September 27, 2023

Example Report

Computerized Analysis of Polypeptides Resolved by 2D Electrophoresis and Western Blotting for sample:

K12 MG1655 *E. coli* versus Cygnus Goat Anti-*E. coli* antibody, Cat. # AP117, Lot # 99

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Samples Compared:

1. Silver-stained 2D gel of K12 MG1655 *E. coli* (LF762 #12-13)
   vs. western blot film from Cygnus Goat Anti-*E. coli* antibody against K12 MG1655 *E. coli* (LF762 #8).

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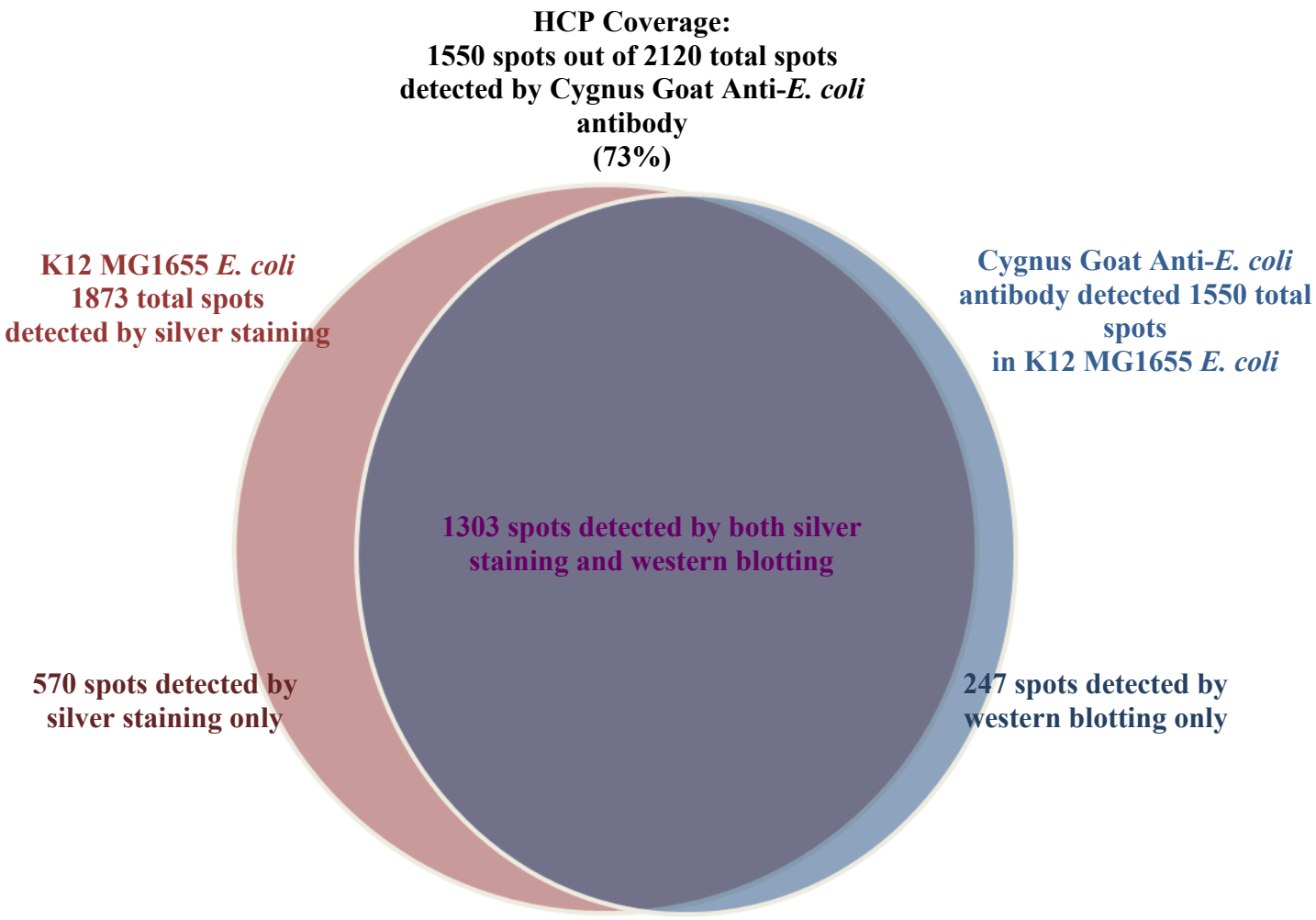


Figure 1. Summary of Results. Venn representation of the HCP coverage of sample **K12 MG1655 *E. coli*** with **Cygnus Goat Anti-*E. coli* antibody**. The number of spots detected by silver staining only, western blotting only, and both silver staining & western blotting are provided. Coverage is calculated as the percentage of spots detected by western blotting divided by total spots detected by silver staining + spots detected by western blotting only. Spot data including pI, MW, and spot percentages are given in the Excel file (HCPAnalysisExampleReport.E.coli.0823.xlsx) for all polypeptide spots. Spot percentages are given to indicate relative abundance.

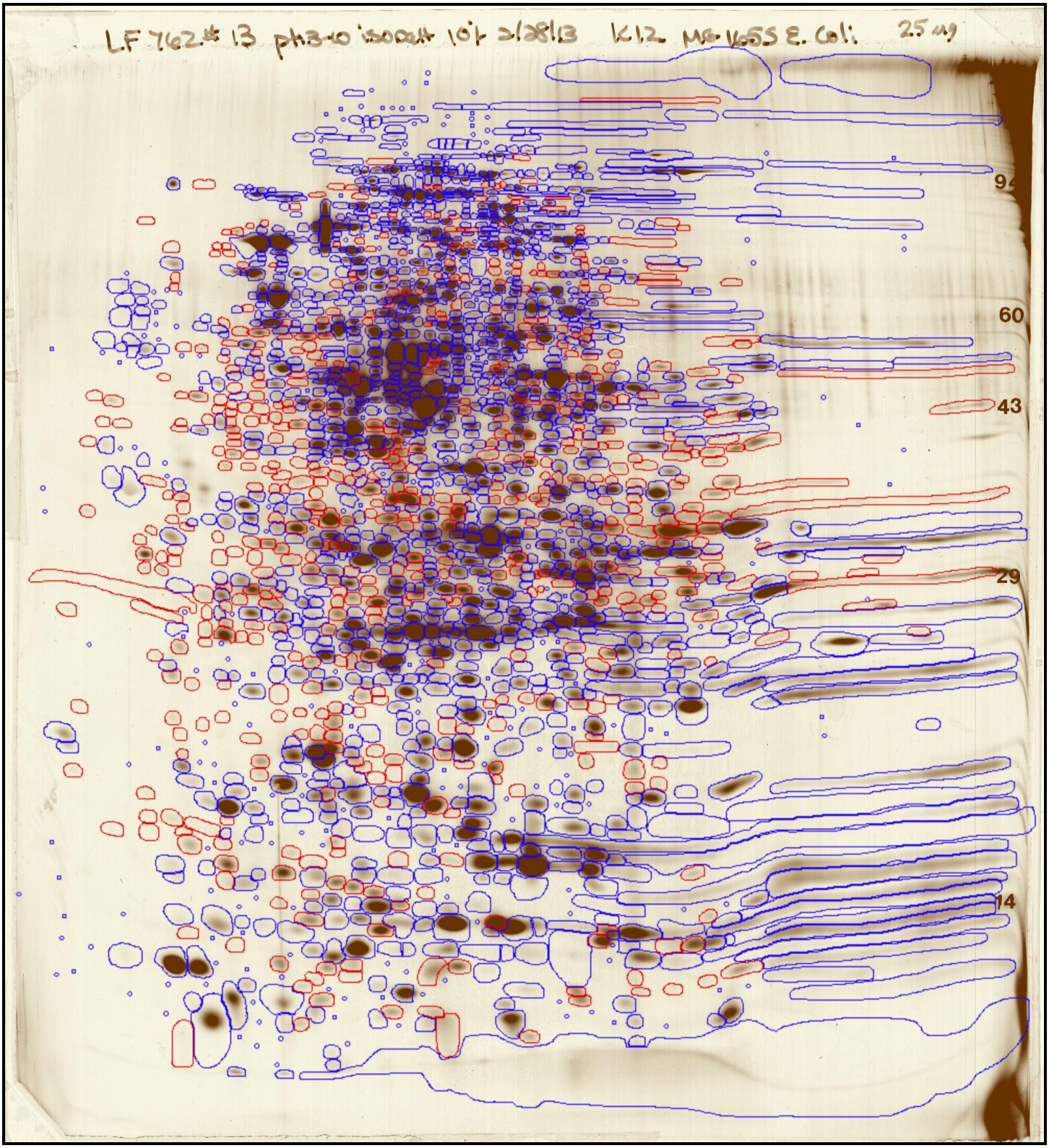


Figure 2. Image of K12 MG1655 *E. coli* silver-stained gel (LF762 #12-13) showing Cygnus Goat Anti-*E. coli* antibody western blot (LF762 #8) matches. Spots present on the silver-stained gel but missing from the western blot are outlined in **red**. Spots present in both the silver-stained gel and the western blot are outlined in **blue**. Spots detected with the antibody but not detectable by silver staining are indicated with small blue dots on the silver-stained gel and added to the total spot number. The Cygnus Goat Anti-*E. coli* antibody detected **1550 spots out of 2120 spots** (73%) found. Spot numbering is provided in Figures 5 and 6; spot data is provided in Table 1.

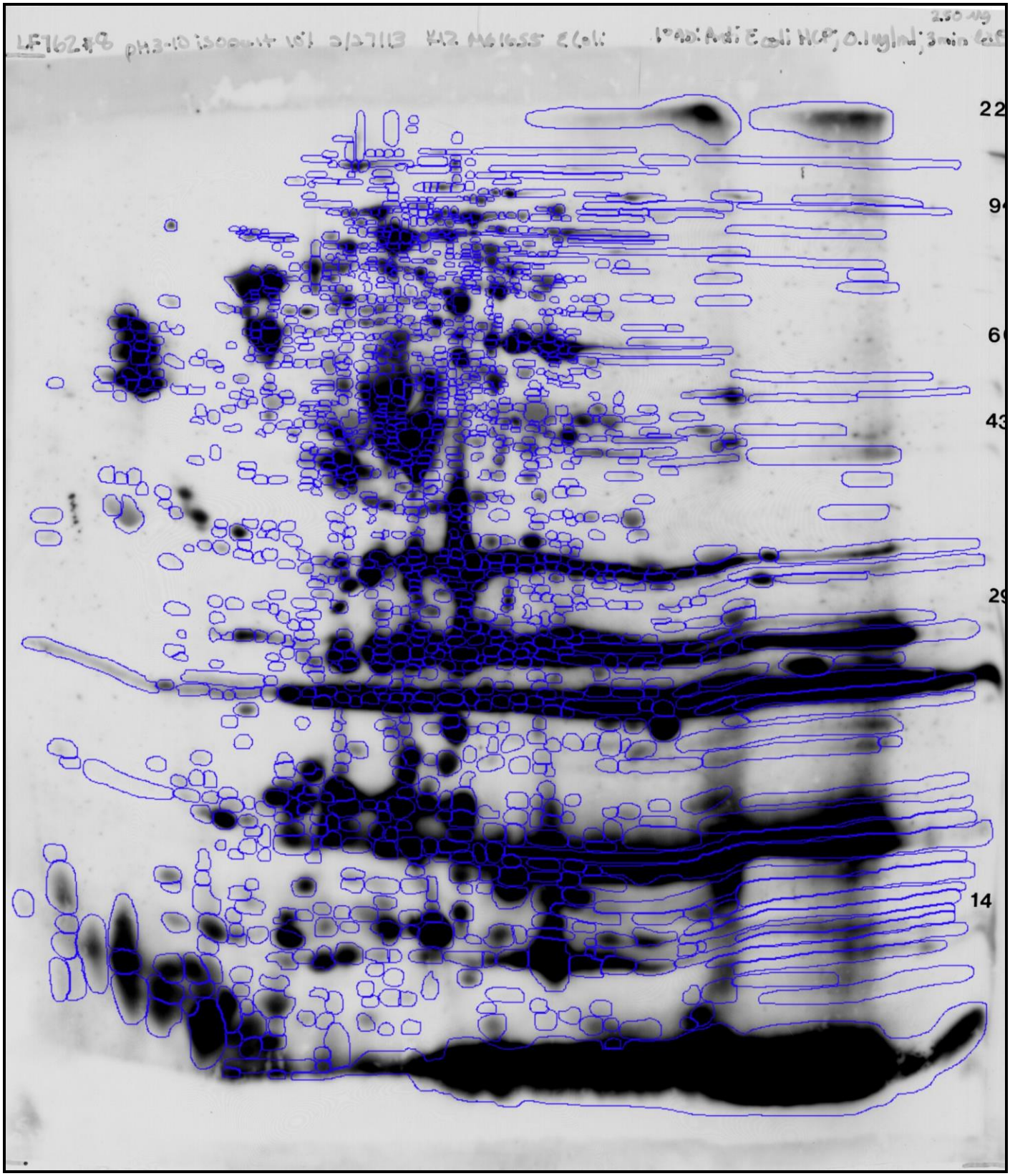


Figure 3. Dark western blot film image of Cygnus Goat Anti-*E. coli* antibody against K12 MG1655 *E. coli* (LF762 #8, 3-minute exposure). All spots detected on the dark and light exposures of the western blot are outlined in **blue**. The Cygnus Goat Anti-*E. coli* antibody detected **1550 spots**. Spot circling of the overexposed areas of the film can be more clearly seen on the light exposure film (Figure 3). Spot numbering is provided in Figures 5 and 6; spot data is provided in Table 1.

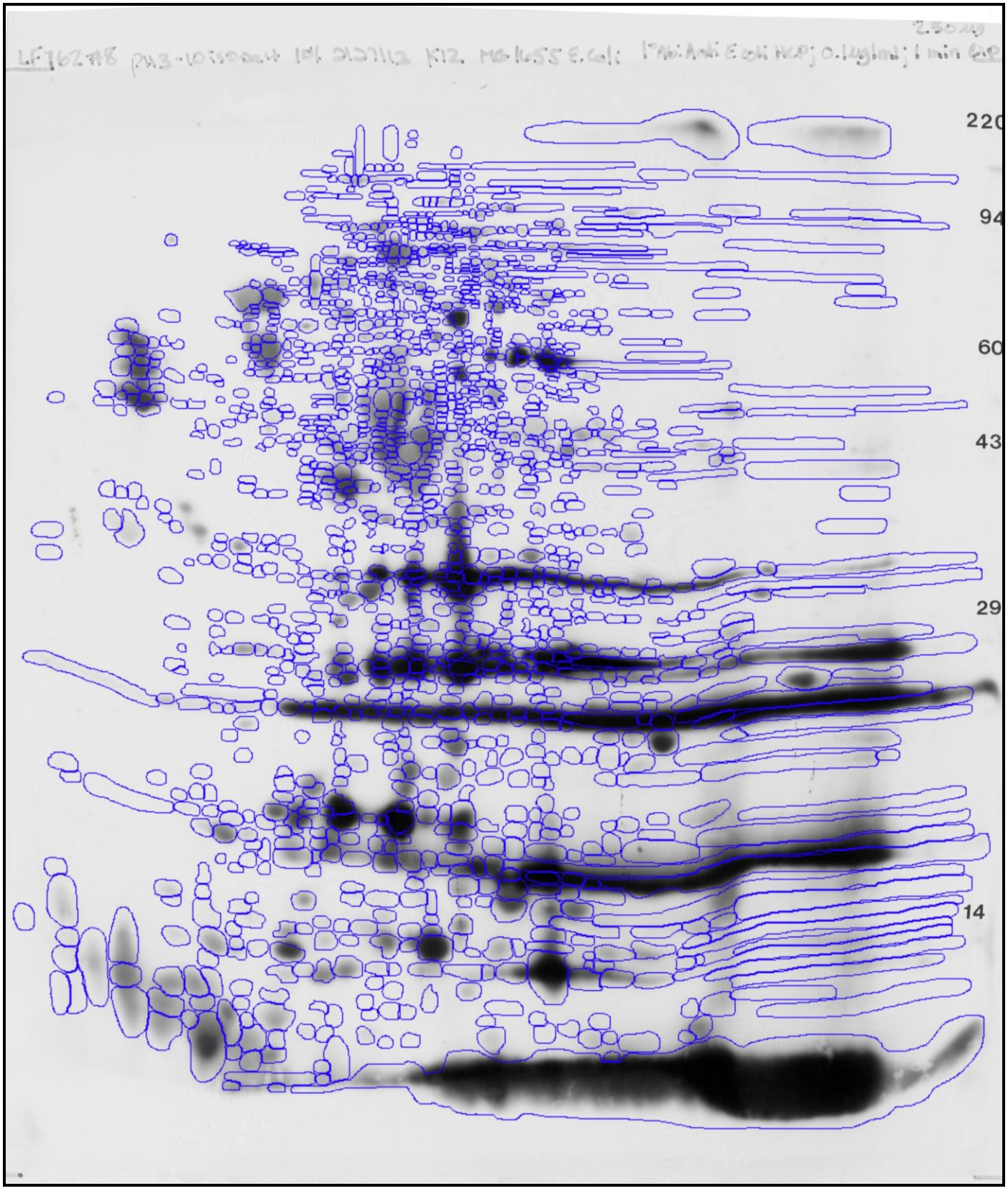


Figure 4. Light western blot film image of Cygnus Goat Anti-*E. coli* antibody against K12 MG1655 *E. coli* (LF762 #8, 1-minute exposure). All spots detected on the dark and light exposures of the western blot are outlined in **blue**. The Cygnus Goat Anti-*E. coli* antibody detected **1550 spots**. Spot numbering is provided in Figures 5 and 6; spot data is provided in Table 1.

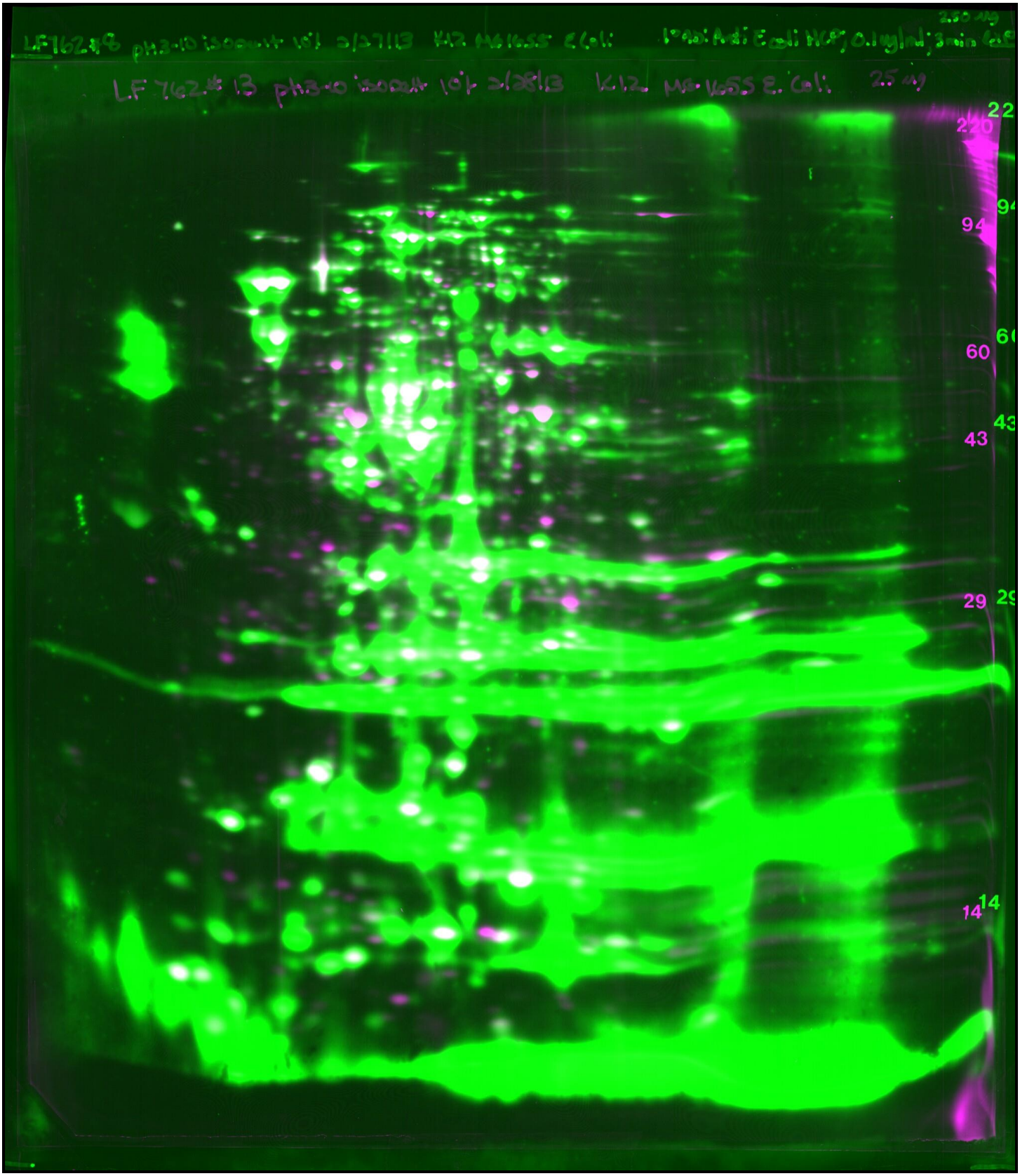


Figure 5. Overlay image showing silver-stained 2D gel of K12 MG1655 *E. coli* (LF762 #13) in magenta overlaying western blot film image of Cygnus Goat Anti-*E. coli* antibody against K12 MG1655 *E. coli* (LF762 #8, 3-minute exposure) in green. Image appears white where spots of similar intensity overlay each other.

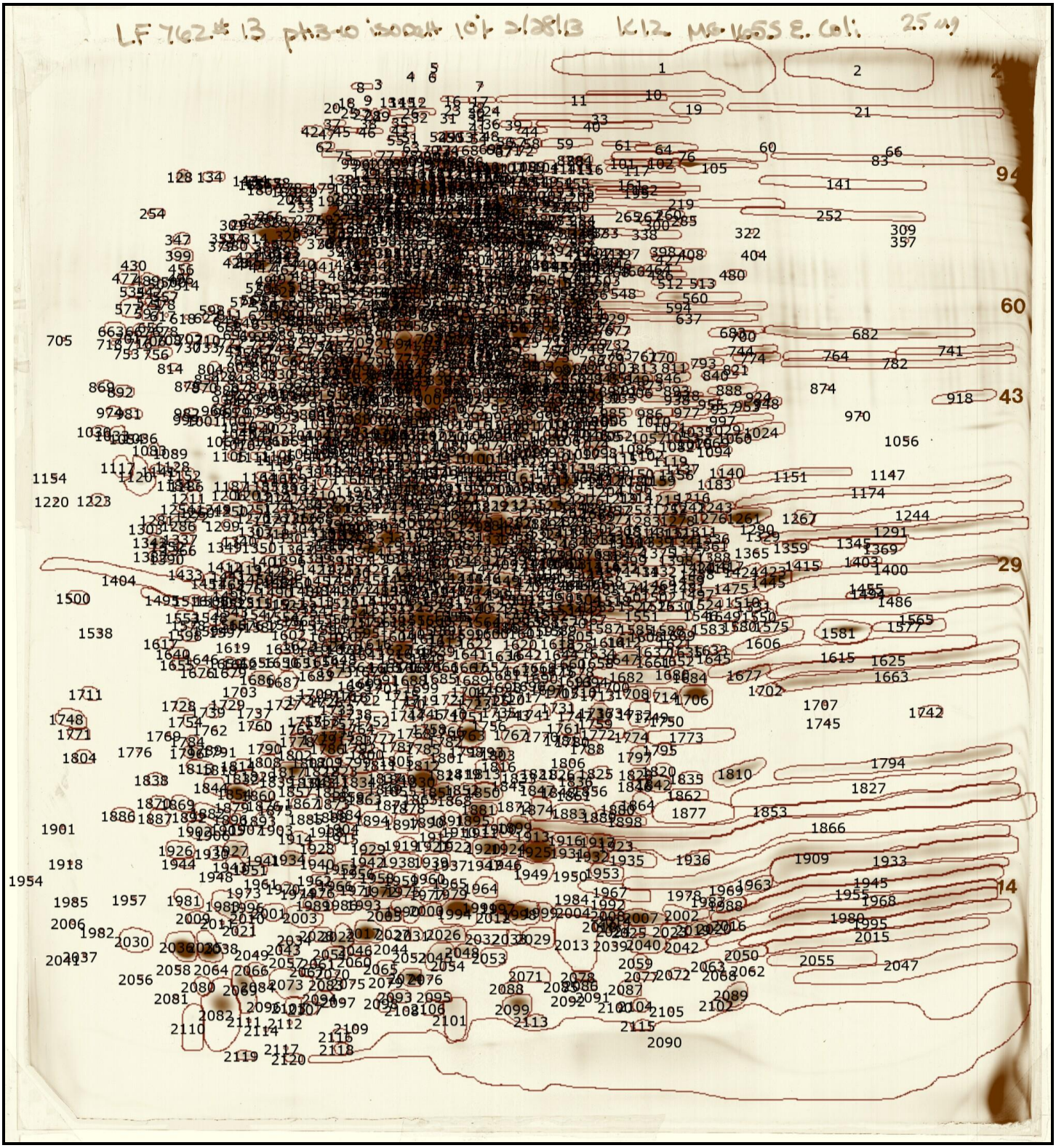


Figure 6. All spot numbering for silver-stained gel image of K12 MG1655 *E. coli* (LF762 #13). Spots detected with the antibody but not detectable by silver staining are indicated with small dots on the silver-stained gel. Spot numbers are best viewed on the computer screen. Magnified images to show greater detail will be provided on request. Spot data, including pI and MW can be found in Table 1.

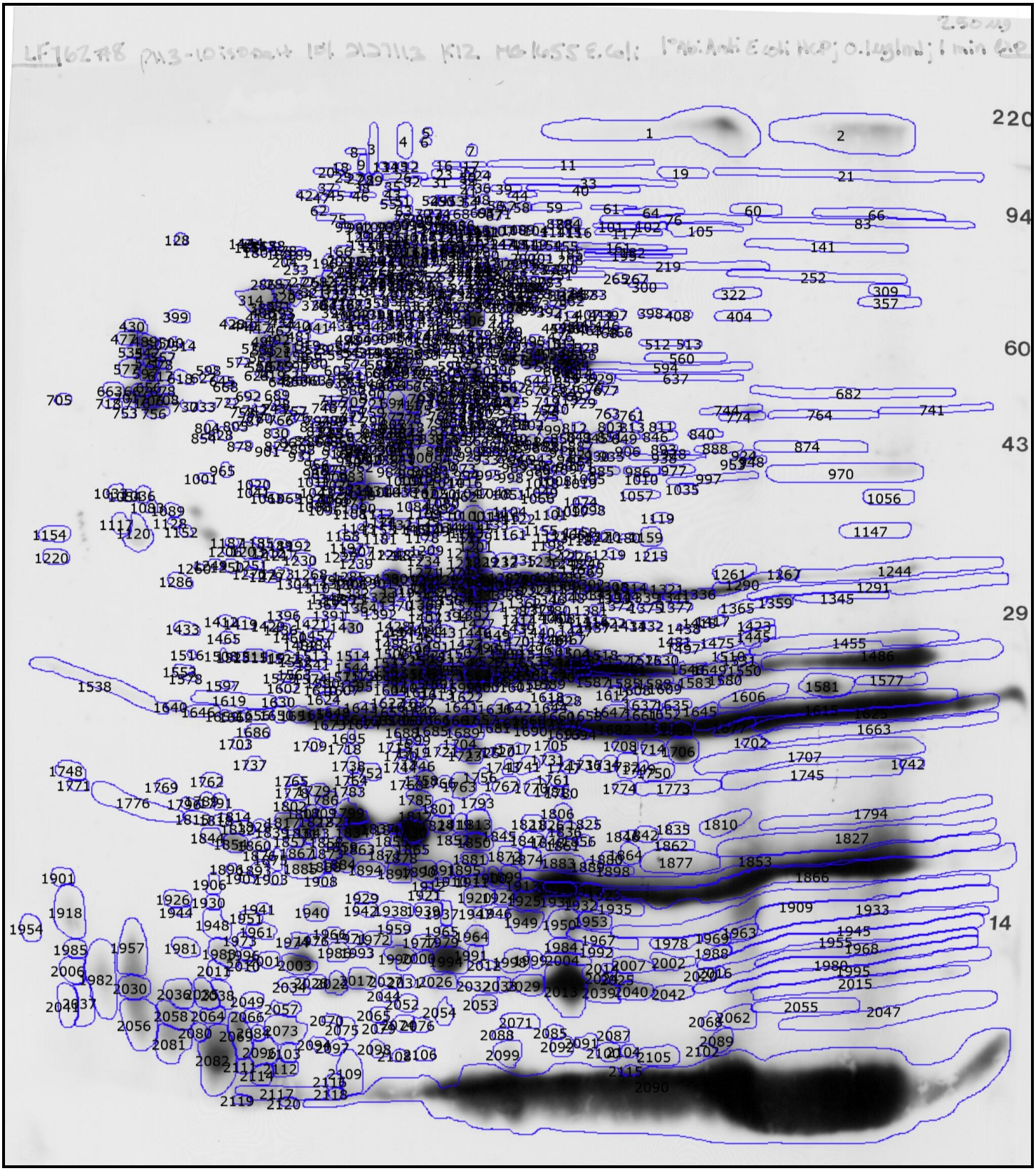


Figure 7. All spot numbering for western blot film image of Cygnus Goat Anti-*E. coli* antibody against K12 MG1655 *E. coli* (LF762 #8, 1-minute exposure). Spot numbers are best viewed on the computer screen. Magnified images to show greater detail will be provided on request. Spot data, including pI and MW can be found in Table 1.

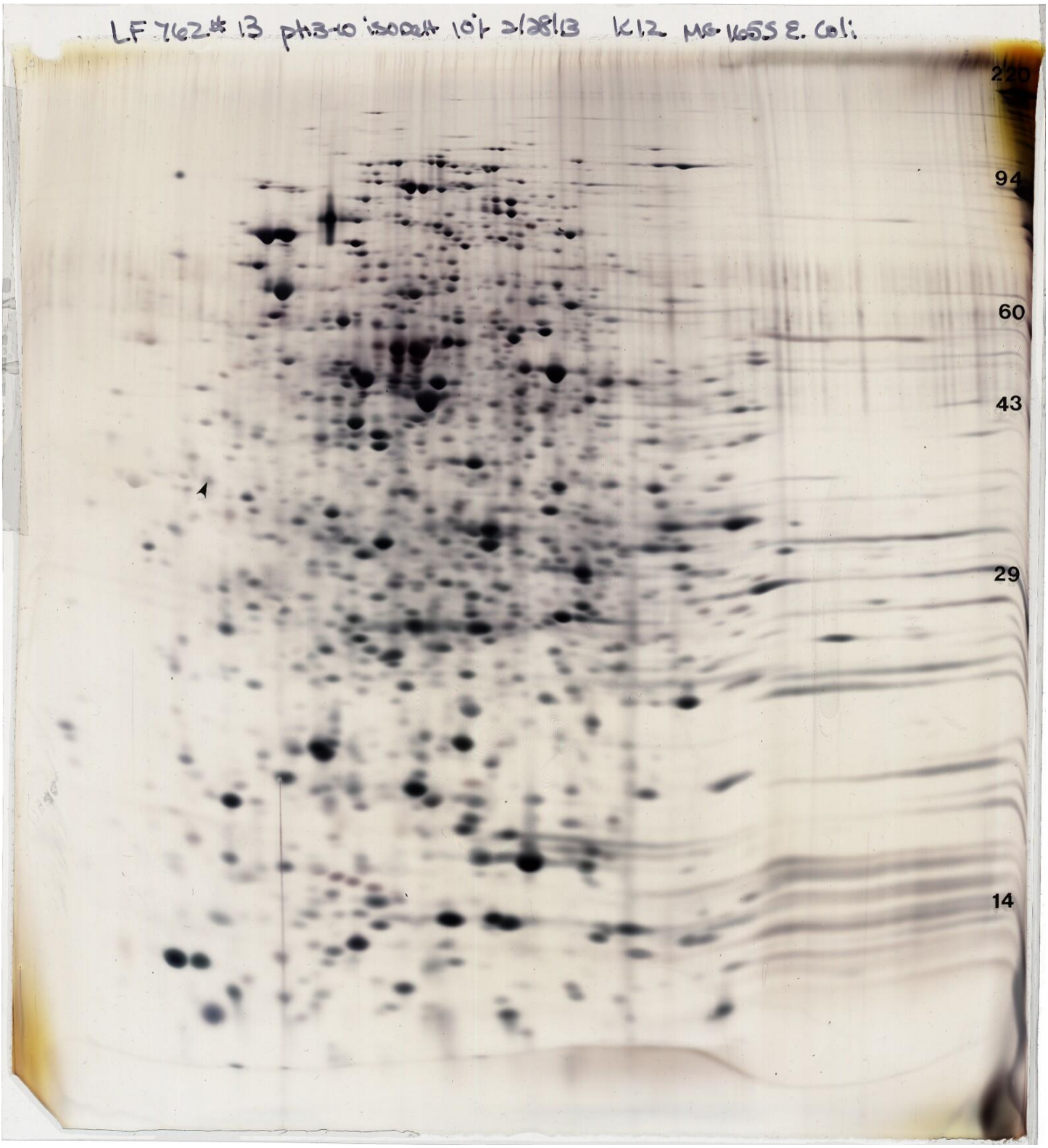


Figure 8. Image of silver-stained gel of K12 MG1655 *E. coli* (LF762 #13). Arrow marks the lower spot of internal standard tropomyosin, pI 5.2, MW 33 kDa.

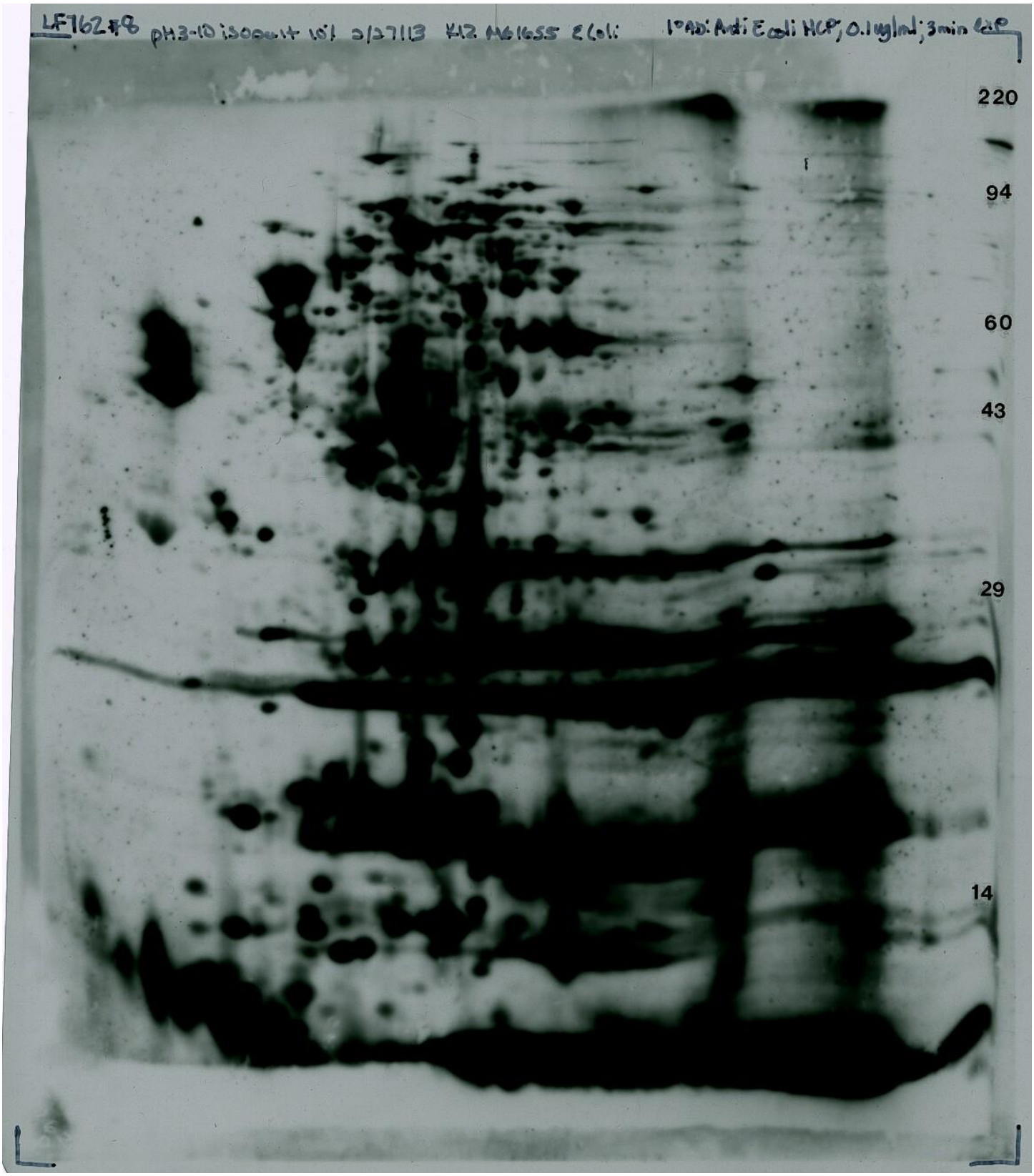


Figure 9. Dark image of western blot film Cygnus Goat Anti-*E. coli* antibody against K12 MG1655 *E. coli* (LF762 #8, 3-minute exposure). Film contrast was adjusted using SpotMap software to distinguish protein spots in dark areas.

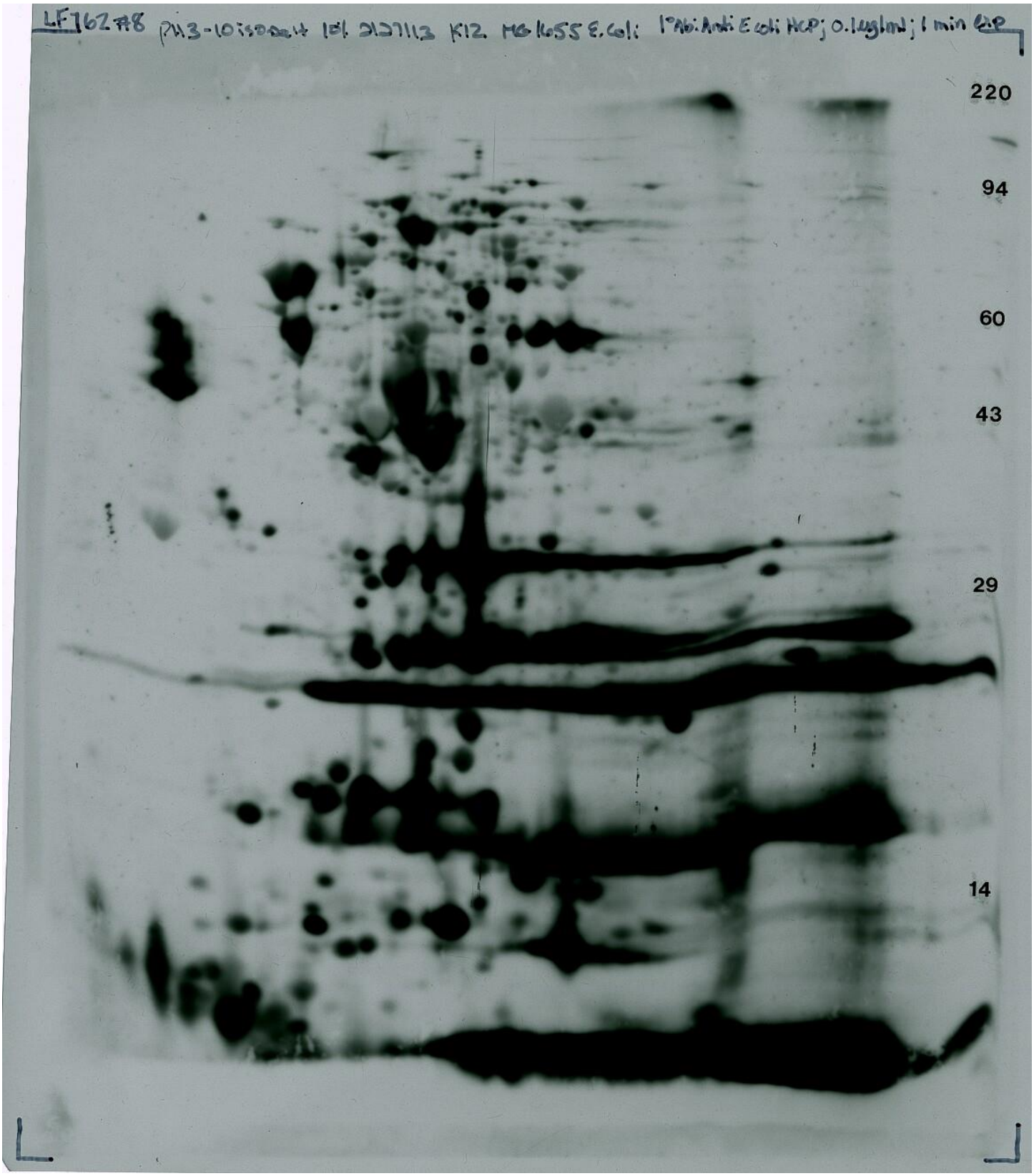


Figure 10. Light image of western blot film Cygnus Goat Anti-*E. coli* antibody against K12 MG1655 *E. coli* (LF762 #8, 1-minute exposure).

Materials and Methods
Two-dimensional electrophoresis was performed according to the carrier ampholine method of isoelectric focusing (Kendrick, N., G. Powers, J. Johansen, M. Hoelter, A. Koll, S. Carlson, D. Channaveerappa, and C.C. Darie. PLoS One, 2020. 15(6): p. e0234645.) by Kendrick Labs, Inc. (Madison, WI). Isoelectric focusing was carried out in a glass tube of inner diameter 3.3 mm using 2.0% pH 3-10 Isodalt Servalytes (Serva, Heidelberg, Germany) for 20,000 volt-hrs. One µg (PVDF) or 100 ng (silver) of an IEF internal standard, tropomyosin, was added to each sample. This protein migrates as a doublet with lower polypeptide spot of MW 33,000 and pI 5.2; an arrow on the stained gels marks its position. The enclosed tube gel pH gradient plot for this set of Servalytes was determined with a surface pH electrode.

After equilibration for 10 min in buffer "O" (10% glycerol, 50mM dithiothreitol, 2.3% SDS and 0.0625 M tris, pH 6.8), each tube gel was sealed to the top of a stacking gel that overlaid a 10% acrylamide slab gel (1.0 mm thick). SDS slab gel electrophoresis was carried out for about 5 hrs at 25 mA/gel. The following proteins (MilliporeSigma) were used as molecular weight standards: myosin (220,000), phosphorylase A (94,000), catalase (60,000), actin (43,000), carbonic anhydrase (29,000), and lysozyme (14,000). These standards appear as bands at the basic edge of the silver-stained (Oakley, B.R., Kirsch, D.R. and Moris, N.R. *Anal. Biochem. 105:*361-363, 1980) 10% acrylamide slab gels. The gels were dried between sheets of cellophane paper with the acid edge to the left.

After slab gel electrophoresis, the gel for blotting was placed in transfer buffer (10 mM CAPS, pH 11.0, 10% methanol) and transblotted onto a PVDF membrane overnight at 225 mA and approximately 100 volts/two gels. The same proteins (MilliporeSigma) were used as molecular weight standards: myosin (220,000), phosphorylase A (94,000), catalase (60,000), actin (43,000), carbonic anhydrase (29,000), and lysozyme (14,000). These standards appear as bands at the basic edge of the Coomassie Brilliant Blue R-250-stained membrane.

Western Blotting Methods
The blot was stained with Coomassie Brilliant Blue R-250, desktop scanned, destained in 100% methanol, and rinsed briefly with Tween-20 tris buffer saline (TTBS). The blot was blocked for two hours in 5% Nonfat Dry Milk (NFDM) in TTBS. The blot was then incubated in primary antibody solution (Goat Anti-*E. coli* [Cygnus, Cat. # AP117, and Lot # 99] diluted to 0.5 µg/ml in 2% NFDM TTBS) overnight and rinsed 3 x 10 minutes in TTBS. The blot was then placed in secondary antibody (Anti-Goat IgG-HRP [Sigma, Cat. # A5420, and Lot # 090M4822] 1:10,000 diluted in 2% NFDM TTBS) for two hours, rinsed as above, treated with ECL, and exposed to GE Amersham Hyperfilm ECL (1-minute, 3-minute, and 10-minute exposures).

Computerized Comparisons
Western blot films (1-minute and 3-minute exposures) and duplicate silver-stained gels were obtained from the sample and scanned with a laser densitometer (Model PDSI, Molecular Dynamics Inc, Sunnyvale, CA). The scanner was checked for linearity prior to scanning with a calibrated Neutral Density Filter Set (Melles Griot, Irvine, CA). The images were analyzed using SpotMap software (version 5.4, 2023, TotalLab, UK). The general method of computerized analysis for these pairs included image warping in conjunction with detailed manual checking. The lighter exposure of the western blot films was used to aid in spot matching for the overexposed areas of the dark exposure. Spots detected with the antibody were added to the master spot set even if not detectable by silver staining.

Spot % is equal to spot integrated density above background (volume) expressed as a percentage of total density above background of all spots measured.

MW and pI Measurements
Note that the isoelectric point (pI) measurements are approximate being based on the pH gradient plot found on the next page for this batch of ampholines for conditions of 9M urea and room temperature of 22ºC. Since the samples themselves may perturb the pH gradient, internal pI standards should be included if more exact pI measurements are required. The molecular weight and pI values for each spot are determined from algorithms applied to the reference image.

pH Gradient Plot LF 3-10 Iso-dalt

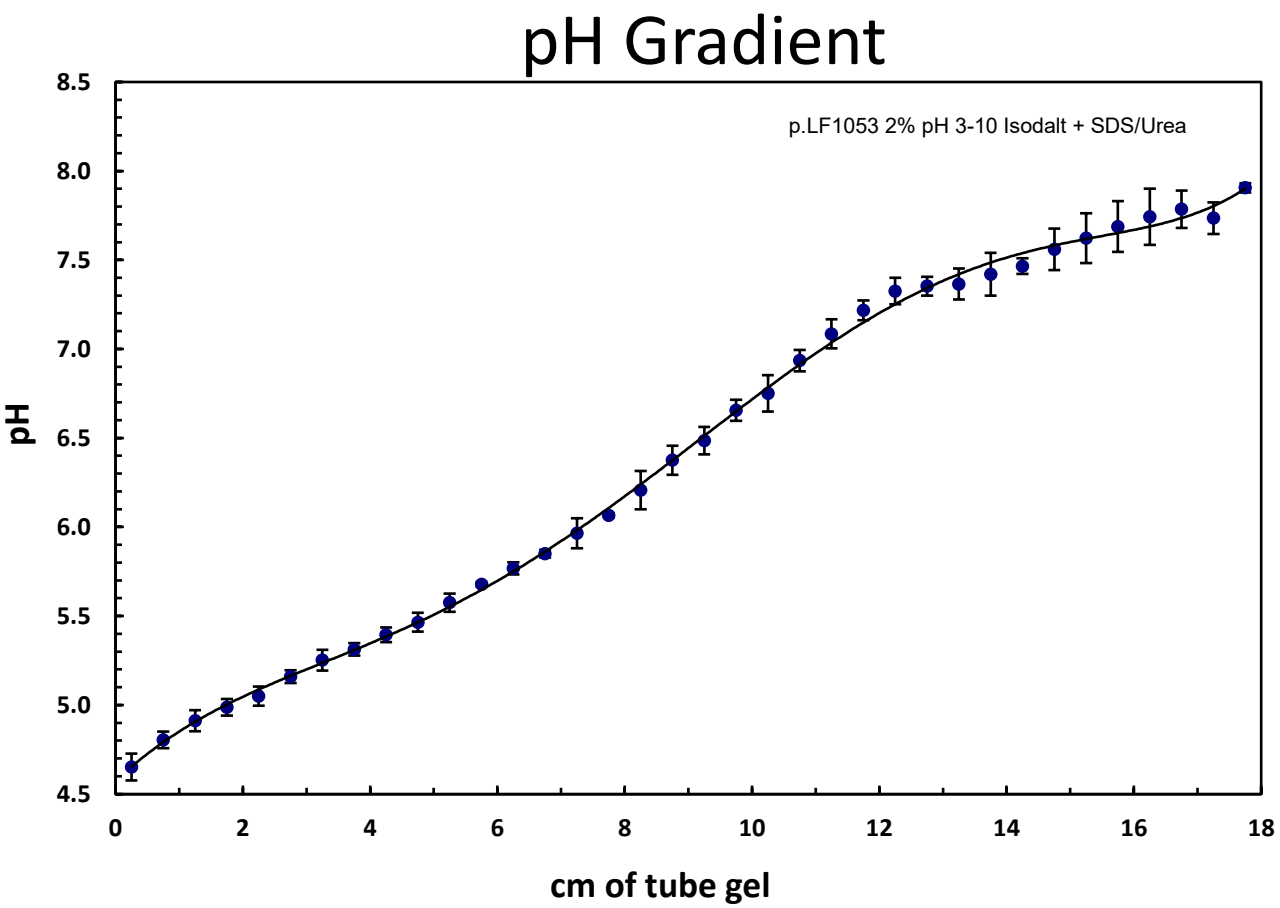
Name: Kendrick Laboratories, Inc. **Date: 9/27/23**

Sample(s): K12 MG1655 *E. coli*

Ampholines: 2% pH 3-10 Iso-dalt Servalytes

Conditions of IEF: 20,000 VHrs. (20 Hrs. at 1,000 V)

Comments:



This pH gradient was measured using a surface pH electrode for 6 blank IEF tube gels.

The dried slab gel for the second dimension is 20 cm wide with a 2D pattern about 18.5 cm wide. If SDS has been added to the sample, an SDS-NP-40 micelle migrates to the extreme acid end of the tube gel, constricting the pH gradient. In this case, the tube gels are poured long and the SDS bulb is cut off and discarded. The dried 2D pattern for samples containing SDS is also about 18 cm wide.

The black arrow on your 2D gel indicates our internal standard, Tropomyosin, pI 5.2 and molecular weight 32,700. The molecular weight standard lines are due to myosin (220,000), phosphorylase A (94,000), catalase (60,000), actin (43,000), carbonic anhydrase (29,000), and lysozyme (14,000) which have been added to the sealing agarose.

Western Blotting Checksheet

Client: Kendrick E. coli HCP Test **Company:** Kendrick Labs **Date:** 3/4/13

Samples: K12 MG1655 E. coli

Date Rcvd: 2/27/13 **Gel ID:** LF762 #6-11 **Analyst:** MH

Reagents:

Reagent	Lot #
Tris Buffered Saline (TBS)	
Tween 20	
Block: Non Fat Dry Milk (NFDM)	

Block

Blocking Solution: 5% NFDM **Incubation Time:** 2 hrs

Primary Antibody

Gel ID	1° Antibody	Dilution	Company	Cat #	Lot#	Rcvd	Stored
LF762 #6	Goat anti-E. coli	0.5 µg/mL	Cygnus	AP117	99	1/15/13	Fridge B2
LF762 #7	Goat anti-E. coli	0.5 µg/mL	Cygnus	AP117	99	1/15/13	Fridge B2
LF762 #8	Goat anti-E. coli	0.5 µg/mL	Cygnus	AP117	99	1/15/13	Fridge B2
LF762 #9	Goat anti-E. coli	0.5 µg/mL	Cygnus	AP117	99	1/15/13	Fridge B2
LF762 #10	Goat anti-E. coli	0.5 µg/mL	Cygnus	AP117	99	1/15/13	Fridge B2
LF762 #11	Goat anti-E. coli	0.5 µg/mL	Cygnus	AP117	99	1/15/13	Fridge B2

Buffer: 2% NFDM **Incubation Time:** Overnight

Secondary Antibody

Gel ID	2° Antibody	Dilution	Company	Cat #	Lot#	Stored
LF762 #6	Anti Goat IgG-HRP	1:10,000	Sigma	A5420	090M4822	Freezer I
LF762 #7	Anti Goat IgG-HRP	1:10,000	Sigma	A5420	090M4822	Freezer I
LF762 #8	Anti Goat IgG-HRP	1:10,000	Sigma	A5420	090M4822	Freezer I
LF762 #9	Anti Goat IgG-HRP	1:10,000	Sigma	A5420	090M4822	Freezer I
LF762 #10	Anti Goat IgG-HRP	1:10,000	Sigma	A5420	090M4822	Freezer I
LF762 #11	Anti Goat IgG-HRP	1:10,000	Sigma	A5420	090M4822	Freezer I

Buffer: 2% NFDM **Incubation Time:** 2 hours

ECL Film Development

ECL: Pierce ECL (Thermo) **Cat#:** 32106 **Lot#:**

Film: GE Amersham Hyperfilm ECL **Cat#:** 28906838 **Lot#:**

Gel ID	Exposure Time	Results
LF762 #6-11	1st: 1 min	Good Light Exposure
	2nd: 3 min	Good Exposure
	3rd: 10 min	Good Dark Exposure

Comments: 11/20/13 - films say 0.1 µg/ml primary, however recent blots suggest (LF821 #13-14) suggest the actual dilution used was 0.5 µg/ml as it says above.

Matt Hall 4/1/15
Person Completing Form Date

Reviewed By 4/1/15
Date