

## DNA Quantification Using SYBR Green I Dye and a Micro-Plate Reader

### Purpose

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

### Equipment and Reagents

#### Equipment

1. Micro-plate reader (e.g. TECAN - M200 96-well plate reader).

#### Materials

1. 96-well black plate (e.g. Greiner – 96-well plate, black. VWR cat # 82050-784).

#### Reagents

1. **1x TE** (10mM Tris HCl, 1mM• EDTA, pH8).
  - (i) Store at room temperature.
2. **SYBR Green I dye** (Invitrogen cat # S7563).
  - i) 100x working stock prepared by diluting 10 µL of SYBR Green dye with 990 µL TE buffer.
  - ii) Store in 10 or 20 µL aliquots in 0.2 mL PCR tubes at -20°C.
  - iii) Before each use, thaw at room temperature.
3. **dsDNA for Standard Curve – Lambda DNA** (Invitrogen cat # 25250-010).
  - i) Serially dilute the DNA to give a total of 7 dilutions plus a no-DNA point.
  - ii) 50 µl aliquots of each standard are stored in 0.2 mL PCR tubes at - 20°C (See table 2. Standards A-H).
  - iii) Thaw one tube of each standard.

### Procedure

#### 1. Preparation of Master Mix

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

	Volume (µL)	20 + n n=number of unknown samples
TE	94	
100X SYBR Green 1	1	
Total	95	

Table 1.

## 2. Standard Curve:

i) For each standard (A-H):

(a) In duplicate, add 5  $\mu\text{L}$  of each standard to a well of a black 96-well plate.

(b) Add 95  $\mu\text{L}$  of the master mix to each well.

Standard	Concentration (ng/ $\mu\text{l}$ )	Volume ( $\mu\text{l}$ )	Total DNA (ng)
A	10.0	5	50.0
B	5.0	5	25.0
C	2.51	5	12.5
D	1.25	5	6.25
E	0.625	5	3.12
F	0.3125	5	1.56
G	0.156	5	0.78
H	0.0	5	0.0

Table 2.

## 3. Unknown Purified Saliva Samples

i) For each unknown sample(n):

(a) Dilute purified DNA 1:50 in 1x TE (4  $\mu\text{L}$  sample + 196  $\mu\text{L}$  1x TE).

(b) Add 5  $\mu\text{L}$  of unknown sample to a well of a black 96-well plate.

(c) Add 95  $\mu\text{L}$  of Master Mix.

## 4. Read Fluorescence of Samples

(a) Excitation 485 nM.

(b) Emission 535 nM.

## Safety and Environmental Information

The use of gloves is required to avoid sample contamination.

## END OF PROCEDURE