

DNA Quantification using the Rotor-Gene™ Real-time PCR Instrument

Purpose

To quantify purified total double-stranded (ds) DNA by fluorescence.

Introduction

Measurement of absorbance at 260 nm (A260nm) is commonly used for quantifying DNA. Disadvantages of using A260nm include (i) insensitivity of the assay, and (ii) interference by non-DNA components such as RNA, particularly in samples that are not highly purified. The RFL assay, developed by DNA Genotek, uses a fluorescent dye such as SYBR Green I, which has specificity for dsDNA. The RFL assay was developed to take advantage of the high sensitivity of real-time PCR instruments such as the Corbett Rotor-Gene.

Equipment and Reagents

Equipment

1. Corbett Rotor-Gene RG-3000A or RG-6000.

Reagents

1. **10× RFL buffer** -[500 mM BES, 1 mM EDTA, 5% w/w TritonX-100, pH 7.5]
 - i) Store at 4°C in refrigerator.
2. **SYBR Green I dye** – (Invitrogen cat # S7563, 10,000×).
 - i) Prepare a 100x working stock by diluting the SYBR Green dye in TE buffer.
 - ii) Stored in 10 or 20 µL aliquots in 0.2 mL PCR tubes at -20°C.
 - iii) Before each use, thaw at RT. Discard unused portion.
3. **dsDNA for Standard Curve, Lambda DNA** – (Invitrogen cat # 25250-010)
 - i) Prepare dilutions for the standard curve in TE buffer.
 - ii) 20 µl aliquots of each standard (See Table 2 Standards A-F) are stored in 0.2 mL PCR tubes at -20°C .
 - iii) Thaw one tube of each Standard. Discard unused portion.
4. **Reduced TE (rTE)** – 10 mM Tris HCl, 0.1 mM EDTA

Procedure

1. Preparation of Master Mix

- i) Prepare a master mix solution, sufficient for all tubes to be assayed.
- ii) Master Mix:

	Each (µl)	18 + n n=number of unknown samples
Water	17.25	
10 X RFL Buffer	2.5	
100 X Sybr Green 1	0.25	
Total	20	

Table 1.

2. Unknown Purified Saliva Samples

- i) For each unknown sample(n):
 - (a) Label a PCR tube.
 - (b) Dilute purified DNA 1:50 in rTE (4 μ L sample + 196 μ L rTE).
 - (c) Add 5 μ L of unknown sample to the corresponding tube.
 - (d) Add 20 μ L of Master Mix. Mix by vortexing.

3. Standard Curve

- i) For each standard (A-F):
 - (a) Label 0.2 mL PCR tubes (in duplicate).
 - (b) Add 5 μ L of DNA standard to the corresponding tube.
 - (c) Add 5 μ L of rTE to 2 separate PCR tubes for a “no DNA” Standard.
 - (d) Add 20 μ L of Master Mix. Mix by vortexing.

Standard	Concentration (ng/ μ L)	Volume (μ L)	Total DNA (ng)
A	7.5	5	37.5
B	5.0	5	25
C	2.5	5	12.5
D	1.25	5	6.25
E	0.625	5	3.125
F	0.3125	5	1.56
rTE	0	5	0

Table 2.

4. Read Fluorescence of Samples

- i) Use RFL program on the Rotor-Gene to read fluorescence of samples.
 - (a) Typical program is 30°C for 20 seconds x 6 cycles.

5. Results

- i) In the RFL program, click on ‘Analysis’, ‘Other’, ‘Concentration’, and choose the channel that did not show saturation (i.e., approach 100%).
- ii) Check the linearity of the standard curve (R2 value > 0.99). Visually inspect curve, remove any outlying points and ensure the standard curve fits the data. If standard curve has an R2 < 0.99, redo assay.
- iii) If standard curve has an R2 > 0.99, change the analysis to Spline and use this data to analyze the results.
- iv) Copy and paste results into an Excel sheet.
- v) All results produced by the RFL program have a unit of “ng/reaction”.
- vi) Calculation of Results:
 - (a) Concentration of Diluted sample [CD] = RFL results [RFL] divided by the volume [V] of DNA added to the RFL rxn
 $[CD] = [RFL] / [V]$
 - (b) Concentration of Undiluted sample [CUD] = Concentration of Diluted samples [CD] multiplied by the Dilution Factor [DF] (typically DF = 50)
 $[CUD] = [CD] * [DF]$
 - (c) Amount of Purified DNA [PD] = Concentration of Undiluted sample [CUD] multiplied by the re-hydration volume [RV] (typically 100 μ L when purifying 500 μ L of sample)
 $[PD] = [CUD] * [VD]$

(d) Total DNA [TD] expected from a 4 mL sample = Amount of Purified DNA [PD] multiplied by the Total Volume [TV] of Oragene-DNA/Saliva sample (typically 4 mL) divided by volume of sample purified (i.e. 500 μ L).
[TD] = [CUP] * [TV]

Safety and Environmental Information

The use of gloves is required to avoid sample contamination.