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Quantification of DNA Extracted from Libyan Buccal Swabs by Using Equation of Standard Curve

Samir Elmrghni*

Department of Forensic Medicine and Toxicology, Faculty of Medicine, University of Benghazi-Libya, Benghazi, Libya

Corresponding Author:** ***Samir Elmrghni, *Department of Forensic Medicine and Toxicology Faculty of Medicine University of Benghazi Libya,* ***Email:*** *samir3272@yahoo.ie*

Abstract: *Since DNA is the blueprint for life, everything living contains DNA. DNA isolation is one of the most basic and essential techniques in the study of DNA. The extraction of DNA from cells and its purification are of primary importance to the field of biotechnology and forensics. Extraction and purification of DNA are the first steps in the analysis and manipulation of DNA that allow scientists to detect genetic disorders, produce DNA fingerprints of individuals, and even create genetically engineered organisms that can produce beneficial products such as insulin, antibiotics, and hormones. DNA can be extracted from many types of cells. The standard curve Calibration was used to calculate the DNA quantities extracted from bucal swabs in samples From Benghazi population which included in different research studies.*

Keywords: *standard curve, DNA quantification, Libyan*

1. Introduction

Libya, a Northern African country, was first inhabited by Berbers, followed by Phoenicians, Greeks, Romans, Arabs and Ottomans. Libya became independent in 1951 after a brief period as an Italian colony; it had been invaded by Italy in 1911. In February 2011 an uprising against the Libyan government occurred. Benghazi is the second largest city in Libya and the main city (or capital) of the Cyrenaica region (or ex-Province), located in the North of Africa. Benghazi is located half way between Tripoli in the West (a distance of approximately 1000 Km between these cities) and Cairo in the East (also approximately 1000 Km). Cyrenaica is surrounded by desert on three sides; hence in ancient times the most accessible civilization was to the North, across the Mediterranean, in Crete and Greece, only 400 km away.

The population of Benghazi was 500,120 in 1995 (census) and increased to 670,797 in the 2006 census. As with other cities in Libya, there is a reasonable amount of ethnic diversity in Benghazi. The people of eastern Libya, Benghazi included, have in the past always been of predominantly Arab descent. In recent times, however, there has been an influx of African immigrants into Benghazi. There are also many Egyptian immigrants in Benghazi and a small Greek community also exists in Benghazi; the Greek island of Crete is a short distance away from Benghazi and many families in Benghazi today bear Cretian surnames. In modern times, Benghazi has seen a lot of Libyans from different parts of the country move into the city, especially since the Kingdom era (1951-1969). Many Libyans came to Benghazi from Misrata (About 60% of the population have roots from Misrata, West of Benghazi).

2. Quality Control

The laboratory has participated in the Y-STR Haplotyping Quality Assurance Exercise (Certified at 2010-5-20). The data were submitted to YHRD (www.yhrd.org) and received the accession number: YA003680

DNA was quantified using a method developed in the laboratory. Lambda DNA (New England BioLabs, Hitchin, UK) was serial diluted to prepare standard solutions with known quantities of DNA in 10µl volumes: 200 ng, 100 ng, 20 ng, 2 ng, 0.2 ng and 0.02 ng. The 10 µl volumes of the DNA standard solutions and 10 µl volumes of volunteer samples were added in triplicate to a Fast Optical 96-well reaction plate (Applied Biosystems). A volume of 10 µl SYBR® Green I nucleic acid gel stain 10,000x

(SIGMA) diluted to 20x concentrated was then added to each well. The plate was then centrifuged at 3,000 rpm for 1 min and maintained at room temperature for 5 min before reading (protected from light). The plate was read using a StepOnePlus™ Real-Time PCR System (Applied Biosystems) under the following conditions: initial holding of 30 seconds at 25ºC and 1 cycle of 10 seconds at 25ºC with data collection. The raw data was then analysed as shown in table below. A screen capture print of the raw data obtained for the standard DNA quantities is shown in the figure 1 below.

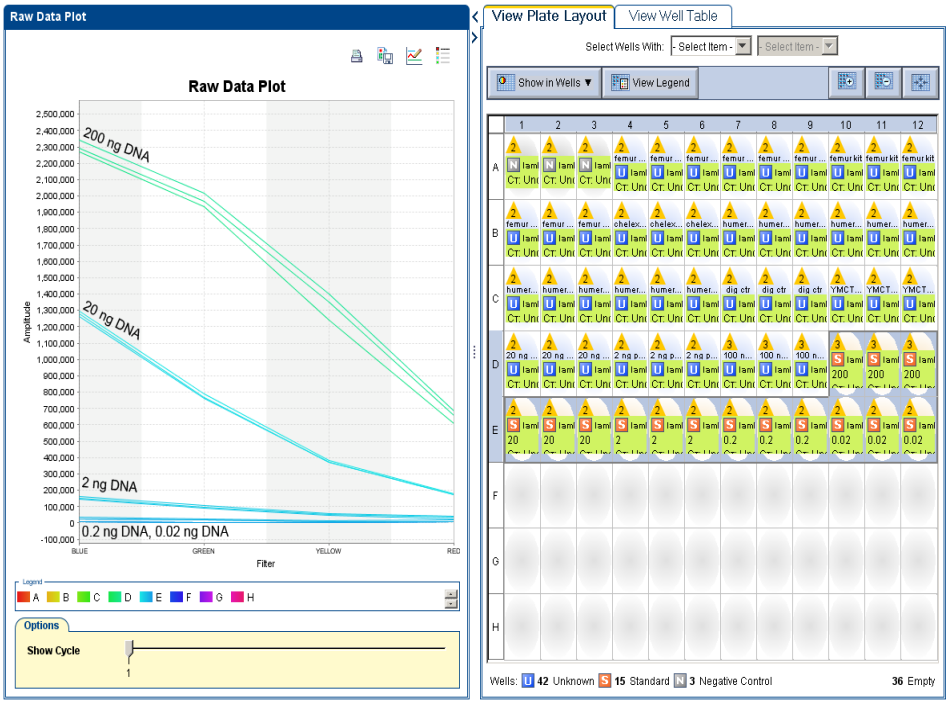

Figure1. *A screen capture of the raw data obtained for the standard DNA quantities*

A screen capture of the raw data obtained for the negative controls is shown in the figure 2 below

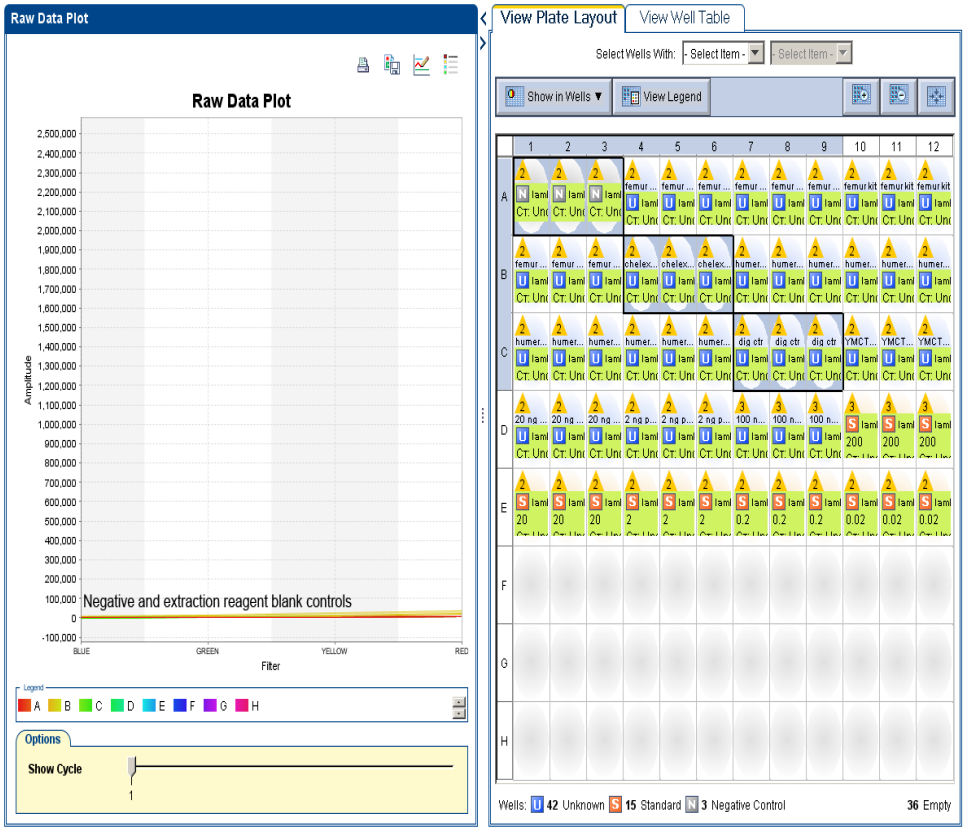

Figure2. *A screen capture of the raw data obtained for the negative controls*

The raw data was then exported as an Excel file and a standard curve drawn (with logarithmic scale for the X and Y axis and adding a trendline) using the blue amplitude values Table 1. (RFU) obtained for the DNA quantities 20 ng, 2 ng and 0.2 ng (200 ng and 0.02 ng not included due to saturation and below threshold, respectively) Table 1. The standard curve is shown in the figure3 below.

Table1. *Calculation of the quantity of some volunteer samples from buccal swabs by using equation of standard curve.*

Well	BLUE(data)	Average	Corrected average	DNA quantity x=(y-10009)/53782
NC	-171	-446.333	-0.33333	0
NC	-679			
NC	-489			
sample1	2384717	2396433	2396879	44.38046186
sample2	2734098	2682524	2682970	49.86725422
Sample3	553044	584474.7	584920.7	10.85700916
PC 20	1108157	1102261	1102707	20.48450535
PC 20	1051740			
PC 20	1146885			
PC 2	113862	120830.7	121276.7	
PC 2	127680			
PC 2	120950			
100 ng	2408656	2402978	2403424	
100 ng	2404571			
100 ng	2395706			
200 ng	2601678	2621263	2621709	
200 ng	2610616			
200 ng	2651495			
20 ng	1123312	1084241	1084687	
20 ng	1069325			
20 ng	1060086			
2 ng	131930	127344	127790	
2 ng	131209			
2 ng	118893			
0.2 ng	16564	15793	16239	
0.2 ng	15866			
0.2 ng	14949			
0.02 ng	10773	5920.333	6366.333	
0.02 ng	1932			
0.02 ng	5056			

(NC) negative control and positive control (PC).

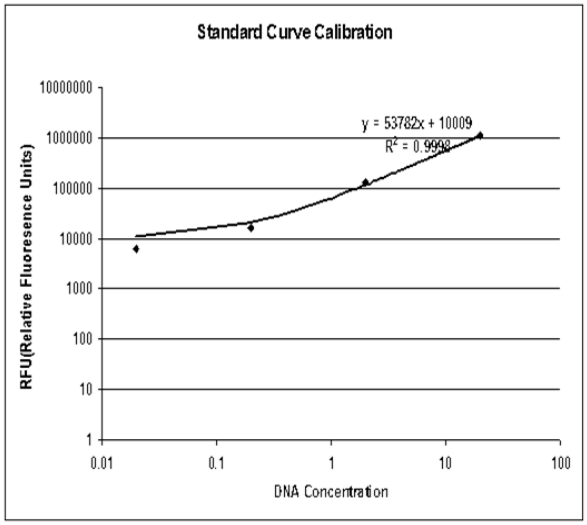

Figure3. *The equation of the standard curve used for quantification of DNA from swab samples Benghazi samples*

The standard curve Calibration was then used to calculate the DNA quantities in the unknown samples (the ideal concentration used to type Autosomal and Y- filer kits is 0.1ng) by substituting Y for the blue amplitude values. DNA concentrations were then calculated noting that 10 µl had been applied to each well. The values obtained for the positive controls (20 ng and 2 ng) indicated the accuracy of the equation.

For Benghazi population samples which included in different research studies (1, 2, 3&4) it was discovered that a 10 fold dilution of the samples gave an optimum amount of target DNA in the amplifications.

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