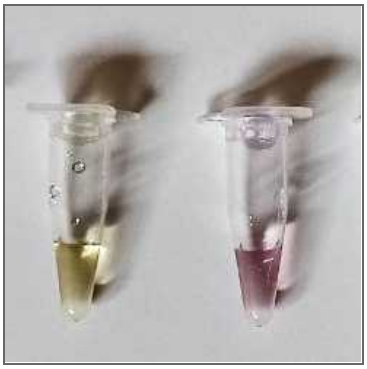

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Protocol status: Working
We use this protocol and it's working

Created: Aug 03, 2023

The Quantitative Loop-Mediated Isothermal Amplification Colorimetric-Phenol Red (QLAMP-PhR) Protocol

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ABSTRACT

QLAMP-PhR Protocol

The LAMP technique was originally developed in 2000 by (Notomi, *et al.*).

In this proposed protocol, we aim to **develop a new biomedical technique for performing quantitative colorimetric LAMP** on various samples. This protocol enables the specificity and sensitivity of the detection of the DNA copy number of any pathogen in any sample (serum, plasma, saliva, stool, urine, etc.) by the LAMP test.

The QLAMP-PhR technique enabled fast, specific, and sensitive quantification, and it can be applied as an alternative molecular diagnostic tool for detecting pathogens.

(we detection of *Fusobacterium nucleatum* ATCC 25586 as an example)

Expected result

We expected the Quantitative results

MATERIALS

Primers set:

- tow inner primers (forward inner primer; and backward inner primer)
- two outer primers (forward outer primer and backward outer primer)
- two loop primers (forward loop primer and backward loop primer) (Option)

Master Mix:

Tris/HCl (pH=8.8) , KCl , $(NH_4)_2SO_4$, Tween 20 , betaine , $MgSO_4$, dNTPs

Last Modified: Aug 04, 2023

PROTOCOL integer ID: 85917

Keywords: LAMP , QLAMP , Phynol Red , Quantitative LAMP

color change indicator: **Phynol Red**
Bacillus stearothermophilus **(Bst)** DNA polymerase

Positive controls: DNA titrate dilutions with distilled water as ($1:10^1$, $1:10^2$, $1:10^3$, $1:10^4$, $1:10^5$, $1:10^6$, $1:10^7$)
Negative control: distilled water

Equipment

NanoDrop™ One/OneC Microvolume UV-Vis Spectrophotometer	NAME
UV-Vis Spectrophotometer	TYPE
Thermo Scientific	BRAND
ND-ONE-W	SKU

Equipment

General Purpose Digital Water Bath	NAME
PolyScience	BRAND
WBE20A11B	SKU

1 Samples :

start with stool samples.
The acquired samples were stored in a freezer at −50 °C.

2 DNA extraction :

Stool samples were removed from the freezer and kept at 37 $^{\circ}C$ for 20 min prior to use.
Genomic DNA was extracted from the stool samples.
All DNA samples were stored at −50 °C until used.

3 Primer design :

LAMP primers were designed for fadA (specific Fusobacterium spp. gene) based on GenBank accession number DQ012971.1. Primer Explorer V5 software (https://primerexplorer.jp/e/) was used to design 3 sets of primers. The first set of primers was chosen and synthesized.

4 LAMP reaction :

A 25µl reaction volume was used for the LAMP technique with final concentrations of:
2mM Tris/HCl (pH=8.8)
10 mM KCl
10 mM $(NH_4)_2SO_4$
0.1 % Tween 20
0.8M betaine
8mM $MgSO_4$
1.4mM each dNTP
8U **Bst DNA polymerase**
2 µl of 15 µg / 100 µl phenol red as a pH quantitative colorimetric indicator **"by experimental result"**

4.1 For each reaction :

40 p.mol FIP and BIP
20 p.mol LB or LF
5 p.mol F3 and B3
Positive controls: 1 µl DNA *F. nucleatum* [ATCC 25586] titrate dilutions with distilled water as ($1:10^1$, $1:10^2$, $1:10^3$, $1:10^4$, $1:10^5$, $1:10^6$, $1:10^7$)
Negative control: 1 µl distilled water was used as a no-template control.
The reactions were performed at **63 °C for 60 min and then cooled at room temperature for 6 min.**

4.2 Absorbance (A) :

The peak absorbance of phenol red was read at **560 nm** by the spectrophotometer.
"experimental result"
The absorbance of each tube was read at **560 nm** with a spectrophotometer.

5 Quantitative colorimetric calibration curve :

Fusobacterium nucleatum subsp. nucleatum [ATCC 25586] was used as a reference strain, and a standard curve was made using DNA extracted from this [ATCC 25586] reference strain.
F. nucleatum has a genome size of 2.4 Mb

A single *F. nucleatum* genome weighs 2.43fg (2.4Mb/987Mb [1pg of double-stranded DNA]=0.00243pg).
1ng of *F. nucleatum* DNA contains approximately 411,523 copies of the genome (1000 pg/0.00243 pg)
The *F. nucleatum* DNA concentration was estimated by measuring the absorbance at **560nm** and plotting the calibration curve **(absorbance against concentration)**.
Samples were considered positive if a **phenol red color change (from violet/fuchsia to yellow/orange)** with amplified *F. nucleatum* DNA was observed.

6 **Quantitative analysis of the colorimetric QLAMP reaction :**
The quantitative LAMP reaction was carried out with a serially diluted copy number of genomic DNA templates as a control samples colored with phenol red, and by correlating the absorption value and the logarithm of DNA concentrations, a linear regression plot was conducted, producing the standard quantitative analysis curve. Then, an exact digital value of the F. nucleatum DNA copy number amount in each sample was obtained.
At **560 nm**, we observed **a linear relationship between Log F. nucleatum concentrations (F. nucleatum DNA copies/sample) and Absorption (A)** by function:
x= - (10.181×A)+3.1409 "experimental result"
10X = F. nucleatum DNA copies/sample "experimental result"

7 **Number of replicates :**
3 replicates have been done.