

# 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 HCI, 1mM• EDTA, pH8).
- (i) Store at room temperature.
- 2. SYBR Green I dye (Invitrogen cat # S7563).
  - i) 100x working stock prepared by diluting 10  $\mu L$  of SYBR Green dye with 990  $\mu L$  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  $\mu L$  of each standard to a well of a black 96-well plate.
  - (b) Add 95 µl of the master mix to each well.

Standard	Concentration (ng/µl)	Volume (µl)	Total DNA (ng)
А	10.0	5	50.0
В	5.0	5	25.0
С	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
Н	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  $\mu$ L sample + 196  $\mu$ L 1x TE).
  - (b) Add 5  $\mu$ L of unknown sample to a well of a black 96-well plate.
  - (c) Add 95 μL 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**