# INTRODUCTION TO REAL TIME PCR

**Ref.PCR8**

## 1.INTRODUCTION

PCR allows the amplification of a target region from a DNA template by using specifics oligonucleotides. In real-time PCR (qPCR), the accumulating amplified product can be detected at each cycle with fluorescent dyes.

**This kit is an introduction to real-time PCR. Students will be able to prepare standard curves and quantify human DNA problem samples. The accumulated amplified product can be detected in each cycle with fluorescent dyes.**

**It is presented as ready microtubes and contains all the necessary components to perform the quantitative PCR analysis.**

The target is a multiple-copy gene, 200 copies per genome, with a slow evolutionary rate. Due to the multiple-copy and the high degree of conservation can be used for quantification of low amounts Human DNA

## 2. COMPONENTS

- **cfhDNA MONODOSE dtec-qPCR tests (INDIVIDUAL TUBES),** contains a mixture of specific primers and labelled probe, dNTPs, BSA, polymerase and buffer at optimal concentrations and lyophilized after synthesis.
- **DNase/RNase free water (green cap)**, 1.5 ml.
- **Positive control (orange cap)**, dehydrated target copies for positive control.
- **Standard Template (red cap** dehydrated target copies for standard curve.
- **Standard buffer (black cap),** exclusive for resuspension of the positive control or standard template.

### 2.1 Storage conditions

All the components of cfhDNA MONODOSE dtec-qPCR Tests are stable at room temperature for transport, but should be stored at -20 ºC if not immediately used. Individual tests are stable for one year under this conditions.

For **Standard Template (red cap)** we recommend, once disolved, store in a exclusive box at -20ºC.

### 2.2 Materials required but not provided

- DNA isolation kit. We recommend our DANAGENE SALIVA KIT.
- DNase/RNase free water to prepare standard curve dilution.
- Micropipettes.
- Sterile pipette tips with filters.
- Vortex.
- Centrifuge.
- Real Time PCR device

## 3. PRACTICE

### 3.1 DNA isolation

The previous step to any genetic study is usually the isolation of genomic DNA, this can be carried out in different ways (homemade methods, commercial kits, etc.) and from different samples (blood, tissue, etc).

For the realization of this practice it is recommended that the source of the **DNA come from the student's saliva,** since it is the most accessible source of DNA and does not supose any risk such as blood collection. For this, the use of **DANAGENE SALIVA KIT is recommended**, which allows genomic DNA to be obtained from a saliva sample.

### 3.2 Preparation of standard curve dilution series

1. Pipette 900 ul de **DNAse/RNase free water** into 5 tubes and label as 2 to 6.

2. Pulse –spin the **Standard Template (red cap)**, reconstitute with 120 ul of **Standard buffer (black cap)** and vortex thoroughly. **Label as n.1.**

3. Pipette 100 ul of diluted **Standard Template (red cap)** into tube 2.

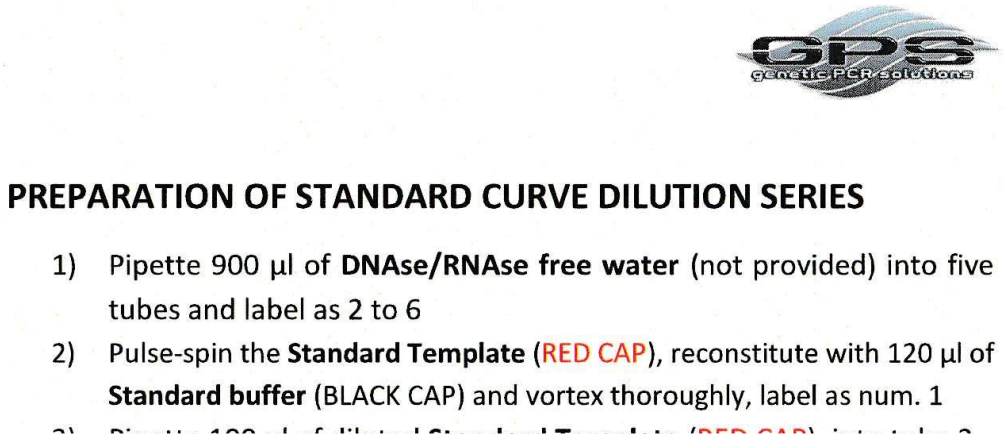
4. Vortex thoroughly and pulse-spin.

5. Change tip and pipette 100 ul from tube 3 into tube 3.

6. Vortex thoroughly and pulse-spin.

7. Repeat steps 5 y 6 with the  tubes 4 a 6 to complete serial dilution.

---




**Pipette 5 ul** of each dilution (1 a 6) + **15 ul** DNase/RNase free water (green cap)into different **Microtubes MONODOSIS dtec-qPCR**.

### 3.2 Preparation of positive control

The **Positive control (orange cap)** must be prepared only when a detection analysis is required (no quantification). Pulse-spin the **Positive control (orange cap)**, reconstitute with 120 ul of **Standard buffer (black cap)** and vortex thoroughly.

Pipette 5 ul of template into the well according to your palte set-uo. The final volume in each qPCR reaction well is 20 ul.

### 3.2 Amplification Protocol

1. Add the desired amount of sample ranging from of 5 ul to a maximum of the total volume of the qPCR of 20 ul, when necessary, complete the final volume up to 20 ul with DNase / RNase free water (green cap), for example 5 ul sample + 15 ul water). Vortex thoroughly and pulse-spin.

2. **For calibration curve**, **Pipette 5 ul** of each dilution (1 a 6) + **15 ul** DNase/RNase free water (green cap) into different **Microtubes MONODOSIS dtec-qPCR**.

3. **Negative control**, add 20 ul DNase/RNase free water (green cap) to one microtube. Accordingly, this reaction should be negative. A positive result may be considered as a symptom of DNA contamination in the water, makong the test inconclusive. Water must be replaced.

4. **Program: 40 cycles**

| | Step | Time | Temperature |
|---|---|---|---|
| | Activation | 2 minutes | 95ºC |
| **40 CYCLES** | Denaturation | 5 seconds | 95ºC |
| | Hybridization/Extension and data collection[1] | 20 seconds | **60ºC** |

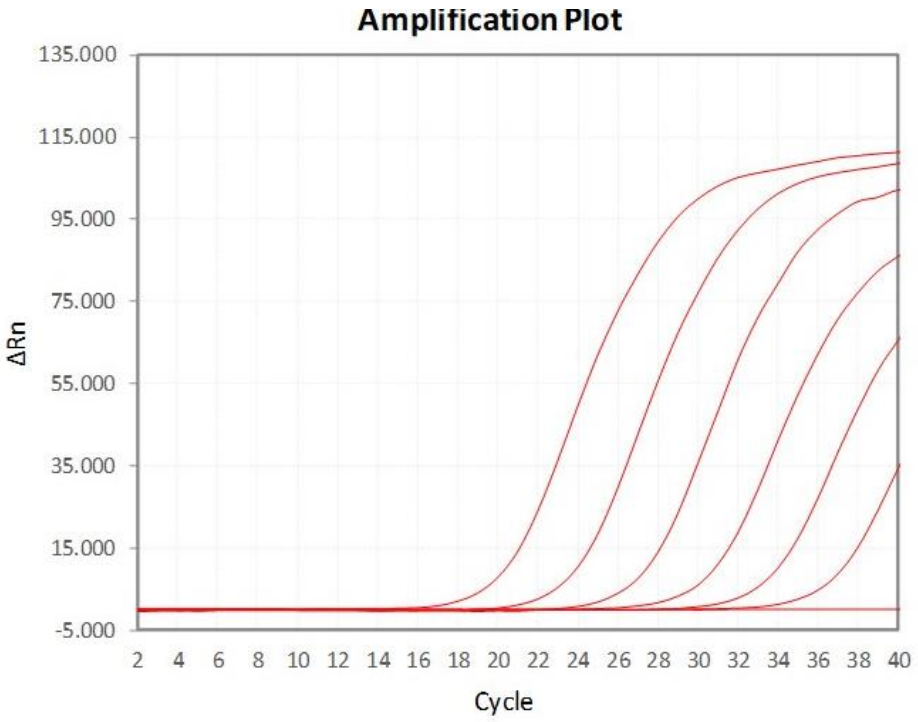
1 Fluorogenic signal should be collected during this step by using the **FAM channel for the target.**

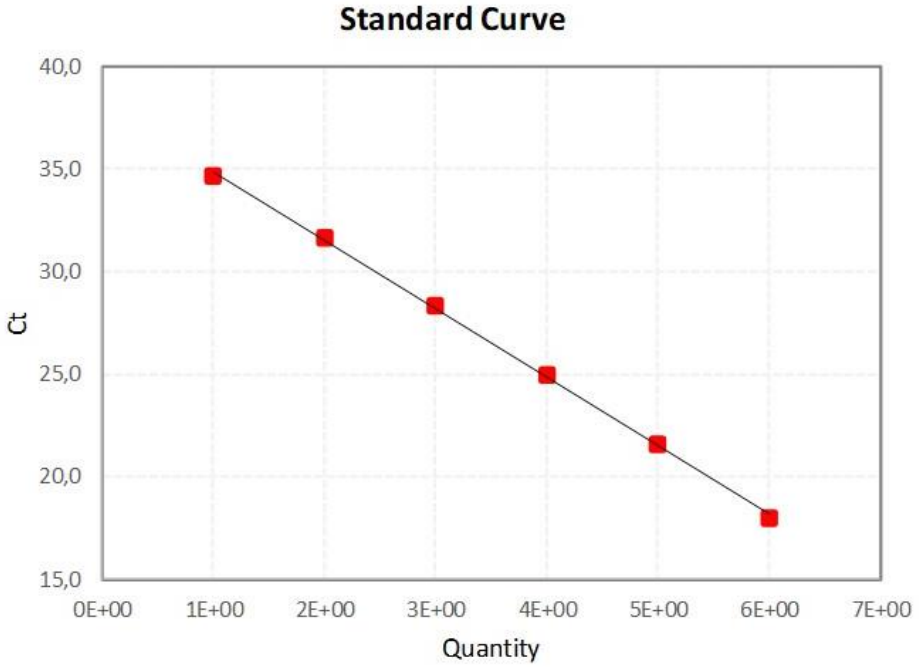
The reagents contains BSA and are **compatible with all real-time PCR thermal cyclers.** Plastic of the generic tube is compatible with: StepONe, StepOnePlus, ABI 7500 Fast, LightCycler 96, LightCycler Nano, CFX96, PikoReal 24-well, DNA Engine systems, MiniOpticon 48-12 and Opticon 2. **For other devices, please, transfer the content of the monodose (20 ul) to appropriate tubes.**

## 4. RESULTS

We present a example of graph with a decimal dilution standard template amplification ( 10-$10^6$ copies).

| Copies | Ct |
|---|---|
| **$10^6$** | 18,0 |
| **$10^5$** | 21,6 |
| **$10^4$** | 25,0 |
| **$10^3$** | 28,3 |
| **$10^2$** | 31,6 |
| **10** | 34,7 |


Amplification Plot
135.000
115.000
95.000
75.000
55.000
35.000
15.000
-5.000
ΔRn
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40
Cycle


Standard Curve
40,0
35,0
30,0
25,0
20,0
15,0
Ct
0E+00 1E+00 2E+00 3E+00 4E+00 5E+00 6E+00 7E+00
Quantity