Australian Government
Department of Health and Aged Care
Therapeutic Goods Administration

Laboratories Branch

Type: Biotherapeutics\PCR\Methods	**Number:** Bio-PCR-Method-16 / **Version:** 1
Owner: s22	**Approver:** s22
Active: 17/10/2023	**Review:** 17/04/2025
Title: Method - rDNA quantitation in Pfizer mRNA Vaccines by qPCR	

Residual DNA Quantitation in Pfizer mRNA Vaccines by qPCR.

Purpose

This document describes the method used for quantitating residual DNA in Comirnaty 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 Pfizer Comirnaty 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 Pfizer in-process control test "Quantitative Polymerase Chain Reaction" as provided in section 3.2.S.4.2 of the Comirnaty dossier, with minor modifications to the method accommodate our laboratory equipment and reagents.

The primary means in which our implementation differs from the original method is in the use of a synthetic oligonucleotide ssDNA 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 method, along with the unmodified sample dilution and reaction mixture preparation details which are reiterated for the reader's convenience.

This method has been verified for use with an alternate reference material which is a synthetic ssDNA oligonucleotide bearing the sequence that is targeted for qPCR

amplification with the assay primers.

The genetic sequence of this reference material is:

s47

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 master stock dilution, which is retained at -20°C.

From this master stock dilution, working aliquots are prepared by serial s47 dilution to form the concentrations as provided in the table below. s47

Reaction mixture preparation

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s47	s47
s47	s47
s47	s47

Test sample preparation

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Standard curve preparation

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Calculation and reporting

As per the method below, but including calculation of the per dose amount of rDNA (ng/dose) as determined from the dosage of the presentation and the concentration of rDNA in ng/mL.

Original In-Process Control Method – Comirnaty Analytical Method

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.

3.2.S.4.2. QUANTITATIVE POLYMERASE CHAIN REACTION (qPCR)

3.2.S.4.2.1. Principle and Scope

The purpose of this analytical procedure is to quantify the residual DNA template in BNT162b2 drug substance (DS) samples.

The level of residual DNA template in test samples is determined by quantitative polymerase chain reaction (qPCR) using fluorescence technology. A qPCR master mix containing target specific primers and fluorescent qPCR quantitation reagent is added to all the sample wells. Samples are prepared in a series of dilutions and are analyzed by qPCR. During extension, primers anneal and PCR product is generated. When polymerization is complete, the fluorescent dye binds to the double-stranded product, resulting in an increase in fluorescence. The fluorescence signal is proportional to the amount of PCR product. The quantitation of DNA is performed during the exponential phase of the reaction at a cycle threshold (Ct) where amplification of a target sequence is first detected above the established signal threshold. This Ct point is dependent on the amount of DNA originally present in the sample. The concentration of DNA in the test sample is interpolated from the linear regression of the standard curve, taking into account the dilution factor.

3.2.S.4.2.2. Apparatus and Equipment

The apparatus and equipment are provided in Table 3.2.S.4.2-1.

Table 3.2.S.4.2-1. Apparatus and Equipment

Real time PCR system
96 well optical reaction PCR plates
Microcentrifuge tubes, DNA LoBind
15 mL and 50 mL nonpyrogenic polystyrene or PET tubes

3.2.S.4.2.3. Reagents and Standards

The reagents and standards are provided in Table 3.2.S.4.2-2 and are of sufficient quality as to be suitable for this analytical procedure.

Table 3.2.S.4.2-2. Reagents and Standards[a]

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3.2.S.4.2.4. Sample Preparation

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3.2.S.4.2.5. Standard and Control Preparation

3.2.S.4.2.5.1. Blank and No-Template Control (NTC)

If blank is included in the assay, the assay diluent is used as blank.

Water (RNase/DNase free) is used as the no-template control (NTC) for the PCR plate. The same lot of water is used to prepare master mix.

3.2.S.4.2.5.2. Standard Curve Preparation

A qualified lot of linearized plasmid standard is used to generate the standard curve. An intermediate dilution of a qualified lot of linearized plasmid standard is prepared at a concentration of approximately s47 . From this intermediate dilution, a standard curve is prepared by serial dilution: s47

3.2.S.4.2.6. Procedure

3.2.S.4.2.6.1. Preparing the PCR Plate

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3.2.S.4.2.6.2. PCR Analysis

Typical instrument operating parameters are shown in Table 3.2.S.4.2-3. s47 [redacted]

Table 3.2.S.4.2-3. Instrument Operating Parameters

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s47	s47
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3.2.S.4.2.7. System Suitability, Assay, and Sample Acceptance

s47 [redacted]

Table 3.2.S.4.2-4. System Suitability, Assay, and Sample Acceptance

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s47

3.2.S.4.2.8. Calculations

The slope efficiency (%) of the standard curve is calculated using the formula below:

$$\text{Slope Efficiency (\%)} = [10^{-1/\text{slope}} - 1] \times 100$$

s47 [redacted]

s47 [redacted]

s47 [redacted]

s47 [redacted]

s47 [redacted]

s47 [redacted]

s47 [redacted]

s47 [redacted]

3.2.S.4.2.9. Data Reporting

Provided the system suitability, assay, and sample acceptance criteria are met, the content of residual DNA template in the TS is reported

Associated Documents

Bio-PCR-Form-16	Worksheet – rDNA quantitation in Pfizer mRNA vaccines by qPCR

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Type: Biotherapeutics\PCR\Methods	**Number:** Bio-PCR-Method-15 / **Version:** 1
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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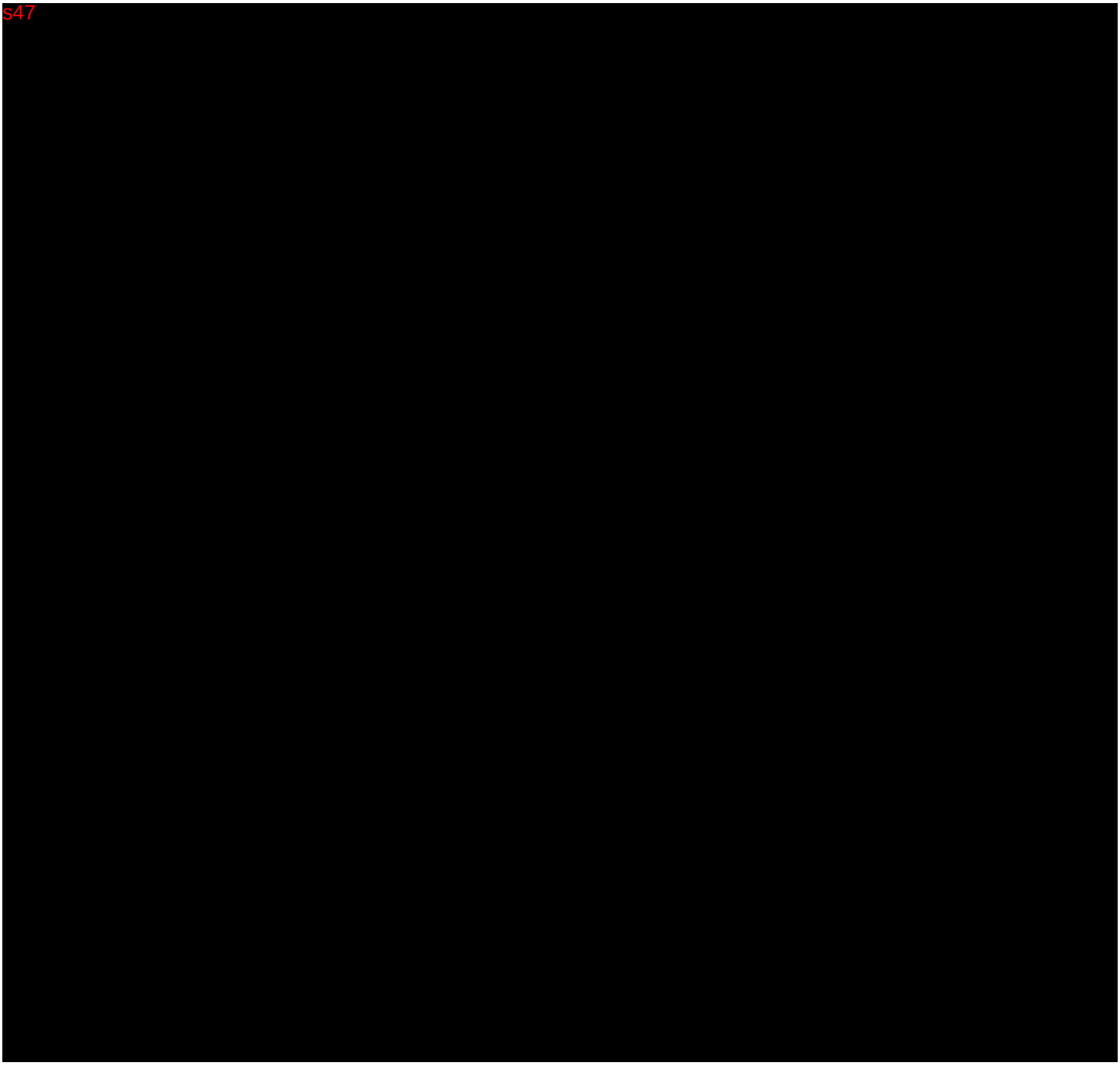

Original In-Process Control Method – Moderna SOP-1020

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.

s47 [redacted]

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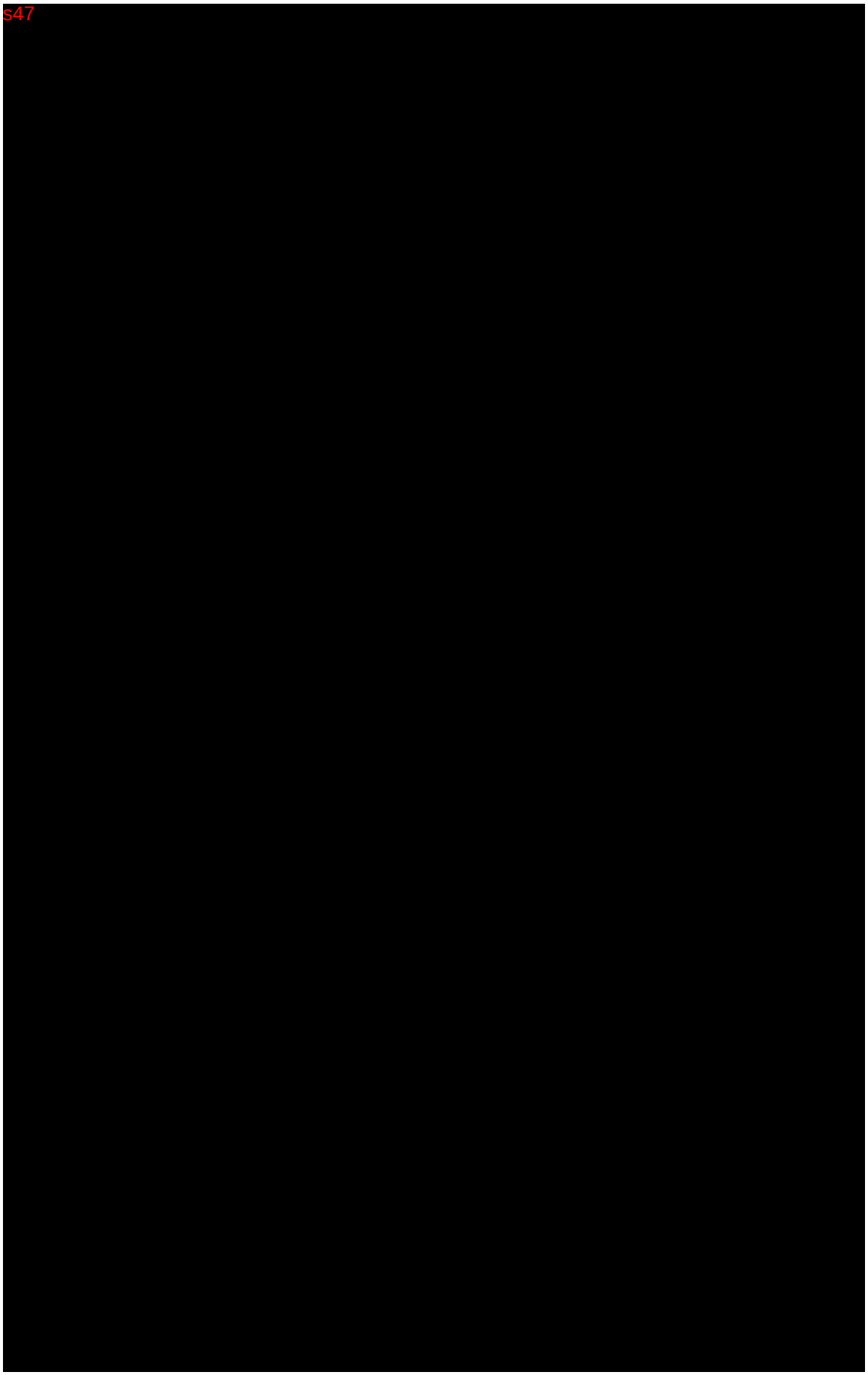

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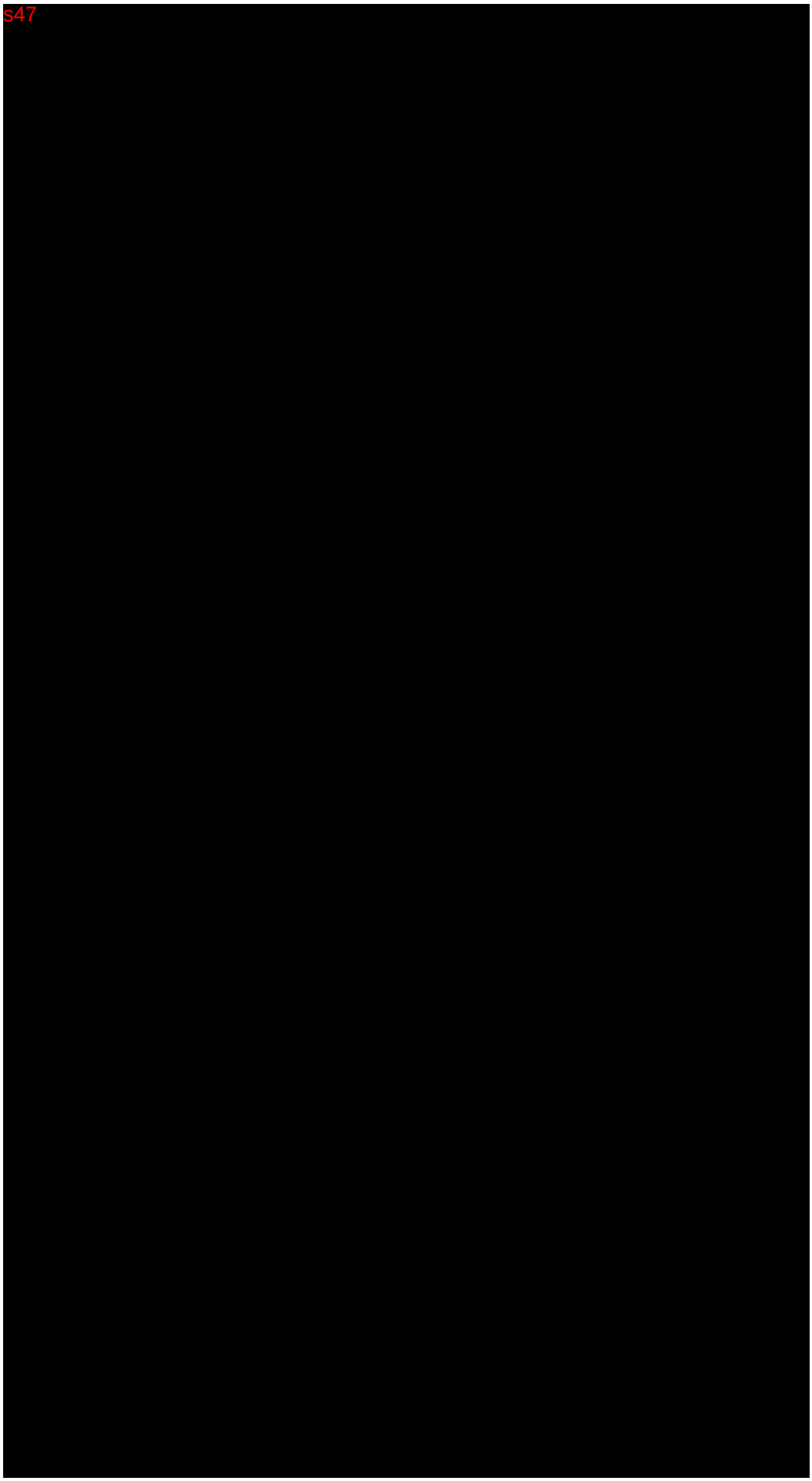

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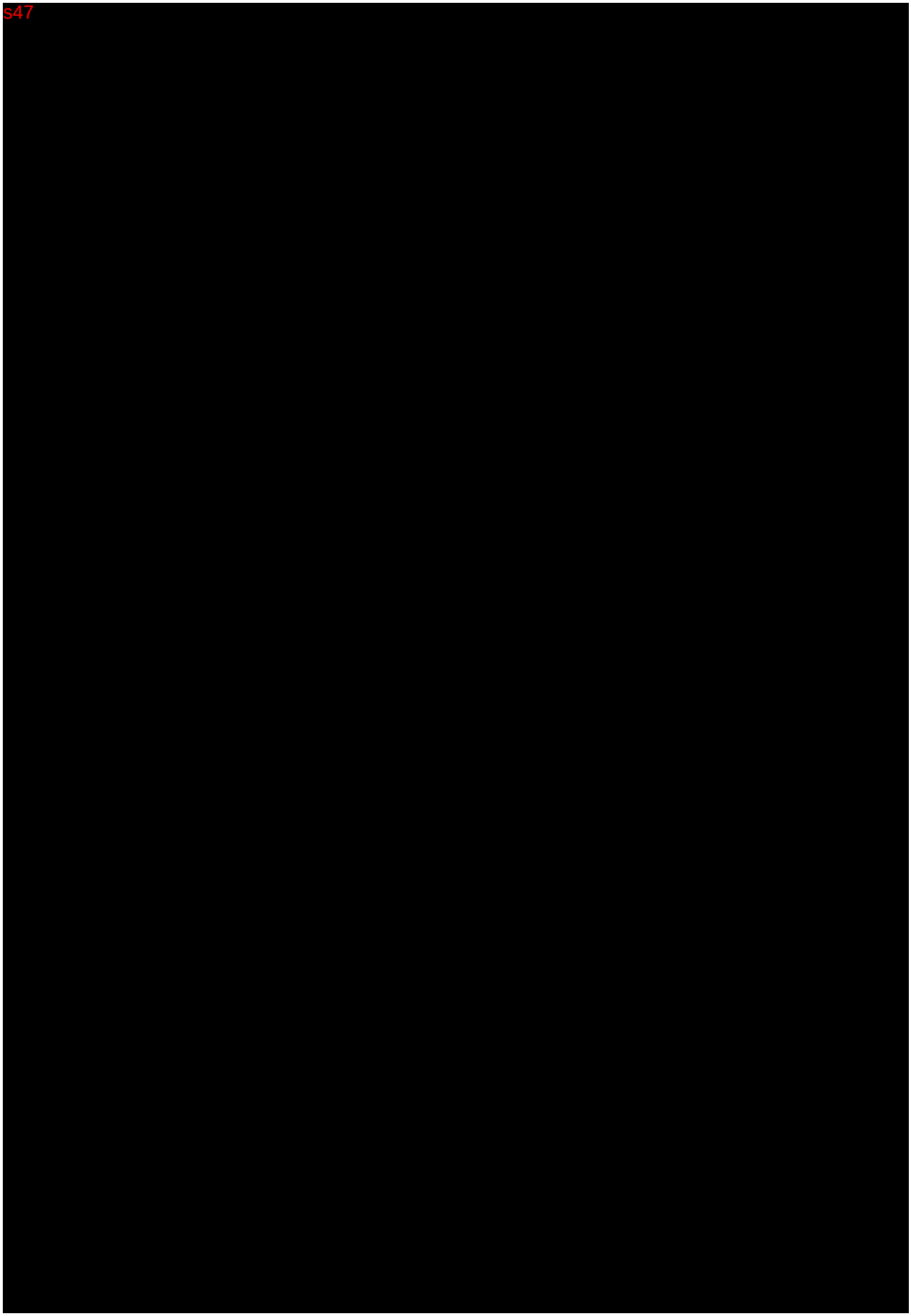

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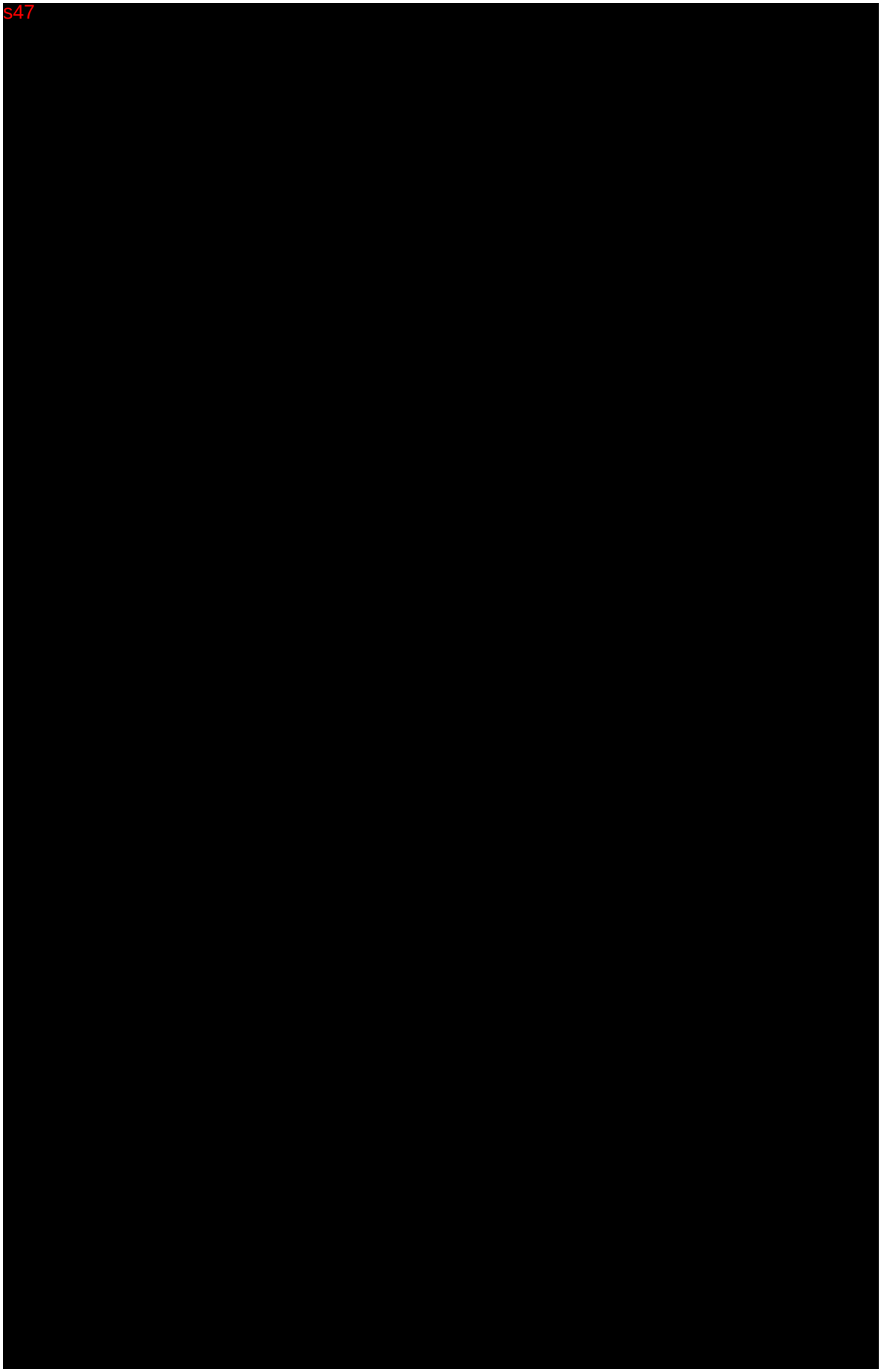

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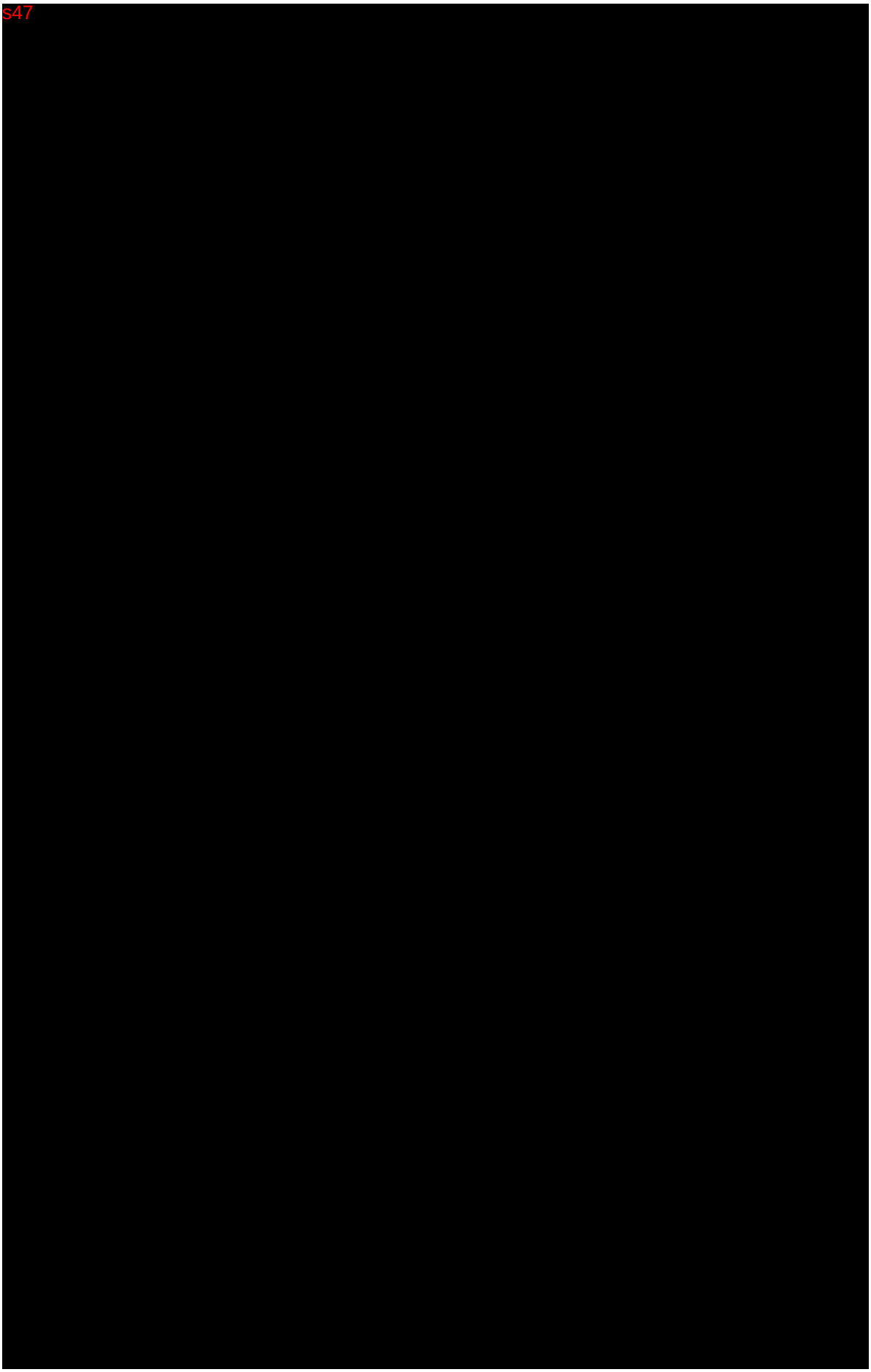

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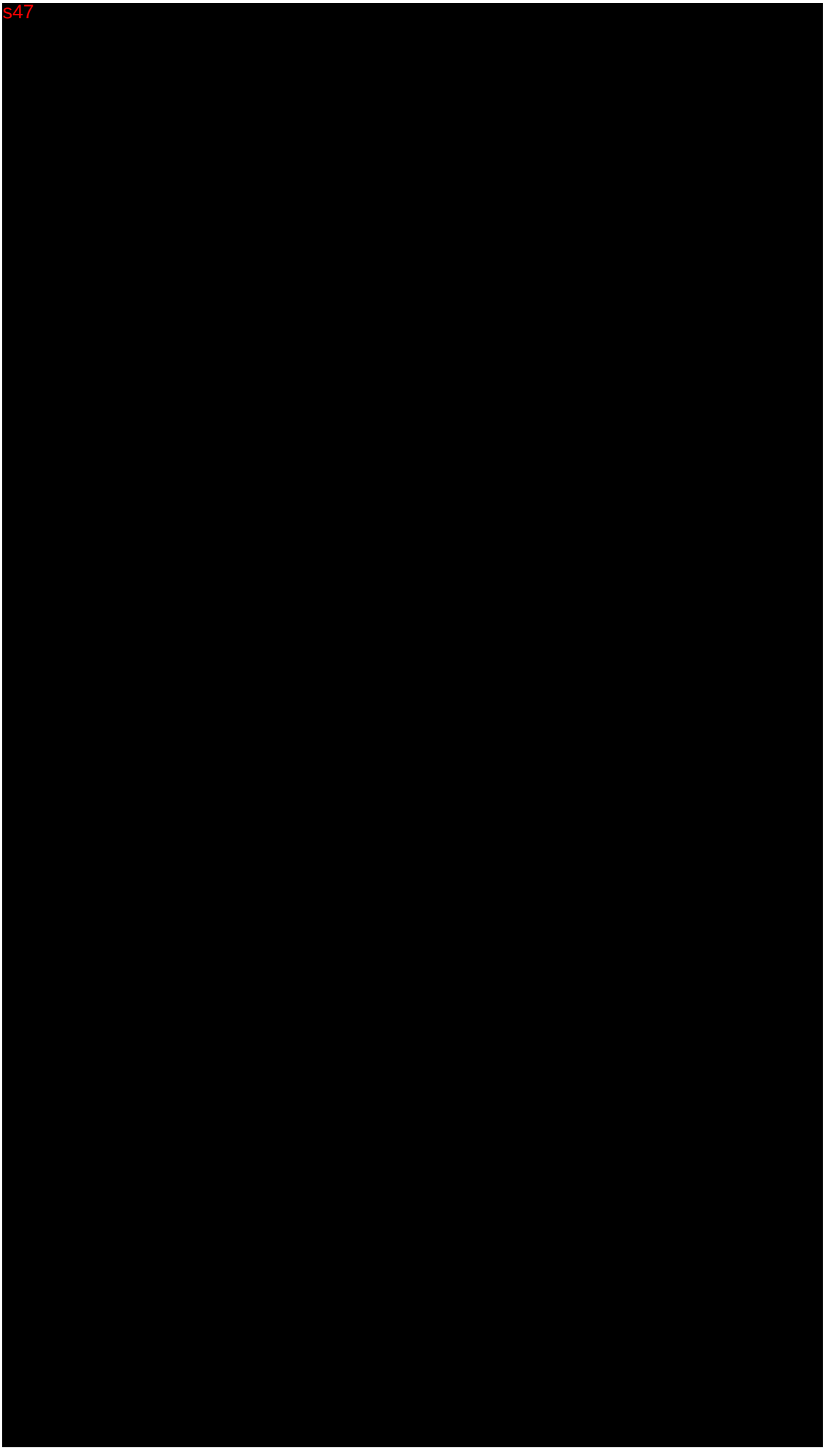

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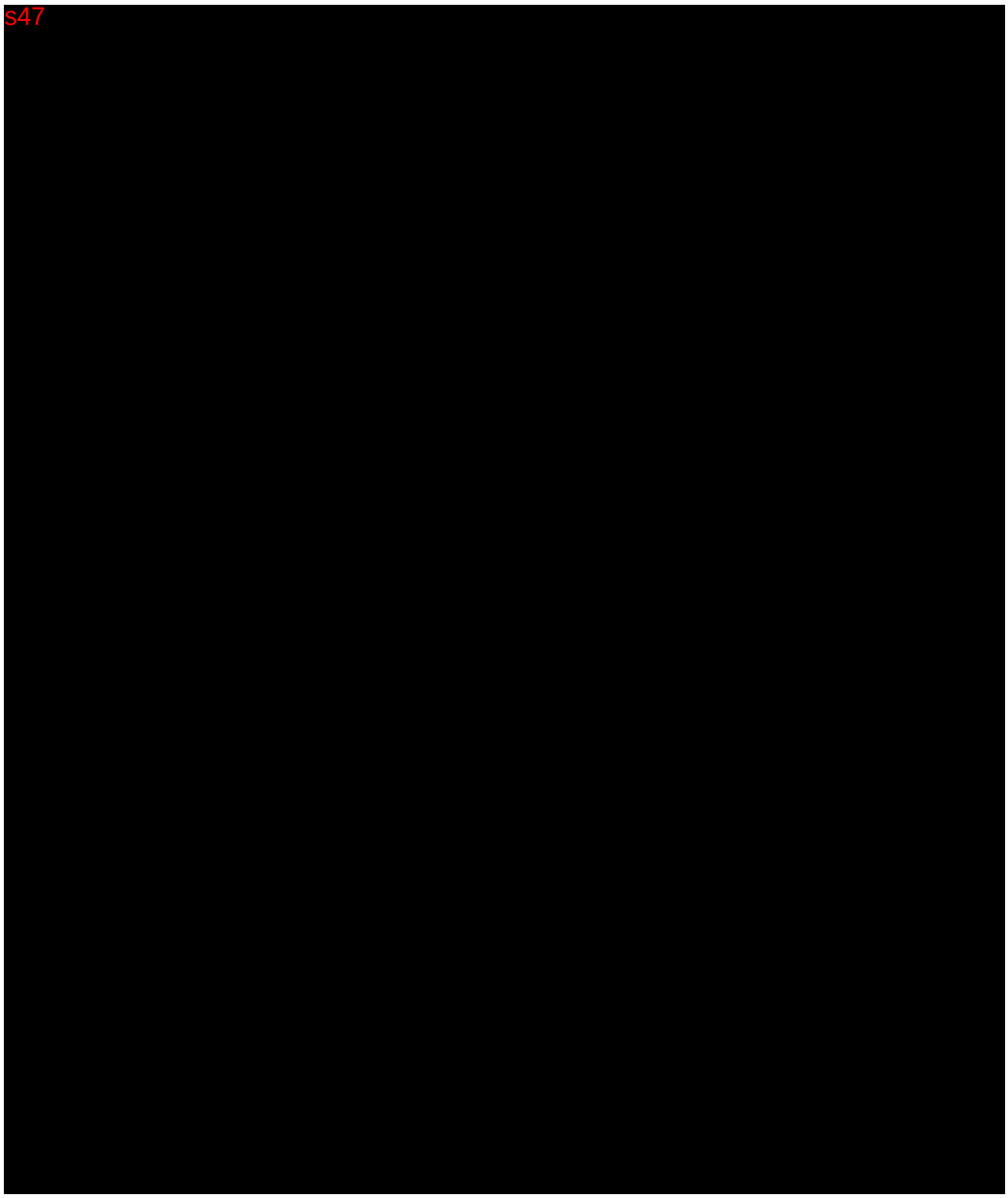

Associated Documents

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