Agarose Gel Electrophoresis Result Sheet

Vertex-CFTR Project — Target Amplification & Digest Verification

Meridian BioSciences Inc. ▪ Molecular Assays Core Facility ▪ Run ID: GEL-20260804-01 ▪ 04 August 2026

1 Run Overview & Metadata

Purpose & Scope

This document records the results of the horizontal agarose gel electrophoresis run performed at Meridian BioSciences. The purpose of this run is to analyze the PCR amplification products of target gene regions (exon 4 and exon 5 of the *CFTR* gene) and verify the restriction enzyme digest of plasmids *pMD-102* and *pMD-103*. All procedures were carried out in accordance with standard operating protocol SOP-ML-GEN-012, based on established molecular cloning guidelines [1, 2]. Fictional control assays and ladder profiles are documented below for quality assurance.

Run Information

Gel ID:	GEL-20260804-01
Run Date:	04 August 2026
Start Time:	10:15 AM
End Time:	11:35 AM
Status:	Active
Project:	Project Vertex-CFTR

Personnel & Authorization

- **Run Operator:** Marcus Sterling, Associate Researcher
- **Reviewing Officer:** Dr. Sarah Lin, Principal Investigator
- **Facility:** Molecular Assays Laboratory, Meridian BioSciences

2 Experimental Run Parameters

Detailed settings of the run, casting, and imaging conditions are summarized below.

Gel Casting & Buffer Settings

- **Gel Type:** Molecular Biology Agarose
- **Agarose Conc.:** 1.5% (w/v) in 1× TAE
- **Running Buffer:** 1× TAE
- **Stain:** GelRed™ (1:10,000 in-gel)
- **Gel Vol / Comb:** 100 µL / 8-well
- **Comb Thickness:** 1.5 mm

Electrophoresis & Imaging Settings

- **Running Voltage:** Constant 120 V
- **Current (Start/End):** 84 mA / 89 mA
- **Run Duration:** 80 min
- **Imaging System:** Meridian Imager-3000
- **Excitation:** UV Transilluminator (302 bp nm)
- **Exposure / Filter:** 150 ms / orange filter

Safety Alert & Reagent Handling

GelRed™ is a safer alternative to Ethidium Bromide but must be handled with gloves. Ensure the electrophoresis chamber lid is closed and the power supply is off before touching the buffer. UV radiation is hazardous; always use the protective shield.

3 Lane Loading Protocol

Each well was loaded with a mixture of DNA sample and 6× DNA Loading Dye. Lane 1 contains the reference ladder. The total volume loaded per well is 10 µL.

Lane	Sample ID	Description / Target	Vol (µL)	Mass (ng)	Expected Bands (bp)
1	M-100bp	Meridian 100bp-10kb DNA Ladder	8	500	100, 250, 500, 750, 1k, 1.5k, 2k, 3k, 5k, 10k
2	NC-01	Negative Control (No-Template PCR)	10	0	None (primer dimers expected)
3	PC-04	Positive Control (Plasmid pMD-102)	10	100	3,000
4	SMP-09	PCR Product Exon 4 (Patient 09)	10	150	1,500
5	SMP-10	PCR Product Exon 4 (Patient 10)	10	50	1,500 (low yield)
6	SMP-11	Restriction Digest pMD-103 (EcoRI/HindIII)	10	200	2,000, 1,000
7	SMP-12	Restriction Digest pMD-104 (EcoRI/HindIII)	10	180	2,000, 1,000
8	SMP-13	PCR Product Exon 5 (Patient 13)	10	0	Failed Amplification
9	SMP-14	PCR Product Exon 5 (Patient 14)	10	120	750

4 Agarose Gel Electrophoresis Image (UV Transillumination)

The high-resolution digitized gel image below shows the fluorescent bands after 80 minutes of separation. The lane alignments and size estimations were performed using the Meridian BioSciences Gel Analysis Suite.

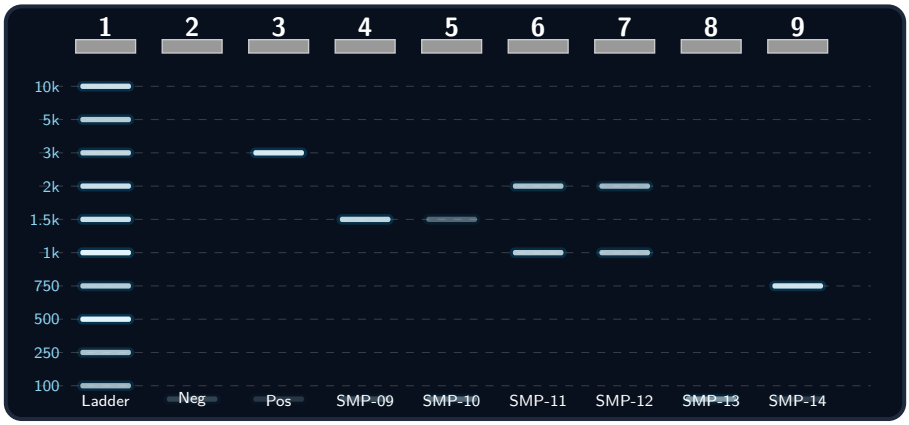

Figure 1: Digital scan of gel GEL-20260804-01 showing fluorescent DNA bands under UV illumination. The migration profile displays the distinct sizes of DNA fragments, which correspond to calculated values in Section 5 using standard logarithmic migration models [3].

5 Quantitative Band Analysis

Lane	Sample ID	Band	Dist (mm)	RF	Est. Size (bp)	Rel. Intensity (%)	Interpretation / Result
2	NC-01	1	71.0	0.95	< 100	8.2	Primer-dimer formation only; no contamination.
3	PC-04	1	24.5	0.33	3,020	94.5	Intact plasmid template band.
4	SMP-09	1	34.0	0.45	1,510	88.0	Strong target PCR product (Exon 4).
5	SMP-10	1	34.2	0.46	1,495	22.4	Target PCR product detected (low yield).
6	SMP-11	1	29.5	0.39	2,040	48.6	Restriction digest fragment 1 (Plasmid backbone).
		2	38.5	0.51	1,010	51.4	Restriction digest fragment 2 (Insert).
7	SMP-12	1	29.7	0.40	2,010	46.2	Restriction digest fragment 1 (Plasmid backbone).
		2	38.8	0.52	990	53.8	Restriction digest fragment 2 (Insert).
8	SMP-13	1	71.2	0.95	< 100	99.0	Primer-dimers only; amplification failure.
9	SMP-14	1	43.5	0.58	760	91.2	Target PCR product detected (Exon 5).

Calibration Curve Equations

The standard molecular weight curve was generated using a semi-logarithmic regression analysis of Lane 1:

$$\log_{10}(\text{Size}) = -0.0245 \cdot (\text{Migration Distance in mm}) + 4.6852 \quad (R^2 = 0.998) \tag{1}$$

Fragments smaller than 250 bp exhibit non-linear migration patterns under the current running conditions and were fitted using a secondary polynomial regression curve.

6 Quality Control & Review Conclusions

Verification & Quality Assessment

The gel electrophoresis run meets all standard quality control thresholds:

- **Resolution Check:** The ladder bands are distinct and well-separated down to 100 bp.
- **Contamination Control:** Negative control in Lane 2 shows no contamination (no bands other than primers).
- **Positive Control:** Positive control in Lane 3 shows a clear, single band of the expected size (3,000 bp).
- **Overall Run Status:** **PASS**

Experimental Observations & Discussion

1. **PCR Yield Variation:** The band in Lane 5 (SMP-10) is significantly fainter than that in Lane 4 (SMP-09), indicating a lower template concentration or suboptimal primer binding. A re-amplification is recommended if quantitative downstream analysis (e.g., sequencing) is required.
2. **Amplification Failure in Lane 8:** SMP-13 (PCR Exon 5) failed to yield any specific product. The intense primer-dimer signal at the bottom of the lane suggests that the PCR reaction was set up correctly, but amplification did not occur. Potential causes include degraded genomic DNA template or a mutation at the primer binding site. A fresh genomic DNA extraction and nested PCR protocol should be scheduled.
3. **Restriction Enzyme Digest Verification:** Restriction digestion profiles in Lanes 6 and 7 (SMP-11 and SMP-12) show two expected bands of 2,000 bp and 1,000 bp, indicating successful dual-enzyme cleavage of the plasmids. The insert sizes match the anticipated values of 1,000 bp [4].

Approvals & Signatures

By signing below, the operator and reviewer certify that the data presented in this report is authentic and has been verified against the raw imaging data.

Marcus Sterling
Run Operator — Research Associate
Date: 04 August 2026

Dr. Sarah Lin
Reviewer — Principal Investigator
Date: 04 August 2026

References

6 References

[1] Joseph Sambrook, Edward F. Fritsch, and Tom Maniatis. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2nd edition, 1989.

[2] Daniel Voytas. Agarose gel electrophoresis. *Current Protocols in Molecular Biology*, 51(1):2–1, 2000.

[3] Phillip A. Sharp, Bill Sugden, and Joseph Sambrook. Detection of two restriction endonuclease activities in Hemophilus parainfluenzae using analytical agarose-ethidium bromide electrophoresis. *Biochemistry*, 12(16):3055–3063, 1973.

[4] C. Aaij and P. Borst. The gel electrophoresis of DNA. *Biochimica et Biophysica Acta (BBA) - Nucleic Acids and Protein Synthesis*, 269(2):192–200, 1972.