PCR amplification of long GC-rich DNA targets

Nadia Assal[1,2], Min Lin[1,2]

[1]Ottawa Laboratory Fallowfield, Canadian Food Inspection Agency, Ottawa, Ontario, Canada;

[2]Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, Ontario, Canada

Jul 17, 2021

1 *Works for me* Share dx.doi.org/10.17504/protocols.io.bbxyippw

Nadia Assal

ABSTRACT

This protocol allows the amplification of long and high GC content genes by PCR without the need for many optimizations. In this protocol, the *Mycobacterium bovis*, a genome with more than 60% GC content, is used as a model and a source of source for a long gene (>1.5 kb) and a GC content of 77.5%. The protocol works well irrespective of the length of the primers.

DOI

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

PROTOCOL CITATION

Nadia Assal, Min Lin 2021. PCR amplification of long GC-rich DNA targets. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bbxyippw

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

Nadia Assal, Min Lin,
PCR procedures to amplify GC-rich DNA sequences of Mycobacterium bovis,
Journal of Microbiological Methods,
Volume 181,
2021,
106121,
ISSN 0167-7012,
https://doi.org/10.1016/j.mimet.2020.106121.
(https://www.sciencedirect.com/science/article/pii/S016770122030837X)
Abstract: Amplification of high GC content genes by PCR is a major challenge during the creation of recombinant GC-rich DNA constructs. This may be due to the difficulty in DNA denaturation or the possibility of forming secondary structures from DNA templates. Tools have been described to address the technical problems associated with the amplification of shorter sequences (<1000 bp). However, obstacles of synthesizing larger-sized GC-rich sequences by PCR continue to exist. This study aims to investigate the amplification of long and high GC content genes by PCR from the Mycobacterium bovis, a genome with GC content >60%, in comparison to amplifying a gene from the Listeria monocytogenes genome, a genome with a 37.8% GC content. Three PCR protocols were designed and experimented at various conditions with two M. bovis genes, Mb0129, a large gene of 1794 bp with 77.5% GC content, mpb83, a smaller gene of 663 bp in length with moderate GC content of 63%, together with LMHCC_RS00060, a large L. monocytogenes gene of 1617 bp with a lower GC content of 41.5%. The result demonstrated the superiority of the 2-step PCR protocol over other protocols in PCR amplification of Mb0129 when specific high fidelity DNA polymerases were used in the presence of an enhancer. The study highlighted the importance of manipulating the cycling conditions to perform the annealing and extension steps at higher temperatures while adjusting the ramp speed at a lower speed for a successful PCR amplification of a large GC-rich DNA template. A final PCR protocol was developed and enabled the amplification of 51 GC-rich targets. This can be a valuable tool for the amplification of long GC-rich DNA sequences for various downstream applications.
Keywords: PCR amplification; Long GC-rich genes; Mycobacterium bovis genes; Optimization; Polymerase

KEYWORDS

long GC-rich, DNA targets, PCR ampification, optimization, Mycobacterial genes

CREATED

Jan 29, 2020

LAST MODIFIED

Jul 17, 2021

PROTOCOL INTEGER ID

32472

GUIDELINES

Thorough cleaning of the work station and the hood using 70% ethanol. Work in the biological safety cabinet.

MATERIALS TEXT

MATERIALS

QIAgen DNeasy Blood and Tissue Kit, 50 rxn **Qiagen Catalog #69504** Step 1

PrimeSTAR GXL DNA Polymerase **Contributed by users Catalog #R050A** Step 2

DMSO **Contributed by users**

BEFORE STARTING

Always work using a lab coat and gloves. Use filtered tips.

1 Extract *M. bovis* genomic DNA using QIAgen DNeasy Blood and Tissue Kit, 50 rxn **Qiagen Catalog #69504** according to the manufacturer's instructions for Gram-positive bacteria.
Use 5 ng/µl of genomic DNA was used in each reaction

2 Prepare the master mix for the polymerase enzyme PrimeSTAR GXL DNA Polymerase **Contributed by users Catalog #R050A** as follows:

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Component	volume in µl
Milliq Water	29.5
Buffer	10
DNTPs (2.5mM)	4
DMSO	2.5
Primers Mix (Forward +Reverse) 25 µM	2
Genomic DNA(5ng/ul)	1
Polymerase	1
Total	**50**

Prepare your mix on ice. Always vortex and centrifuge all reagents before use. Add the enzyme last. Vortex and mix your reaction tubes. Make sure there are no bubbles.

3 Adjust the thermal cycler as follows:

Use a 2 Step Protocol:
35-40 cycles of (Denaturation at 98 °C for 15 seconds then Annealing and extension at 68 °C for 1min / kb)
the reaction is then kept on hold at 4 °C

Adjust the speed of the thermal cycler heating and cooling ramp speed to 2 °C/seconds.

Load the thermal cycler with your tubes. Start your program

4 After finishing your program, run your reactions on agarose gel electrophoresis.

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