SARS PCR/Sequencing Primers

Rachel Graham (rlgraham@ad.unc.edu)
ORFeome

Method Article

Keywords: SARS-CoV, sequencing, genome analysis

Posted Date: July 10th, 2014

DOI: https://doi.org/10.1038/protex.2014.021

Abstract

This protocol details the primers and conditions used for forward and reverse PCR amplification and sequencing of SARS-CoV genomes.

Introduction

This protocol details the steps, reagents, and conditions required to sequence SARS-CoV genomes in the forward and reverse directions. The protocol begins with the RT-PCR step, assuming that SARS-CoV RNA purified using a standard procedure \(i.e., TRIzol extraction) has already been performed.

Reagents

Invitrogen SuperScript III kit dNTPS \(10 mM) Random Hexamers RNasin \(if desired) Thermo Phusion PCR enzyme Primers \(2 mM stock – see Tables 1 and 2) Agarose 1X TAE Buffer Ethidium Bromide

Equipment

70ºC water bath 55ºC water bath Thermal cycler

Procedure

Reverse Transcription: 1. In a 1.5-µL Eppendorf tube, add 1-4 µL RNA and 1 µL Random Hexamers. 2. Incubate at 70ºC for 5 min. 3. Place reaction briefly on ice and assemble the reverse transcription reaction using the kit-supplied reagents \(a master mix can be made if multiple reactions are being run): 4 µL 5X First-Strand Buffer 2 µL DTT 1 µL SuperScript III reverse transcriptase 1 µL dNTPs 1 µL RNasin \(if desired) x µL H2O to 20 µL 3. Incubate at 55ºC for 45 min to 1 h. 4. Inactivate the reverse transcriptase at 70ºC for 15 min. Place reaction on ice after inactivation. 5. Proceed with PCR setup. PCR \(with Phusion PCR kit): 1. Assemble PCR reactions to generate amplicons according to those detailed in Table 2 \(for whole-genome sequencing) or with any combination of forward and reverse primers from Table 1. 2. PCR reaction setup: 2 µL First-strand template 1 µL Forward Primer 1 µL Reverse Primer 5 µL 10X HF Buffer 1 µL dNTPs 0.5 µL Phusion polymerase x µL H2O to 50 µL 3. PCR reactions are run under standard PCR conditions: 98ºC 5 min 35 cycles of: 98ºC 15 sec xºC for 30 sec* 72ºC for ~45 sec/kb 72ºC 10 min 8ºC Hold * Annealing temperature is primer-dependent, but for most SARS-CoV primers in Table 1, annealing temperatures 52-55ºC will work. Confirmation of PCR products and sequencing: 1. Run PCR products \(5 µL/reaction) on a 0.8% agarose/1X TAE gel to verify PCR success. 2. Purify PCR products with PCR purification kit of choice \(the Qiagen PCR Purification Kit works well). 3. PCR products can be diluted to 150-200 µL/reaction to ensure that enough product is present for assembling sequencing reactions. 4. Assemble sequencing reactions according to the primer/amplicon combinations outlined in Table 2.

Timing

Reverse transcription: 1-1.5 h PCR: 2-4 h Sequencing: facility-dependent

Anticipated Results

Primers have been designed to give clean, single-band PCR products. If multiple bands are detected, alternate annealing temperatures may be required. It may also be possible that alternate bands indicate multiple genome patterns at that locus.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplement0.doc
- supplement0.doc