylococcus aureus* ATCC 12600^T, 8000-fold; $\triangle$, *Escherichia coli* ATCC 11775^T 4000-fold; $\blacktriangle$, *Escherichia coli* ATCC 11775^T 8000-fold. dF/dT is the change in fluorescence per change in temperature.

The temperature was gradually increased at a rate of 0.1 , 0.2 , 0.5 , 1 or 2°C s^{-1} in different experiments. However, the rate of $0.1^{\circ}\text{C s}^{-1}$ gave the best result for calculating the T_m . Depending upon the G + C content, the melting curves measured at the transition rates of 0.5 to 2°C s^{-1} could not be differentiated precisely (data not shown).

The concentration of SYBR Green 1 also influenced the measured T_m values. The optimal SYBR Green 1

Fig. 2. T_m of *Staphylococcus aureus* ATCC 12600^T is influenced by the DNA concentration. The SYBR Green 1 was diluted 8000-fold for this experiment. □, 400 ng DNA; ●, 200 ng DNA; ○, 100 ng DNA; ▲, 50 ng DNA.

concentration for these experiments was from 1:8000 to 1:4000 dilution of the stock solution (Fig. 1). However, the 1:4000 dilution of the SYBR Green 1 solution was selected for further experiments because the melting curves were sharper (Fig. 1). The T_m of bacterial DNA with high G+C contents could not be determined in SYBR Green 1 concentrations lower than this range. In contrast, it was still possible to determine the T_m of low G+C content DNA from

Staphylococcus aureus ATCC 12600^T even in 20000-fold dilutions of SYBR Green 1 (data not shown).

The concentration of DNA affected the melting curve, especially in DNA with a high G+C content. We used five different concentrations of chromosomal DNA from bacterial strains with low or high G+C content and found that the optimal concentration was 25–100 ng μl^{-1} . If the DNA concentration was higher than 400 ng μl^{-1} , the T_m of bacterial strains with a high G+C content, such as *P. aeruginosa* ATCC 10145^T, could not be calculated because of the distorted shape of the melting curve. No visible influence of DNA concentration in this range was detected when DNA from a low G+C-containing organism, such as *Staphylococcus aureus*, was used. However, at high concentrations of DNA (200–400 ng μl^{-1}), the measured T_m value was affected when 8000-fold-diluted SYBR Green 1 was used (Fig. 2). This phenomenon also suggested that the concentration of DNA had an influence on the observed T_m value even if the optimal SYBR Green 1 and SSC concentrations were selected. In addition, the DNA used in this method did not require extensive treatment with RNase, and apparently accurate T_m values were obtained from preparations of DNA containing large amounts of RNA. The insensitivity to contamination by RNA was probably due to the fact that SYBR Green 1 combines only with double-stranded DNA.

Derivation of a suitable formula for calculation of the G+C content

Given the above findings, 50 ng DNA μl^{-1} and a 4000-fold dilution of SYBR Green 1 in 0.1 × SSC solution were selected as the optimal conditions. Under these conditions, the apparent T_m values of DNAs from 37 strains were measured, and the G+C content was calculated using a previously reported formula

Fig. 3. New formula for the capillary method. Five reference DNAs from *Staphylococcus aureus* ATCC 12600^T, *H. influenzae* NCTC 8143^T, *Escherichia coli* ATCC 11775^T, *Mycobacterium tuberculosis* ATCC 27294^T and *P. aeruginosa* ATCC 10145^T were prepared and the T_m of each DNA was determined by a Light Cycler. The G+C contents determined by the HPLC are plotted. Regression analysis was carried out to derive a new formula for the capillary method.

(Mandel *et al.*, 1970; Formula A in Table 1): $\text{mol \% G+C}_x = \text{mol \% G+C}_r + 1.99(T_m x - T_m r)$, where, x is the unknown organism and r is the reference organism. However, the T_m values of the reference organisms were lower than previously reported values, especially when high G+C-containing organisms were compared. Thus, we measured the G+C content of five reference organisms by an HPLC method (Ezaki *et al.*, 1990) (Table 1). Regression analysis was performed to derive a proper formula relating the T_m measured by the capillary method to the G+C content (Formula B; Table 1), $\text{mol \% G+C}_x = \text{mol \% G+C}_r + 1.4652(T_m x - T_m r) + 0.0063(T_m x^2 - T_m r^2)$ (Fig. 3).

DISCUSSION

The Light Cycler is a microvolume multisample fluorimeter with rapid temperature control, and SYBR Green 1 is a double-stranded-DNA specific dye (Wittwer *et al.*, 1997a). The application of the Light Cycler combined with SYBR Green 1 has made great contributions to diagnosis and taxonomy of micro-organisms in recent years, such as rapid-cycle DNA amplification (Wittwer *et al.*, 1997b) and quantification of low-copy transcripts during amplification, genotyping with hybridization probes and other analyses in molecular biology (Livak *et al.*, 1995; Desilva *et al.*, 1998). It is possible that this new technique will come into widespread use in diagnostic laboratories because of its rapid analysis and multifunctional use.

The G+C content of a bacterial chromosome is an important index for the identification and classification of bacteria. In these experiments, we used the Light Cycler and monitored the denaturing of DNA by the decrease on fluorescence of SYBR Green 1 in a 10 µl capillary tube. Determination of the G+C content of an unknown organism could be completed within 10 min. This rapid estimation of G+C content makes it easy to decide the sequencing conditions because bacteria have wide variability of their G+C content, ranging from 25 to 80 mol %. T_m values determined by the Light Cycler method that we used were influenced by four factors: denaturing speed and the concentrations of SYBR Green 1, SSC and DNA. A slow temperature transition during denaturing is essential to obtain a precise melting curve and to differentiate DNAs with small differences in T_m . Presumably the slow change in temperature allows enough time for SYBR Green 1 to disassociate DNA during the transition from the double-stranded to single-stranded state.

The equation established by Mandel *et al.* (1970) has been used to determine the G+C content based upon the T_m . The T_m is based on the increase in absorption at 260 nm during denaturing. In our experiments, the T_m was obtained with a Light Cycler by monitoring the decrease of SYBR Green 1 fluorescence because the fluorescent dye only binds double-stranded DNA.

We applied Mandel's formula to calculate the G+C content after measuring the T_m of a series of DNAs

with G+C contents in the range 30–70 mol %. However, the G+C content value calculated by HPLC and other methods was different from that determined by this formula, especially when the G+C content of an organism was extremely high. This deviation was pointed out in the original paper by Mandel *et al.* (1970). Our new regression analysis with a two-factor parameter method modified the calculation (Formula B; Table 1). Our new formula works especially well when the G+C content of an organism is extremely high or low, using DNA from *Escherichia coli* ATCC 11775^T as a standard. For instance, the G+C content of *Mycobacterium tuberculosis* H37Rv (lab strain) determined by HPLC and the new method were 65.5 ± 1.4 and 65.2 mol %, respectively. These values agree well with the value of 65.6 mol % determined for another strain by genome sequencing (Cole *et al.*, 1988). In contrast, Formula A estimated the G+C content of strain H37Rv as 61.97 mol %.

The G+C content of *Escherichia coli* ATCC 11775^T is 50–51 mol %. The complete genome sequence of *Escherichia coli* K-12 revealed that the G+C content of the sequenced strain was 50.5 mol % (Blattner *et al.*, 1997). Thus this standard is reasonably close to the actual sequenced data. Because T_m from this capillary method was influenced by differences in the binding capacity of SYBR Green 1 among bacterial chromosomal DNAs of different G+C contents, we recommend the use of three different DNA standards: *Staphylococcus aureus* ATCC 12600^T for low G+C contents, *Escherichia coli* ATCC 11775^T for medium G+C contents and *P. aeruginosa* ATCC 10145^T for high G+C content.

In summary, our SYBR Green 1 based method for determination of G+C content was more rapid and less labour-intensive than traditional techniques. It did not require enzymic digestion of the DNA as does the HPLC method. Although the Light Cycler is not yet common equipment in research laboratories, it is increasingly used in many diagnostic laboratories to quantify PCR amplicons. Because 24 samples can be analysed within 10 min, this assay could contribute to the rapid identification of micro-organisms because of the rapidity and simple procedure.

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REFERENCES

- Blattner, F. R., Plunkett, G., III, Bloch, C. A. & 11 other authors (1997). The complete genome sequence of *Escherichia coli* K-12. *Science* **277**, 1453–1474.
- Buchanan, R. E. & Gibbons, N. E. (editors) (1974). *Bergey's Manual of Determinative Bacteriology*, 8th edn. Baltimore: Williams & Wilkins.
- Campos, J. M. (1995). *Haemophilus*. In *Manual of Clinical Microbiology*, 6th edn, pp. 556–565. Edited by P. A. Murray & others. Washington, DC, American Society for Microbiology.

- Cole, S. T., Brosch, R., Parkhill, J. & 22 other authors (1998). Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nature* **393**, 537–544.
- Colwell, R. R. & Mandel, M. (1964). Adansonian analysis and deoxyribonucleic acid base composition of some gram-negative bacteria. *J Bacteriol* **87**, 1412–1422.
- De Ley, J. (1970). Re-examination of the association between melting point, buoyant density, and chemical base composition of deoxyribonucleic acid. *J Bacteriol* **101**, 738–754.
- De Ley, J., Park, I. W., Tijtgat, R. & Van Ermengem, J. (1966). DNA homology and taxonomy of *Pseudomonas* and *Xanthomonas*. *J Gen Microbiol* **42**, 43–56.
- Desilva, D., Reiser, A., Herrmann, M., Tabiti, K. & Wittwer, C. (1998). Rapid genotyping and quantification on the Light Cycler with hybridization probes. *Roche Mol Biochem* **2**, 12–15.
- Ezaki, T., Hashimoto, Y., Takeuchi, N., Yamamoto, H., Liu, S. L., Miura, H., Matsui, K. & Yabuuchi, E. (1988a). Simple genetic method to identify viridans group streptococci by colorimetric dot hybridization in microdilution wells. *J Clin Microbiol* **26**, 1708–1713.
- Ezaki, T., Takenchi, N., Liu, S. L., Kai, A., Yamamoto, H. & Yabuuchi, E. (1988b). Simple and rapid genetic identification of *Campylobacter* species without radioisotope. *Microbiol Immunol* **32**, 191–199.
- Ezaki, T., Saidi, S. M., Liu, S. L., Hashimoto, Y., Yamamoto, H. & Yabuuchi, E. (1990). Rapid procedure to determine the DNA base composition from small amounts of Gram-positive bacteria. *FEMS Microbiol Lett* **67**, 127–130.
- Fleischmann, R. D., Adams, M. D., White, O., Clayton, R. A., Tomb, E. F., Dougherty, B. A. & Merrick, J. M. (1995). Whole-genome random sequencing and assembly of *Haemophilus influenzae* Rd. *Science* **269**, 496–512.
- Frederick, S. N. & Metchock, B. (1995). *Mycobacterium*. In *Manual of Clinical Microbiology*, 6th edn, pp. 400–437. Edited by P. A. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover & R. H. Tenover. Washington, DC: American Society for Microbiology.
- Gause, G. G., Loshkareva, N. P., Zbarsky, I. B. & Gause, G. F. (1964). Deoxyribonucleic acid base composition in certain bacteria and their mutants with impaired respiration. *Nature* **203**, 598–599.
- Ko, C. Y., Johnson, J. L., Barnett, L. B., McNair, H. M. & Vercellotti, J. R. (1977). A sensitive estimation of the percentage of guanine plus cytosine in deoxyribonucleic acid by high performance liquid chromatography. *Anal Biochem* **80**, 183–192.
- Krieg, N. R. & Holt, J. G. (editors) (1984). *Bergey's Manual of Systematic Bacteriology*, vol. 1, 1st edn. Baltimore: Williams & Wilkins.
- Lee, L. F. & Boezi, J. A. (1966). Characterization of bacteriophage gh-1 for *Pseudomonas putida*. *J Bacteriol* **92**, 1821–1827.
- Livak, K. J., Flood, S. J. A., Marmaro, J., Giusti, W. & Deetz, K. (1995). Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl* **4**, 357–362.
- Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.
- Mandel, M., Igambi, L., Bergendahl, J. & Scheltgen, E. (1970). Correlation of melting temperature and cesium chloride buoyant density of bacterial deoxyribonucleic acid. *J Bacteriol* **101**, 333–338.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Owen, R. J., Hill, L. R. & Lapage, S. P. (1969). Determination of DNA base compositions from melting profiles in dilute buffer. *Biopolymers* **7**, 503–516.
- Ririe, K. M., Rasmussen, R. P. & Wittwer, C. T. (1997). Product differentiation by analysis of DNA melting curves during the polymerase chain reaction. *Anal Biochem* **245**, 154–160.
- Schildkraut, C. L., Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its buoyant density in CsCl. *J Mol Biol* **4**, 430–443.
- Sneath, P. H. A., Mair, N. S., Sharpe, M. E. & Holt, J. G. (editors). (1986). *Bergey's Manual of Systematic Bacteriology*, vol. 2, 1st edn. Baltimore: Williams & Wilkins.
- Takayama, K., Abe, S. & Kinoshita, S. (1965). Taxonomic studies on glutamic acid producing bacteria: on the base composition of DNA. *J Agr Chem Soc Jpn* **39**, 342–346.
- Tamaoka, J. (1986). Analysis of bacterial menaquinone mixtures by reverse-phase high-performance liquid chromatography. *Methods Enzymol* **123**, 251–256.
- Washington, C. W., Jr (1995). *Legionella*. In *Manual of Clinical Microbiology*, 6th edn, pp. 533–544. Edited by P. A. Murray & others. Washington, DC: American Society for Microbiology.
- Wittwer, C. T., Ririe, K. M., Andrew, R. V., David, D. A., Gundry, R. A. & Balis, U. J. (1997a). The Light Cycler: a microvolume multisample fluorimeter with rapid temperature control. *Biotechniques* **22**, 176–181.
- Wittwer, C. T., Herrmann, M., Moss, A. & Rasmussen, R. (1997b). Continuous fluorescence monitoring of rapid cycle DNA amplification. *Biotechniques* **22**, 130–138.