Australian Government
Department of Health and Aged Care
Therapeutic Goods Administration

Laboratories Branch

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Owner: s22	**Approver:** s22
Active: 17/10/2023	**Review:** 17/04/2025
Title: Method - rDNA quantitation in Moderna Vaccines by qPCR	

Residual DNA Quantitation in Moderna mRNA Vaccines by qPCR.

Purpose

This document describes the method used for quantitating residual DNA in Spikevax mRNA vaccines by qPCR. The majority of the method is copied verbatim from an in-process control method provided under commercial in confidence arrangements and should not be distributed beyond the HPRG.

Scope

This method is applicable to all presentations of Moderna Spikevax released prior to October 2023. Before extension of the method to test future presentations, the sponsor's in process control test method must be evaluated to confirm that the molecular weight of the linearised plasmid reference material has not changed from that used in the original test method and subsequent method verification studies (D23-3732273)

Responsibility

This method governs the appropriate application of rDNA testing methods by PCR Unit members of the Laboratories Branch Biotherapeutics Section. Ongoing maintenance of this method document is the responsibility of the PCR Unit Manager and Team Leader

Procedure/Policy

The method here presented is an implementation of Moderna SOP-1020, with minor modifications to the method accommodate our laboratory equipment and reagents.

The primary means in which our implementation differs from SOP-1020 is in the use of a s47 reference material for the construction of quantitative standard curves. Further minor differences arise in the experimental qPCR templates used for our qPCR systems and in the way in which the data is processed as described here.

Details of standard curve dilutions using our ssDNA reference material are detailed prior to a verbatim reproduction of the original SOP-1020, along with the unmodified sample dilution and reaction mixture preparation details which are reiterated for the reader's convenience.

s47

Synthetic ssDNA reference material

SOP-1020 has been verified for use with an alternate reference material which is a synthetic ssDNA oligonucleotide bearing the sequence that is targeted by amplification with the primers and probe.
The genetic sequence of this reference material is:

s47 [redacted]

The yield of oligonucleotide is determined spectrophotometrically at the time of synthesis and is expressed in µg and nmol units. This reference material is resuspended according to the manufacturer's instructions to make a s47 [redacted] master stock dilution, which is retained at -20°C.

From this master stock dilution, working aliquots are prepared by serial s47 [redacted] dilution to form the following concentrations. These concentrations have been determined so that the standard curve reactions prepared during testing will have a number of gene target copies equivalent to the number of gene target copies present in standard curve reactions prepared with s47 [redacted] as per SOP-1020.

s47 [redacted]

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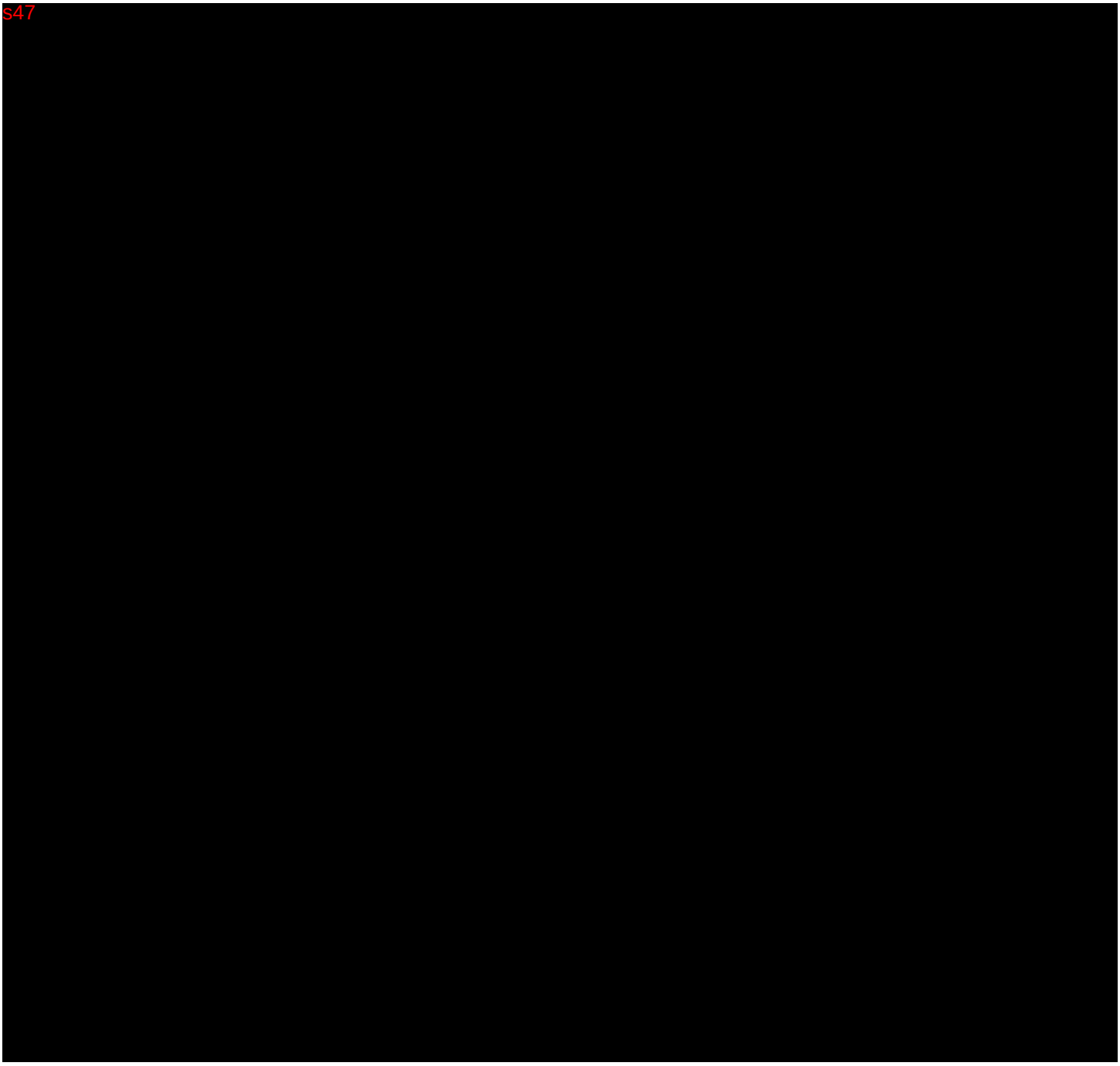

Original In-Process Control Method – Moderna SOP-1020

The following is unmodified presentation of the original Moderna SOP-1020, which was provided under commercial in confidence arrangements and is not to be distributed beyond the HPRG.

1.0 PURPOSE

The purpose of this procedure is to detect and quantify residual plasmid DNA in mRNA Drug Substance (DS) or mRNA Product intermediate (MPI) using a real time quantitative PCR (qPCR) assay designed to amplify the kanamycin resistance gene in the plasmid.

2.0 SCOPE

This procedure applies to detection of the residual plasmid DNA in mRNA DS or MPI samples for validated constructs.

3.0 REFERENCED DOCUMENTS

Document #	Title
FRM-0736	Assay Performance Worksheet: SOP-1020 Determination of Residual DNA by qPCR
FRM-0795	SOP-1020 Residual DNA Calculation Sheet
SOP-0017	Maintaining a RNase Free Work Environment
SOP-0004	Operation and Maintenance of Thermo Class II A2 1300 Series Biological Safety Cabinets (BSC)
SOP-0033	Out of Specification (OOS)
SOP-0081	Preparation of Solutions and Samples in the GMP-Quality Control Laboratory
SOP-0082	Data Review and Reporting in the GMP Quality Control Laboratory
SOP-0210	Assignment of Assay Reference Numbers and use of QC Assay Performance Worksheets
SOP-0409	Quality Control Invalid Assay Procedure
SOP-0451	Operation and Maintenance of the QuantStudioTM 7 Flex Real-Time PCR System
SOP-0452	Personnel Flow and Gowning in the QC Bioassay Laboratories
SOP-0465	Use of the Eppendorf 5424 Microcentrifuge and the Eppendorf 5810R Centrifuge

4.0 RESPONSIBILITIES

Department/ Functional Area	Responsibilities
Quality Control Laboratory Personnel	• Following all procedures outlined in this document, as applicable. • Maintaining a RNase-Free work environment per **SOP-0017.** • Following proper safety measures in the GMP laboratory. • Documenting sample information and preparation in the appropriate laboratory notebook or QC controlled document
Quality Control Manager or Designee	• Ensuring that laboratory personnel are trained in this procedure. • Ensuring that all procedures in this document are followed when applicable. • Ensure that this procedure is revised as necessary • Data Review

5.0 DEFINITIONS

Term	Definition
ABI	Applied Biosystems Instruments
C_T	The PCR cycle at which an increase in reporter fluorescence above the baseline signal can first be detected
°C	Degrees Celsius
DS	Drug Substance
DNA	Deoxyribonucleic acid
FAM	Fluorescein
GMP	Good Manufacturing Practices
IPA	Isopropyl Alcohol
MPI	mRNA Product Intermediate
MW	Molecular Weight
mL	Milliliters
mM	Millimolar
ng	Nanograms
NTC	No Template Control
PPE	Personal Protective Equipment
qPCR	Quantitative Polymerase Chain Reaction
QC	Quality Control
R^2	Coefficient of Determination (square of correlation coefficient (R))
SDM	Second Derivative Maximum
TAM	Tetramethylrhodamine
µg	Micrograms
µL	Microliters

6.0 MATERIALS

NOTE: Alternative vendors or part numbers may be used, provided the reagent grade or classification is maintained.

6.1. Reagents

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[redacted]

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[redacted]

6.2. Consumables

Item	Vendor	Catalog#
Adhesive PCR film	Abgene	AB-0558
MicroAmp™ 8-Tube Strip with Attached Domed Caps (or equivalent)	Thermo Scientific™	A30589
Applied Biosystems™ MicroAmp™ Optical Adhesive Film	Thermo Scientific™	43-119-71
Applied Biosystems™ MicroAmp™ Optical 96-Well Reaction Plate	Thermo Scientific™	14-224-001
1.5 mL Microcentrifuge tubes	SealRite	1615-5510
15 mL Corning™ Polypropylene Centrifuge Tubes	Thermo Scientific™	05-538-53F
5 mL Falcon™ Serological Pipets	Thermo Scientific™	13-675-48
10 µL pipette tips	Thermo Fisher™	022491270
20 µL pipette tips	Thermo Fisher™	022491296
200 µL pipette tips	Thermo Fisher™	022491211
1000 µL pipette tips	Thermo Fisher™	022491253
Lab Armor™ Bath Beads	Thermo Scientific™	10-876-002

6.3. Equipment

Item	Vendor	Model #
Micropipettes, Multichannel Pipettes	Various (see above)	P2, P10, P20, P200, P1000
Thermo Scientific™ S1 Pipet Fillers	Thermo Scientific™	14-387-165
QuantStudio 7	Thermo Fisher™	CON00048
Biosafety Cabinets	Thermo Fisher™	1323
Microcentrifuge	Eppendorf	5424
5810R Centrifuge	Thermo Fisher™	14100143
Mini vortex	Thermo Fisher™	14955151

7.0 SAFETY

7.1. Wear proper PPE (lab coat, gloves, safety glasses). Use Moderna Safety Manual as a reference. Follow all safety information provided on material SDSs.

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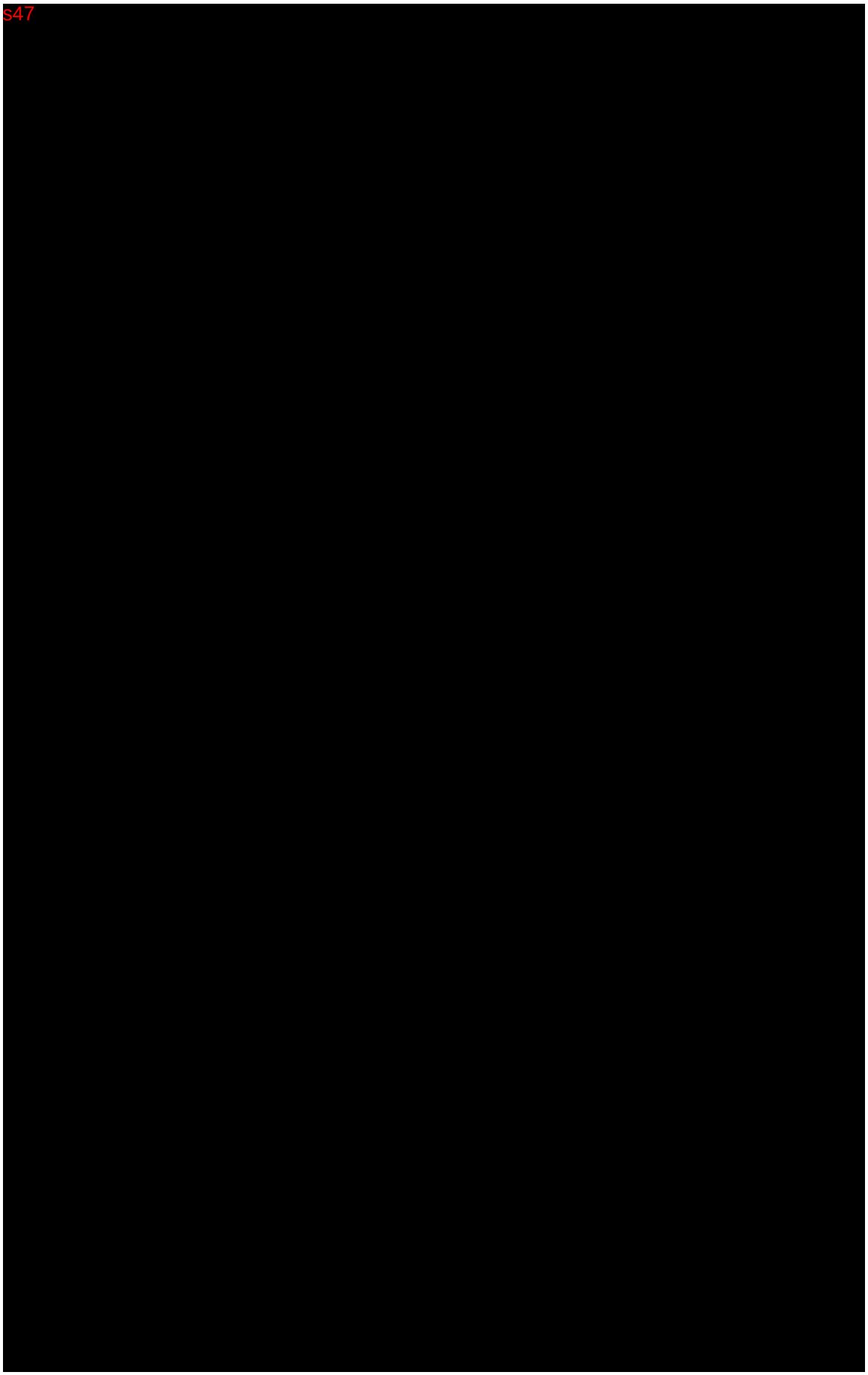

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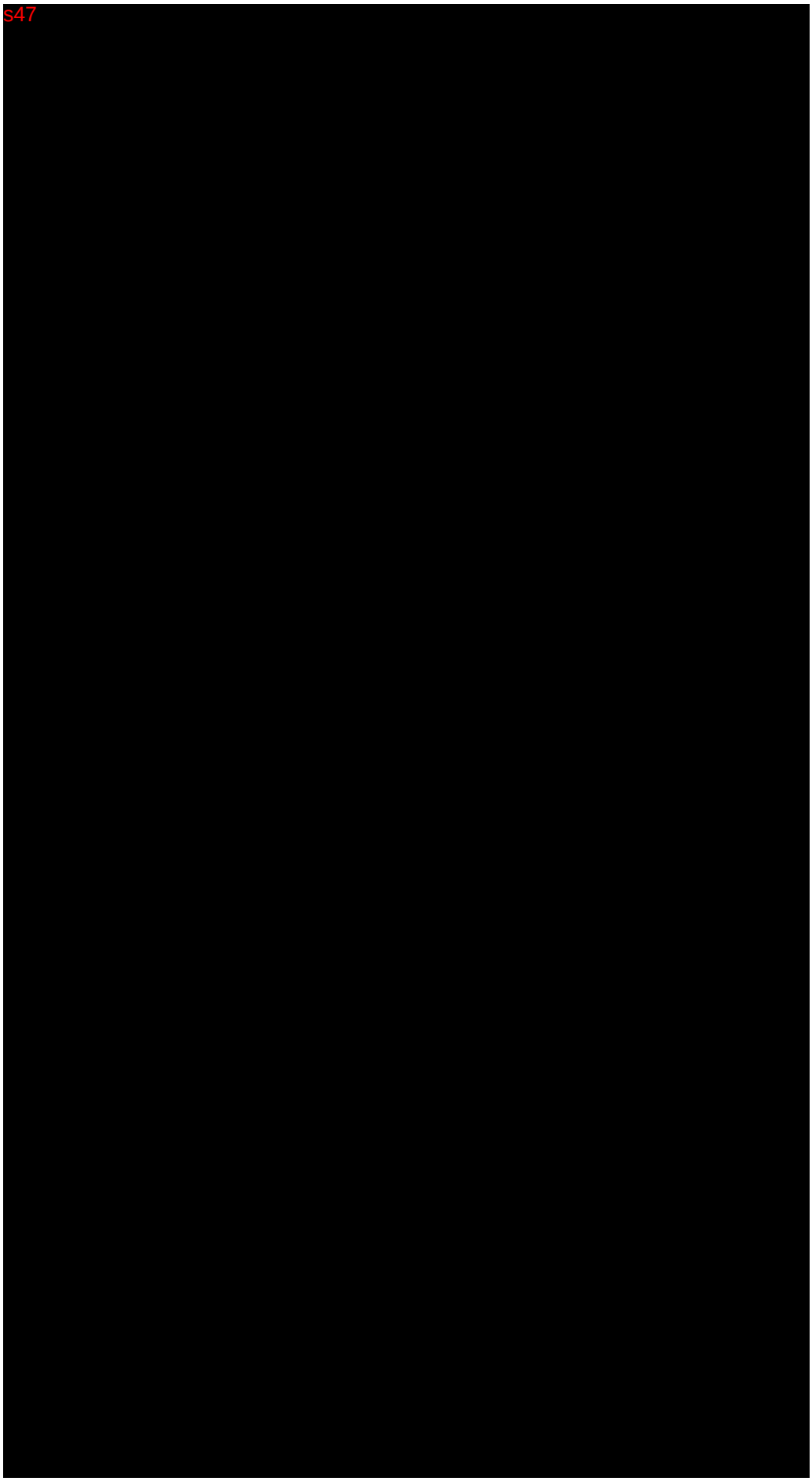

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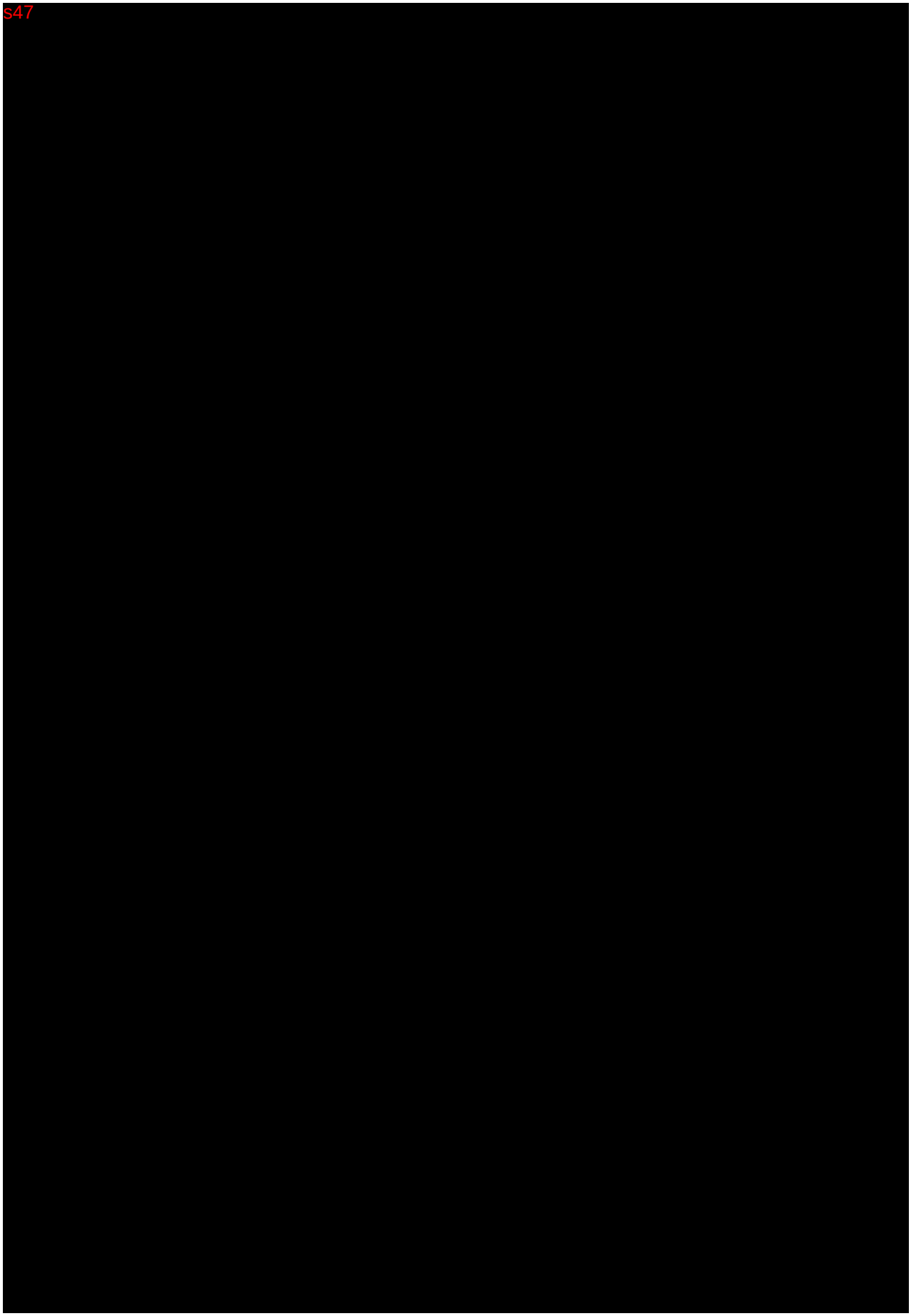

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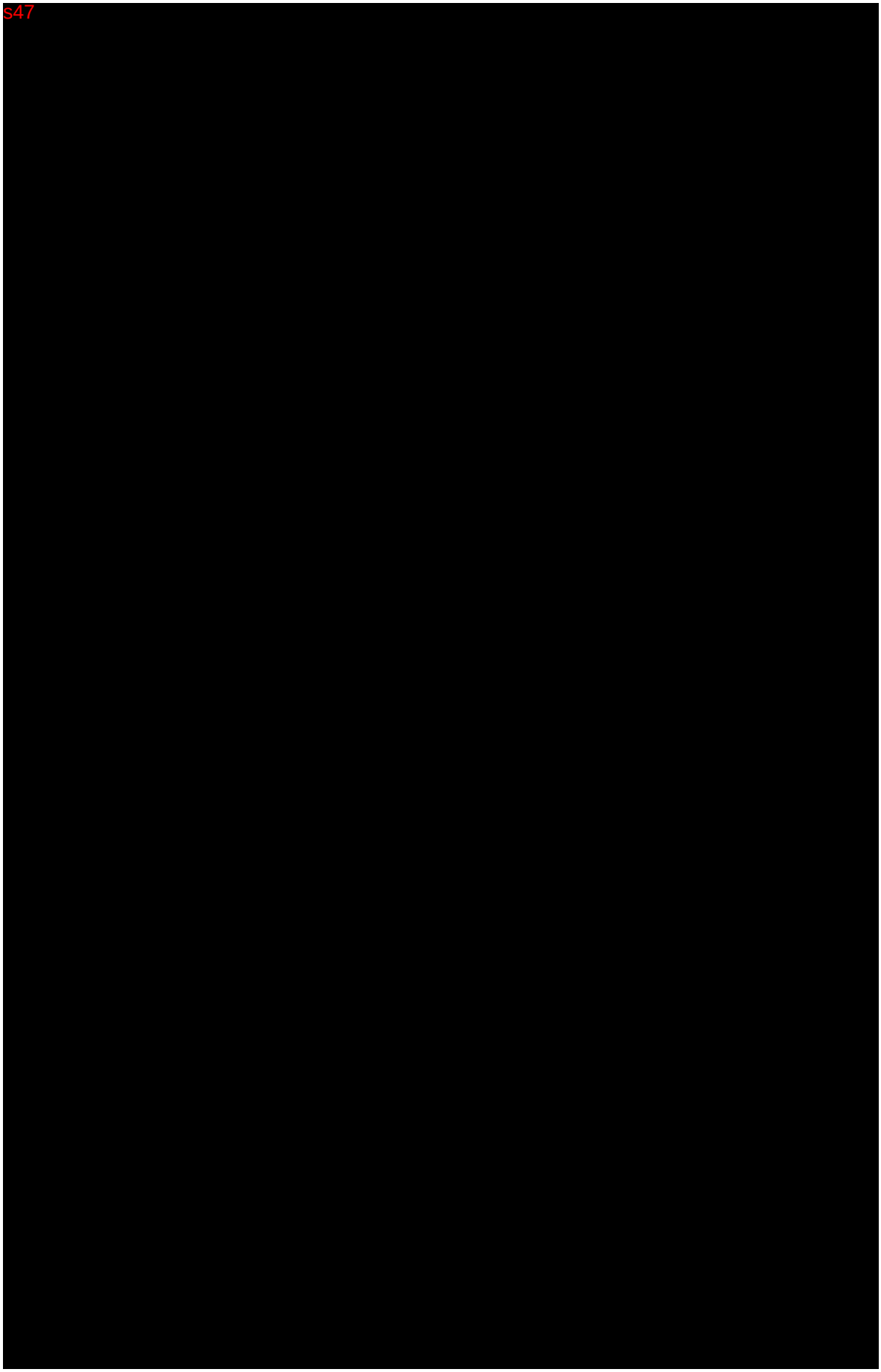

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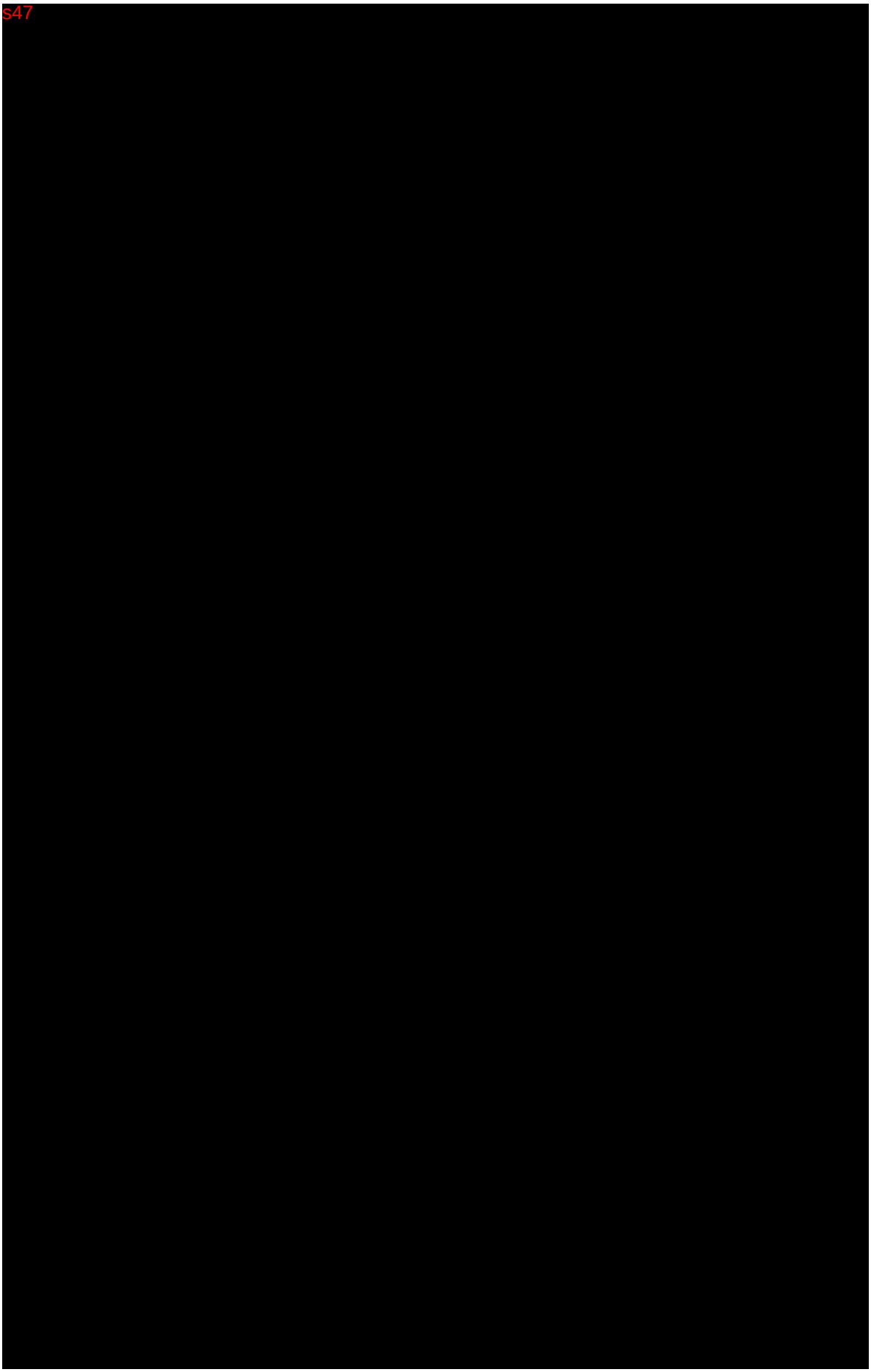

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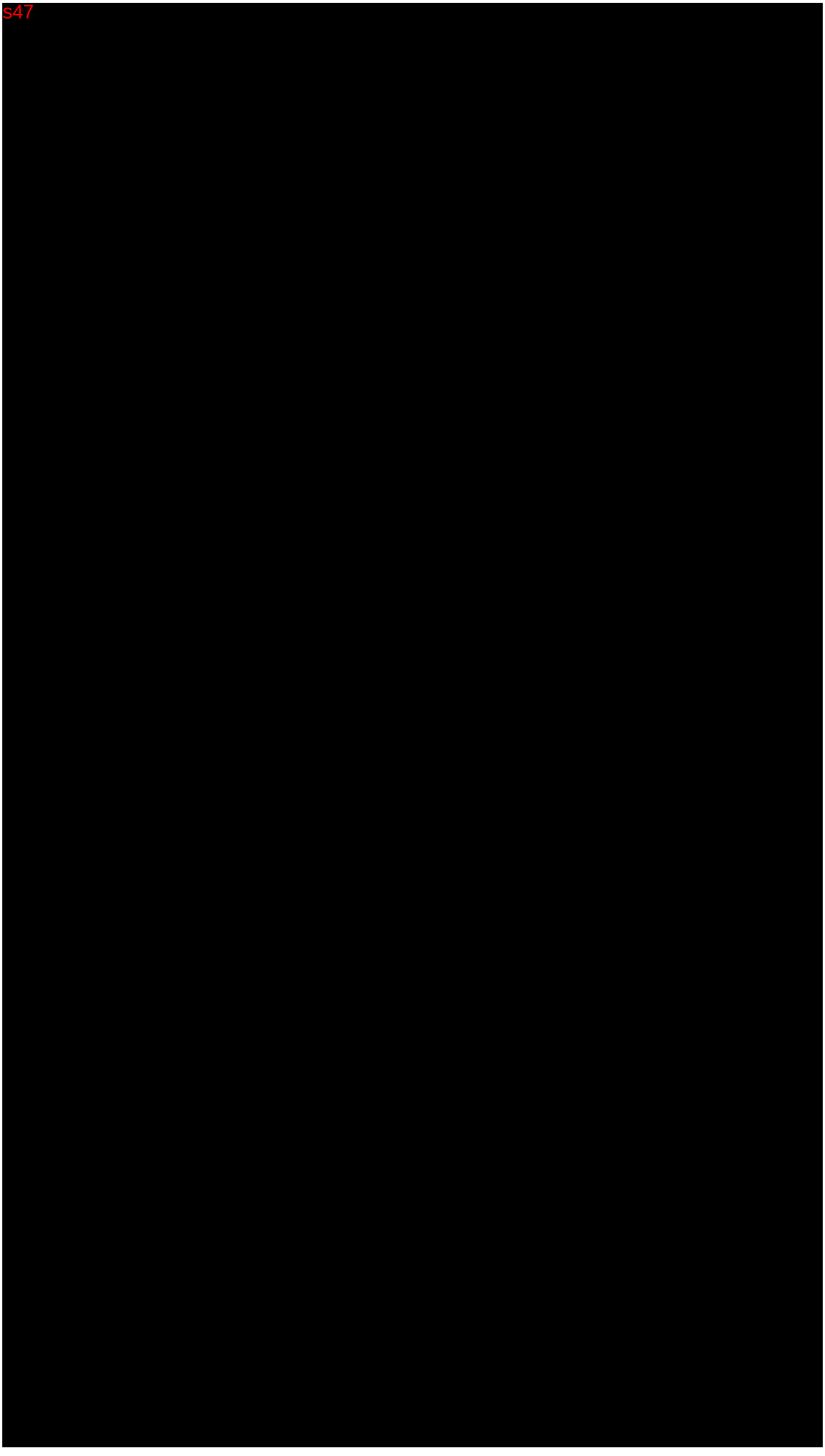

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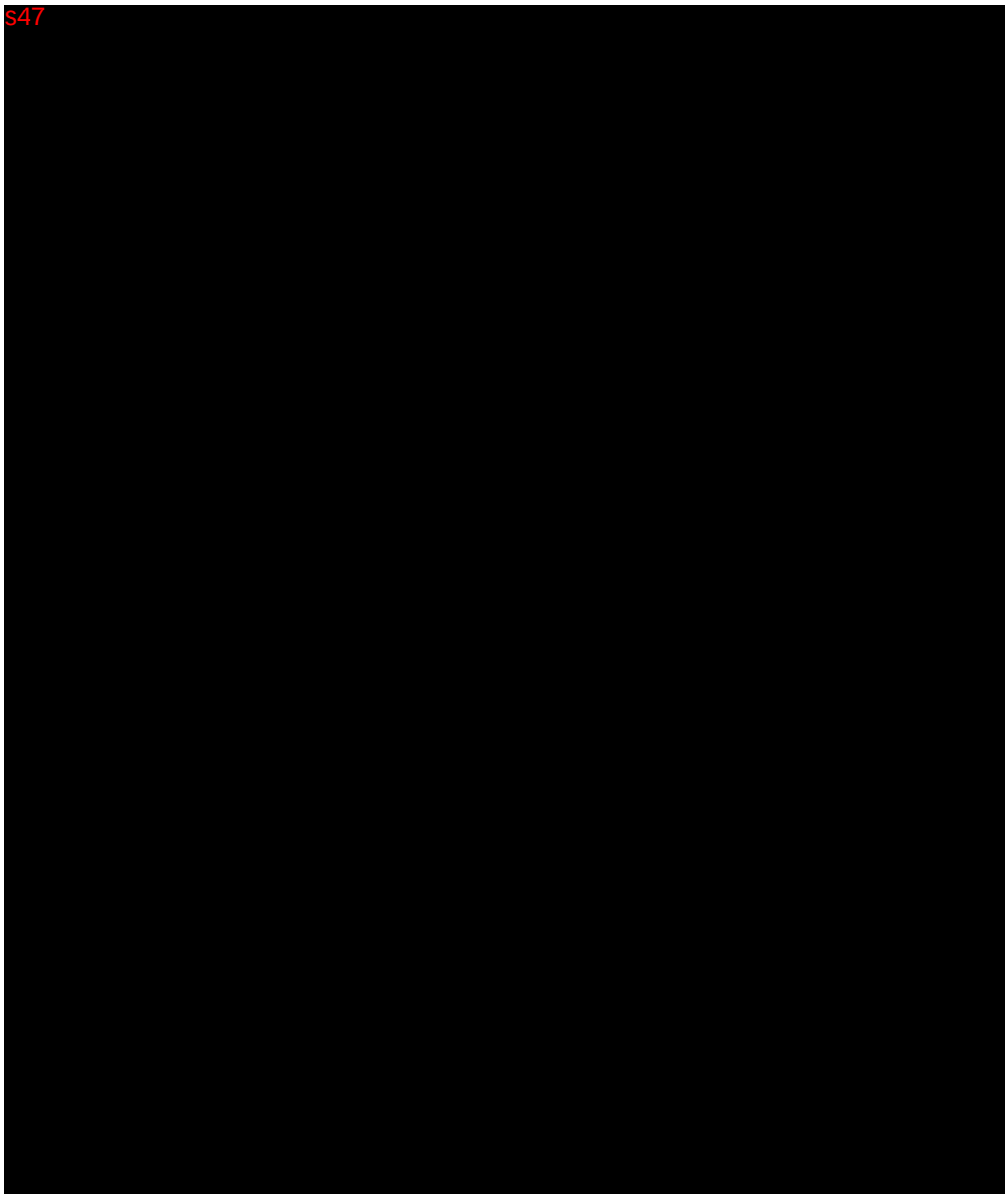

Associated Documents

Bio-PCR-Form-15	Worksheet - rDNA Quantitation in Moderna mRNA Vaccines by qPCR