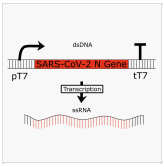

Feb 21, 2021

LAMP/RT-LAMP COVID positive control

In 1 collection

Anibal Arce Medina[1], Isaac Núñez[2], Tamara Matute[2], Fernan Federici[2]

[1]Pontificia Universidad Catolica de Chile; [2]SynBioUC

1 Works for me dx.doi.org/10.17504/protocols.io.bsknncve

Laboratorio de Tecnologias Libres Reclone.org (The Reagent Collaboration Network)

Tamara Matute

SUBMIT TO PLOS ONE

Citation: Anibal Arce Medina, Isaac NÃÂºÃÂ±ez, Tamara Matute, Fernan Federici (02/21/2021). LAMP/RT-LAMP COVID positive control. https://dx.doi.org/10.17504/protocols.io.bsknncve

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

ABSTRACT

This protocol explains how to prepare dsDNA and ssRNA target positive control for LAMP and RT-LAMP reactions. Particularly it describes the pipeline for SARS-Cov-2 Nucleocapsid (N) gene positive control which is included in the ReClone collection. The general procedure involves amplifying the gene of interest with primers which add T7 promoter and T7 terminator to its sequence followed by T7 in vitro transcription to obtain the ssRNA.

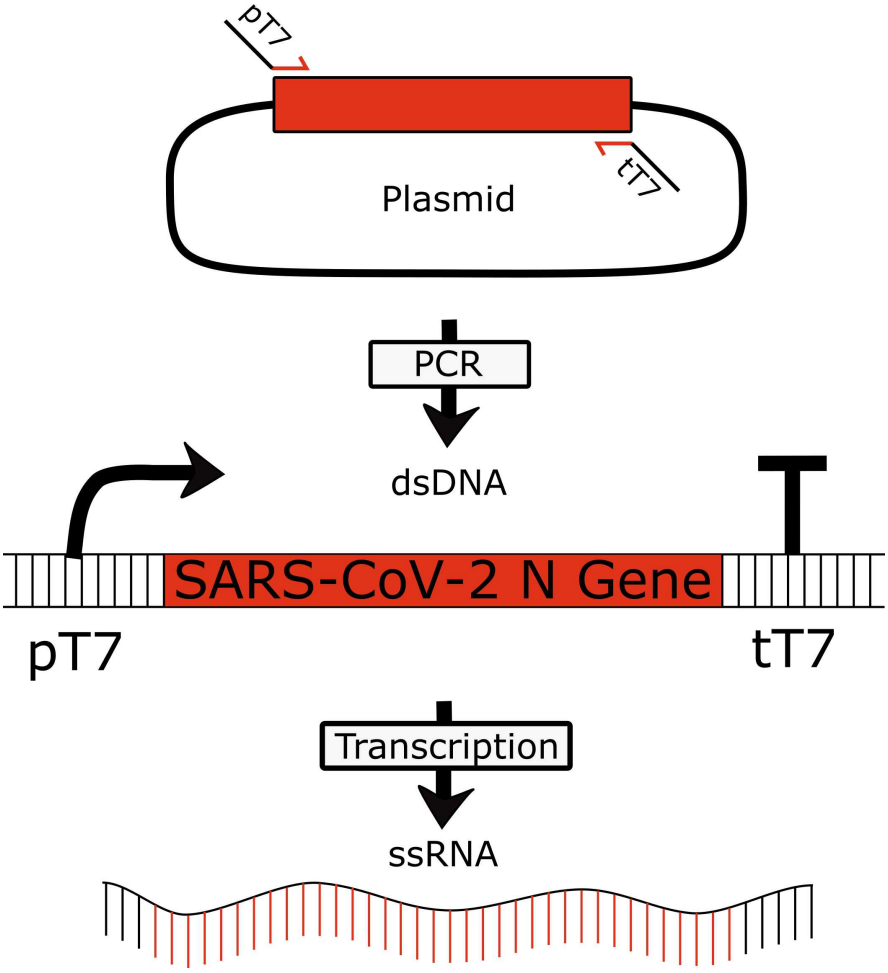

Figure 1: General pipeline. It shows how dsDNA is amplified from control plasmid at the time pT7 and tT7 are incorporated to its sequence which is used to perform the ssRNA T7 in vitro transcription.

Sequence elaboration:

Sequence OrfN_5 was designed and synthesized based on IDT SARS-CoV-2 positive control plasmid (CAT#10006625_2019).

DOI

dx.doi.org/10.17504/protocols.io.bsknncve

Citation: Anibal Arce Medina, Isaac NÃÂºÃÂ±ez, Tamara Matute, Fernan Federici (02/21/2021). LAMP/RT-LAMP COVID positive control. https://dx.doi.org/10.17504/protocols.io.bsknncve

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

PROTOCOL CITATION

Anibal Arce Medina, Isaac Núñez, Tamara Matute, Fernan Federici 2021. LAMP/RT-LAMP COVID positive control. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bsknncve

COLLECTIONS

Low Cost LAMP and RT-LAMP

KEYWORDS

COVID, SARS-Cov-2, ssRNA, dsDNA, positive control, ReClone, in vitro transcription, Reverse Transcription

LICENSE

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

IMAGE ATTRIBUTION

Isaac Núñez. GNU General Public License v3.0.

CREATED

Feb 20, 2021

LAST MODIFIED

Feb 21, 2021

PROTOCOL INTEGER ID

47470

PARENT PROTOCOLS

Part of collection
Low Cost LAMP and RT-LAMP

MATERIALS TEXT

Materials and Tools:

- Pipettes p2, p10, p200
- Pipette tips
- PCR tubes (0.2 uL)
- rNTPS

Kits and enzymes:

- Promega Wizard® SV Gel and PCR Clean-Up System
- Qiagen RNeasy Kit
- HiScribe™ (NEB E2040S)
- PPI (NEB M0361S)
- RNasin® (Promega N2111)
- DNAse I (NEB M0303S)

Equipment:

- Thermocycler
- Centrifuge
- spin
- Nano-Spectophotometer

Citation: Anibal Arce Medina, Isaac NÃÂºÃÂ±ez, Tamara Matute, Fernan Federici (02/21/2021). LAMP/RT-LAMP COVID positive control. https://dx.doi.org/10.17504/protocols.io.bsknncve

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

N gene Amplification

1 Amplify SARS-Cov-2 Nucleocapsid (N) gene fragment dsDNA from ReClone or IDT positive control plasmid with the *ultramers* indicated in Table 1.

Name	Sequence
NT7_Fw	CGA AAT TAA TAC GAC TCA CTA TAG GGG CAA CGC GAT GAC GAT GGA TAG
T7_Nter_Rv	ACT GAT CAA AAA ACC CCT CAA GAC CCG TTT AGA GGC CCC AAG GGG TTA TGC TAG TTA GGC CTG AGT TGA GTC AG

Table 1: Primers/Ultramers used to amplify N-gene positive control

The amplification can be carried out using any standard PCR reaction formulation (e.g. Phusion DNA polymerase with HF buffer), adding **0.01 ng** to **1 ng** of the plasmid template and **1 µl** of each primer at 10 ?M concentration and following this thermocycler program:

Initial denaturation at **98 °C** *for* **00:00:30**

Followed by ***35 cycles*** *of:*

98 °C *per* **00:00:05**

57 °C *per* **00:00:15**

extension at **72 °C** *per* **00:01:00**

End by a final extension at **72 °C** *per* **00:05:00**

dsDNA positive control

2 **dsDNA purification**

Perform PCR reaction purification with any commercially available kit.
We use Promega Wizard® SV Gel and PCR Clean-Up System

Typical yields are around **25 µl** at 30 - 100 ng/?L.

3 Store sample under **-20 °C**

In case you are not making RT-LAMP positive control **go to step 10**.

ssRNA positive control

4 **In vitro transcription preparation**

In vitro transcription has to be done from previously prepared dsDNA. We do it using HiScribe™ (NEB E2040S) system plus PPI (NEB M0361S) and RNasin® (Promega N2111):

Citation: Anibal Arce Medina, Isaac NÃÂºÃÂ±ez, Tamara Matute, Fernan Federici (02/21/2021). LAMP/RT-LAMP COVID positive control. https://dx.doi.org/10.17504/protocols.io.bskkncve

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Compound	Volume (μL)
rATP	2,0
rUTP	2,0
rGTP	2,0
rCTP	2,0
Buffer 10X	2,0
T7 pol	2,0
RNAsin	0,5
PPI (NEB)	0,5
DNA (from PCR)	7,0
Total	***20,0***

Table 2: *In vitro* transcription formulation.

5 Incubate reaction at **37 °C** **Overnight**

6 Treat the reaction with DNAse I (NEB M0303S) by diluting the reaction with **70 µl** H_20 (molecular grade) and **10 µl** 10X buffer DNAse I.

7 Incubated at **37 °C** per **00:15:00** .

Heat inactivation of DNAse is not recommended because it can harm RNA.

8 Perform RNA purification with any available kit. We use Qiagen RNeasy Kit.

Typical yields are around **50 µl** at 1000ng/?L.

9 Perform aliquots and store the samples at **-80 °C**

Copy number computation and dilutions

10 In order to compute the sample **copy number concentration**, it's necessary to measure its **mass concentration** in a nano-spectrophotometer, plate reader, or estimate it by gel electrophoresis imaging.

Citation: Anibal Arce Medina, Isaac NÃÂºÃÂ±ez, Tamara Matute, Fernan Federici (02/21/2021). LAMP/RT-LAMP COVID positive control. https://dx.doi.org/10.17504/protocols.io.bsknncve

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

NanoDrop™ 3300 Fluorospectrometer
Fluorospectrometer

NanoDrop™ 3300 nd-3300

Repeat steps 8 to 10 for ssRNA each time you perform experimentation because samples and aliquots concentration is not stable even though keeping them frozen.

11 Compute the sample copy number concentration [copies/?L] by replacing the length [bp] (**Table 3**) and mass concentration [ng/?L] (C_{mass}) in the next mathematical expressions :

(1.a) $\mathrm{Z}\frac{g}{\mathrm{mol}\,dsDNA} = \text{length}\ dsDNA[bp] * 617.96\frac{g}{mol*bp} + 36.04\frac{g}{mol}$

(1.b) $\mathrm{Z}\frac{g}{\mathrm{mol}\,ssRNA} = \text{length}\ ssRNA[bp] * 321.47\frac{g}{mol*bp} + 18.02\frac{g}{mol}$

(2) $\mathrm{Y}\frac{\text{copies}}{ng} = \left(\mathrm{Z}\frac{g}{\mathrm{mol}\ ssRNA} * \frac{10^9 ng}{g}\right)^{-1} * 6,02210^{23}\frac{\text{copies}}{\mathrm{mol}\,dsDNA}$

(3) $\mathrm{X}\frac{\text{copies}}{\mu L} = C_{mass}\frac{ng}{\mu L} * \mathrm{Y}\frac{\text{copies}}{ng}$

	Length [bp]
dsDNA	1427
ssRNA	1374

Table 3: dsDNA and ssRNA fragment sizes.

use $\text{eq. } 1.\text{a}$ for dsDNA and $\text{eq. } 1.\text{b}$ fo for ssRNA.

Typilcall amounts are:
dsDNA: 10^8 - 10^9 copies/ng
ssRNA: 10^9 - 10^{10} copies/ng

Citation: Anibal Arce Medina, Isaac NÃÂºÃÂ±ez, Tamara Matute, Fernan Federici (02/21/2021). LAMP/RT-LAMP COVID positive control. https://dx.doi.org/10.17504/protocols.io.bskkncve

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

12

Make serial dilutions with molecular grade H_2O to reach the target $\frac{\text{copies}}{\mu L}$ concentration.

As positive control we typically use **1 µl** of sample at $10.000\ \frac{\text{copies}}{\mu L}$

You can store aliquots of dsDNA samples at targets concentrations at **-20 °C** or **-80 °C** .
Freezing and thawing cycles may affect the concentration then use each aliquot just one time.

Citation: Anibal Arce Medina, Isaac NÃÂºÃÂ±ez, Tamara Matute, Fernan Federici (02/21/2021). LAMP/RT-LAMP COVID positive control. https://dx.doi.org/10.17504/protocols.io.bsknncve

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited