February 5, 2013

Report on Computerized Analysis of Polypeptides Resolved by 2D Electrophoresis

Pellets of Neisseria gonorrhoeae:
BB22 FA1090 A25 PB(s) strain vs
BB22R1 FA1090 A25 PB(r) revertant

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Samples Compared:

1. BB22 FA1090 A25 PB(s) strain (gels LF753 #4-5)
   vs. BB22R1 FA1090 A25 PB(r) revertant (gels LF753 #6-7)

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23, 24 Methods and pH Gradient.
CD Containing electronic copies of report, Excel data, and gel images.

Table 1. Summary of Results for 2D gel comparison of Pellets of *Neisseria gonorrhoeae*: **BB22** vs. **BB22R1**. Reference spot numbering, pI, and MW are given for changing polypeptide spots analyzed in samples **BB22 FA1090 A25 PB(s) strain** (gels LF753 #4-5) and **BB22R1 FA1090 A25 PB(r) revertant** (gels LF753 #6-7). Also shown are fold increases or decreases (difference) of the polypeptides for **BB22** vs. **BB22R1**. The differences are calculated from spot percentages (individual spot density divided by total density of all measured spots). Polypeptide spots **increased** by a fold increase of ≥ 1.7 and p value ≤ 0.05 or a fold increase of ≥ 3.0 are highlighted in **blue** in the difference column, while spots **decreased** with a fold decrease of ≤ -1.7 and p value ≤ 0.05 or a fold decrease of ≤ -3.0 are highlighted in **red**. All differences were visually verified using the montage images found on the following pages. Nd = pI/MW not determined. Spot percentages are given to indicate relative abundance. A total of **1137** spots were analyzed. Figures showing spot numbering, sample overlay, and individual montages showing the spots of interest outlined in green are provided on the following pages. All spot data is given on the CD.

Spot #	pI	MW	BB22 LF753 #4 Spot %	BB22 LF753 #5 Spot %	BB22 Average Spot %	BB22R1 LF753 #6 Spot %	BB22R1 LF753 #7 Spot %	BB22R1 Average Spot %	BB22 vs BB22R1 Difference	BB22 vs BB22R1 T-test (p)	Montage Image Page #
15	6.1	149,690	0.003	0.003	0.003	0.001	0.001	0.001	2.6	0.039	7
22	5.9	140,865	0.005	0.005	0.005	0.003	0.002	0.003	2.0	0.024	7
82	5.8	89,524	0.007	0.006	0.006	0.002	0.003	0.003	2.3	0.029	8
106	6.1	110,379	0.007	0.005	0.006	0.002	0.002	0.002	3.0	0.044	8
143	6.3	79,778	0.019	0.017	0.018	0.011	0.011	0.011	1.7	0.022	9
263	6.0	64,327	0.007	0.006	0.006	0.003	0.004	0.003	2.0	0.025	9
297	5.8	58,544	0.006	0.006	0.006	0.002	0.002	0.002	2.7	0.009	10
335	5.6	54,865	0.013	0.012	0.013	0.008	0.005	0.006	2.0	0.046	10
338	5.8	54,477	0.058	0.063	0.061	0.031	0.028	0.030	2.1	0.008	11
386	6.2	52,468	0.021	0.021	0.021	0.013	0.010	0.011	1.9	0.028	11
166	5.5	76,851	0.002	0.000	0.001	0.004	0.004	0.004	-4.0	0.134	12
351	5.8	53,316	0.003	0.010	0.006	0.035	0.032	0.033	-5.2	0.017	12
408	6.5	51,160	0.014	0.004	0.009	0.086	0.079	0.082	-9.1	0.007	13
543	6.3	42,701	0.006	0.007	0.006	0.021	0.018	0.020	-3.1	0.016	13
625	6.9	36,441	0.031	0.021	0.026	0.054	0.058	0.056	-2.2	0.028	14
642	6.7	34,846	0.007	0.009	0.008	0.023	0.018	0.020	-2.5	0.047	14
848	6.0	26,568	0.008	0.008	0.008	0.025	0.025	0.025	-3.0	0.000	15
881	6.6	23,889	0.022	0.018	0.020	0.040	0.038	0.039	-1.9	0.013	15
900	6.9	22,926	0.002	0.010	0.006	0.079	0.084	0.081	-13.6	0.003	16
907	6.0	22,675	0.001	0.007	0.004	0.014	0.013	0.014	-3.4	0.107	16
909	6.4	22,432	0.006	0.004	0.005	0.010	0.010	0.010	-2.1	0.039	17
912	6.6	22,062	0.011	0.032	0.021	0.072	0.079	0.076	-3.6	0.039	17

Spot #	pI	MW	BB22 LF753 #4 Spot %	BB22 LF753 #5 Spot %	BB22 Average Spot %	BB22R1 LF753 #6 Spot %	BB22R1 LF753 #7 Spot %	BB22R1 Average Spot %	BB22 vs BB22R1 Difference	BB22 vs BB22R1 T-test (p)	Montage Image Page #
922	6.0	21,663	0.008	0.011	0.010	0.020	0.019	0.020	**-2.0**	0.014	18
934	5.6	20,964	0.042	0.053	0.047	0.085	0.078	0.081	**-1.7**	0.035	18
1046	6.3	13,237	0.004	0.003	0.003	0.011	0.014	0.012	**-3.5**	0.039	19

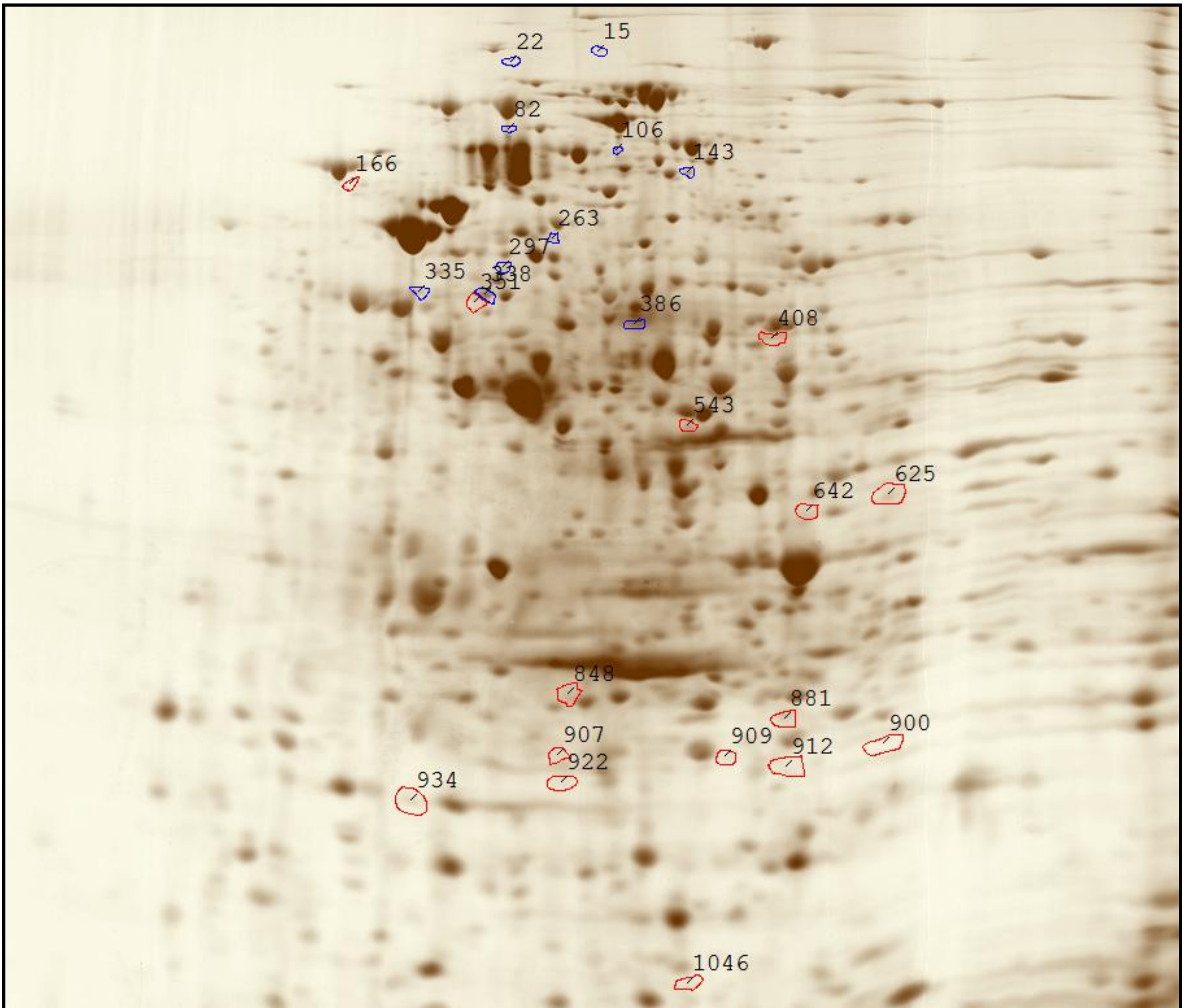


Figure 1. **2D Gel Difference Image of Averaged BB22** (gels LF753 #4-5) versus **BB22R1** (gels LF753 #6-7). Polypeptide spots *Increased* in BB22 vs BB22R1 are outlined in **Blue**, while spots *Decreased* in BB22 vs BB22R1 are outlined in **Red**. See Table 1 for spot measurements. Montage and overlay images of changing spots are provided on the following pages. All spot data is given in the Excel Table on the CD.

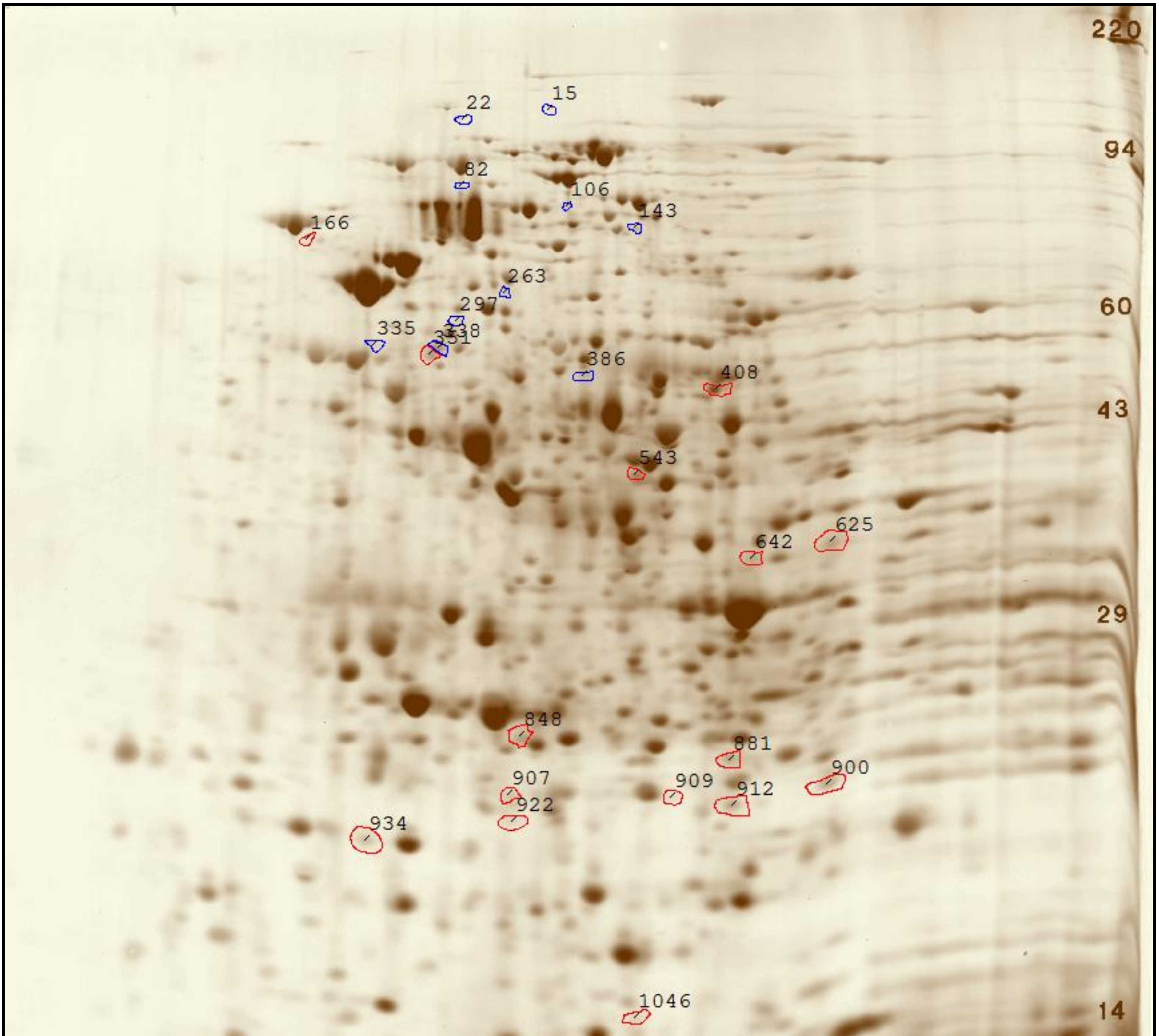


Figure 2. **2D Gel Difference Image of BB22R1** (gels LF753 #6-7) to show spots increased in BB22R1 (in red) versus BB22 strain. Polypeptide spots *Increased* in BB22 vs BB22R1 are outlined in **Blue**, while spots *Decreased* in BB22 vs BB22R1 are outlined in **Red**. Montage and overlay images of changing spots are provided on the following pages. All spot data is given in the Excel Table on the CD.

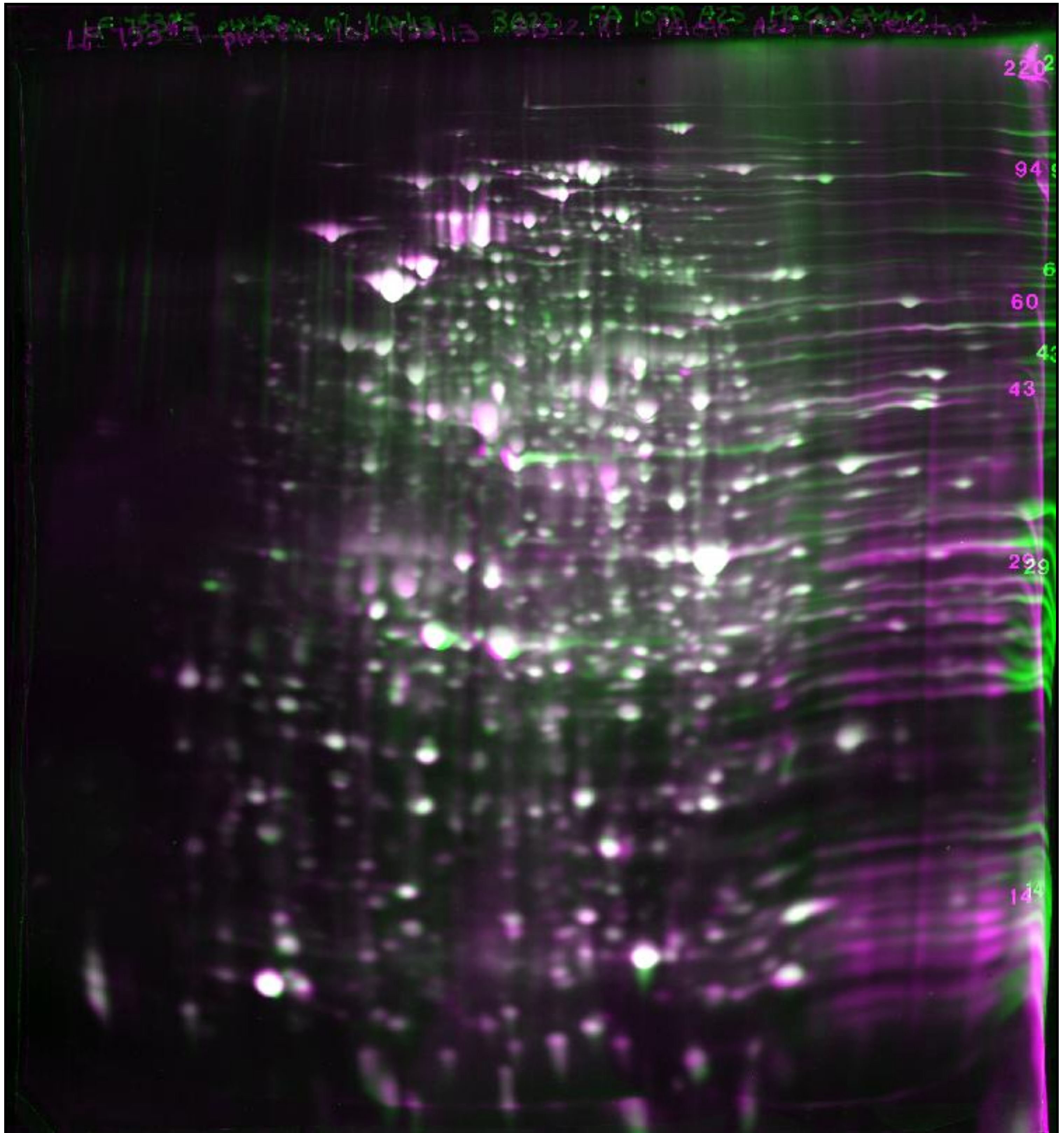


Figure 3. Overlay image showing BB22 (gel LF753 #5) in green overlaying BB22R1 (gel LF753 #7) in magenta. Image appears white where spots of similar intensity overlay each other.

15

PB(s) strain LF753 #5

PB(s) strain LF753 #4

PB(r) revertant LF753 #7

PB(r) revertant LF753 #6

Figure 4. Montage image of spot 15.

22

PB(s) strain LF753 #5

PB(s) strain LF753 #4

PB(r) revertant LF753 #7

PB(r) revertant LF753 #6

Figure 5. Montage image of spot 22.

82

PB(s) strain LF753 #5

PB(s) strain LF753 #4

PB(r) revertant LF753 #7

PB(r) revertant LF753 #6

Figure 6. Montage image of spot 82.

106

PB(s) strain LF753 #5

PB(s) strain LF753 #4

PB(r) revertant LF753 #7

PB(r) revertant LF753 #6

Figure 7. Montage image of spot 106.

Figure 8. Montage image of spot 143.

Figure 9. Montage image of spot 263.

Figure 10. Montage image of spot 297.

Figure 11. Montage image of spot 335.

338

PB(s) strain LF753 #5

PB(s) strain LF753 #4

PB(r) revertant LF753 #7

PB(r) revertant LF753 #6

Figure 12. Montage image of spot 338.

386

PB(s) strain LF753 #5

PB(s) strain LF753 #4

PB(r) revertant LF753 #7

PB(r) revertant LF753 #6

Figure 13. Montage image of spot 386.

166

PB(s) strain LF753 #5

PB(s) strain LF753 #4

PB(r) revertant LF753 #7

PB(r) revertant LF753 #6

Figure 14. Montage image of spot 166.

351

PB(s) strain LF753 #5

PB(s) strain LF753 #4

PB(r) revertant LF753 #7

PB(r) revertant LF753 #6

Figure 15. Montage image of spot 351.

408

PB(s) strain LF753 #5

PB(s) strain LF753 #4

PB(r) revertant LF753 #7

PB(r) revertant LF753 #6

Figure 16. Montage image of spot 408.

543

PB(s) strain LF753 #5

PB(s) strain LF753 #4

PB(r) revertant LF753 #7

PB(r) revertant LF753 #6

Figure 17. Montage image of spot 543.

625

PB(s) strain LF753 #5

PB(s) strain LF753 #4

PB(r) revertant LF753 #7

PB(r) revertant LF753 #6

Figure 18. Montage image of spot 625.

642

PB(s) strain LF753 #5

PB(s) strain LF753 #4

PB(r) revertant LF753 #7

PB(r) revertant LF753 #6

Figure 19. Montage image of spot 642.

848

PB(s) strain LF753 #5

PB(s) strain LF753 #4

PB(r) revertant LF753 #7

PB(r) revertant LF753 #6

Figure 20. Montage image of spot 848.

881

PB(s) strain LF753 #5

PB(s) strain LF753 #4

PB(r) revertant LF753 #7

PB(r) revertant LF753 #6

Figure 21. Montage image of spot 881.

900

PB(s) strain LF753 #5

PB(s) strain LF753 #4

PB(r) revertant LF753 #7

PB(r) revertant LF753 #6

Figure 22. Montage image of spot 900.

907

PB(s) strain LF753 #5

PB(s) strain LF753 #4

PB(r) revertant LF753 #7

PB(r) revertant LF753 #6

Figure 23. Montage image of spot 907.

909

PB(s) strain LF753 #5

PB(s) strain LF753 #4

PB(r) revertant LF753 #7

PB(r) revertant LF753 #6

Figure 24. Montage image of spot 909.

912

PB(s) strain LF753 #5

PB(s) strain LF753 #4

PB(r) revertant LF753 #7

PB(r) revertant LF753 #6

Figure 25. Montage image of spot 912.

922

PB(s) strain LF753 #5

PB(s) strain LF753 #4

PB(r) revertant LF753 #7

PB(r) revertant LF753 #6

Figure 26. Montage image of spot 922.

934

PB(s) strain LF753 #5

PB(s) strain LF753 #4

PB(r) revertant LF753 #7

PB(r) revertant LF753 #6

Figure 27. Montage image of spot 934.

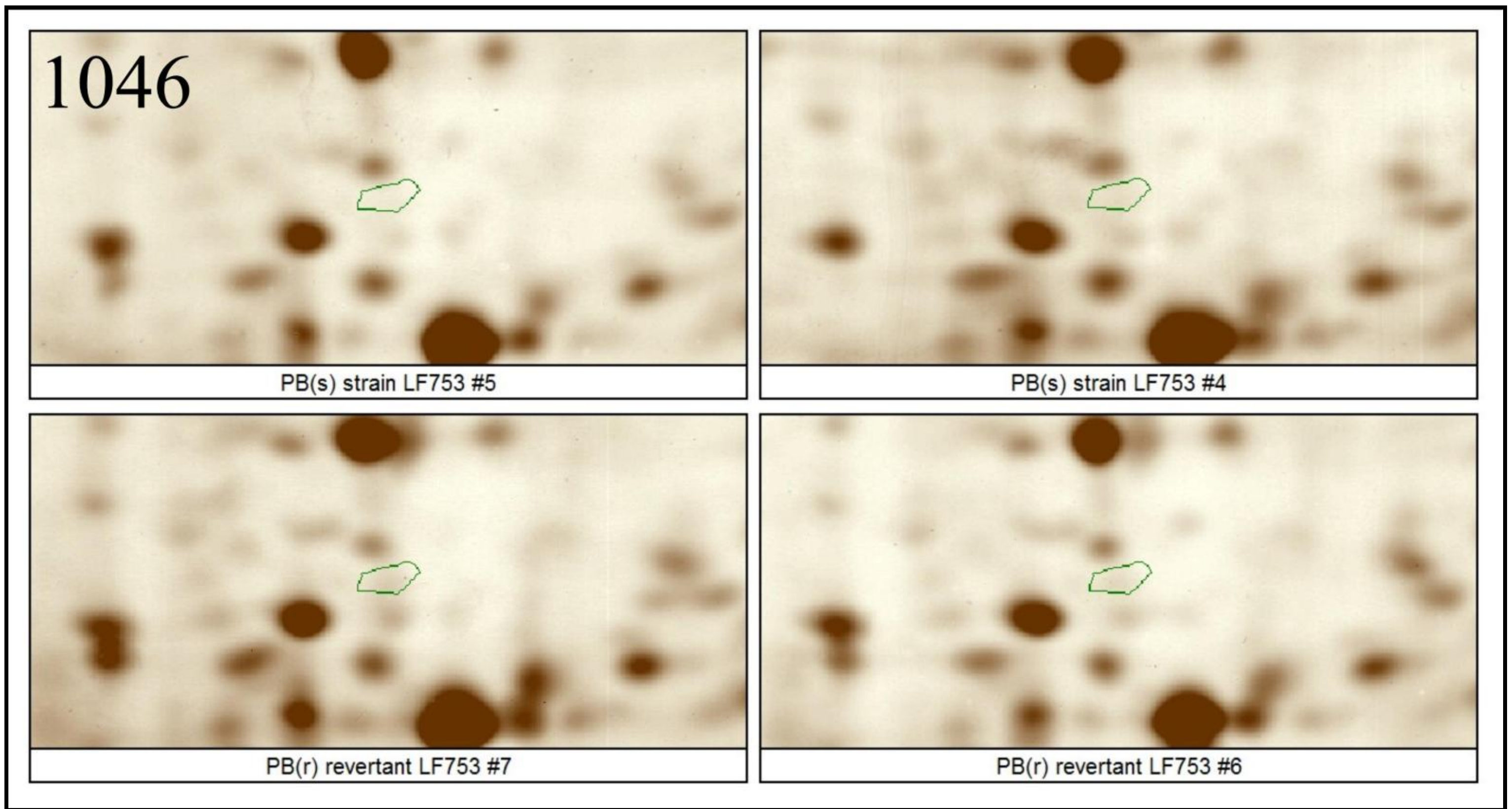


Figure 28. Montage image of spot 1046.

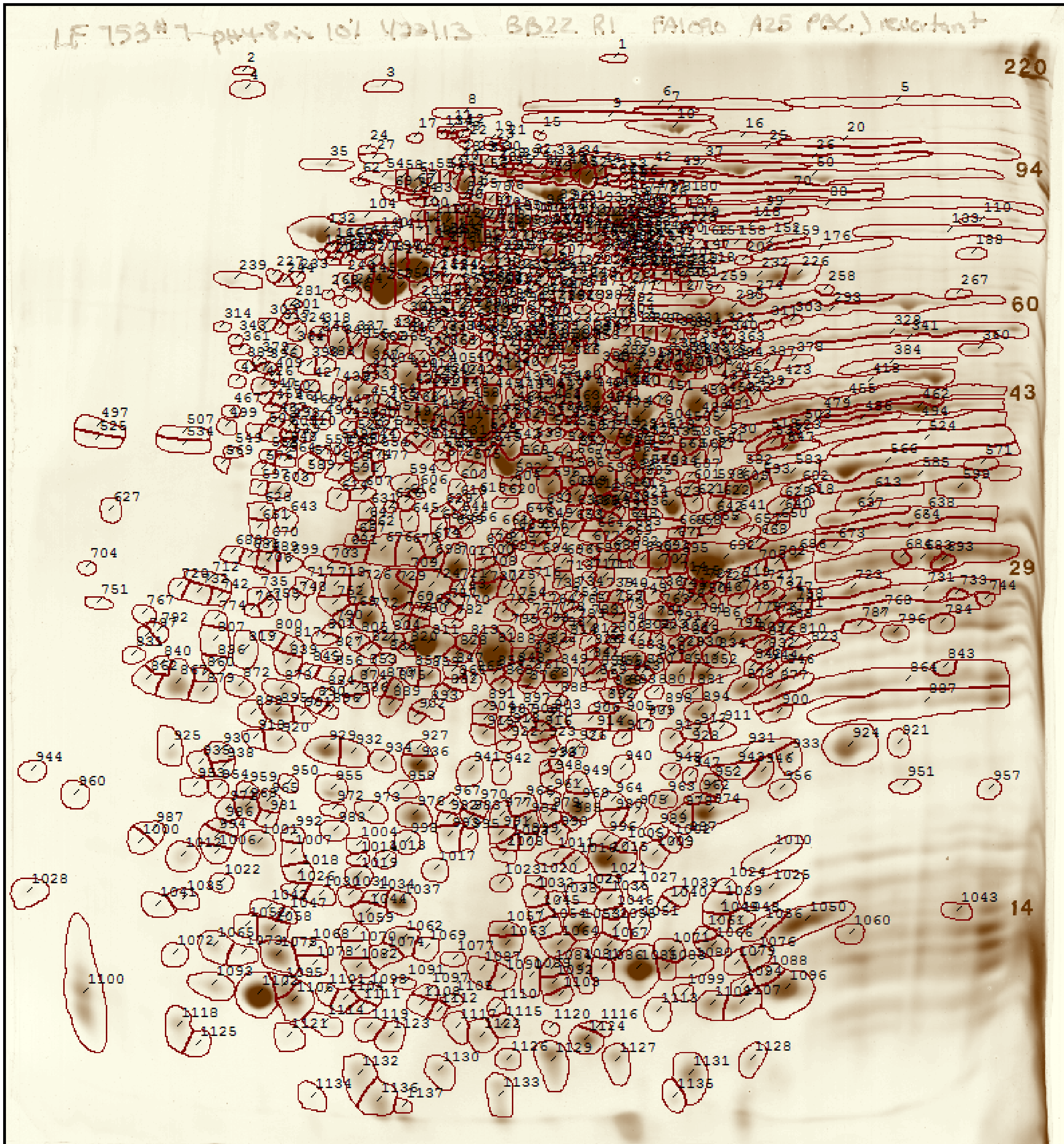


Figure 29. Reference gel showing all spot numbering. Magnified images to show greater detail will be provided on request. All spot data, including pI and MW can be found in the Excel file on the CD.

Figure 30. Image of silver-stained gel of sample BB22(LF753 #5).

Figure 31. Image of silver-stained gel of sample BB22R1(LF753 #7).

Materials & Methods

Two-dimensional electrophoresis was performed using the carrier ampholine method of isoelectric focusing (O'Farrell, P.H., *J. Biol. Chem. 250*: 4007-4021, 1975, Burgess-Cassler, A., Johansen, J., Santek, D., Ide J., and Kendrick N., *Clin. Chem. 35*: 2297, 1989) by Kendrick Labs, Inc. (Madison, WI). Isoelectric focusing was carried out in a glass tube of inner diameter 3.3 mm using 2% pH 4-8 mix Servalytes (Serva, Heidelberg Germany) for 20,000 volt-hrs. One hundred ng of an IEF internal standard, tropomyosin, was added to the sample. This protein migrates as a doublet with lower polypeptide spot of MW 33,000 and pI 5.2; an arrow on the stained gel marks its position. The enclosed tube gel pH gradient plot for this set of ampholines was determined with a surface pH electrode.

After equilibration for 10 min in Buffer 'O' (10% glycerol, 50 mM 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 (1.00 mm thick). SDS slab gel electrophoresis was carried out for about 5 hrs at 25 mA/gel. The following proteins (Sigma Chemical Co., St. Louis, MO) 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 along 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 gel. The silver-stained gels were dried between sheets of cellophane with the acid edge to the left.

Computerized Comparisons

Duplicate gels were obtained from each sample. The gels were 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 Progenesis Same Spots software (version 4.5, 2011, Nonlinear Dynamics, Durham, NC) and Progenesis PG240 software (version 2006, Nonlinear Dynamics, Durham, NC). The general method of computerized analysis for these pairs included image warping followed by spot finding, background subtraction (average on boundary), matching, and quantification in conjunction with detailed manual checking.

Spot % is equal to spot integrated density above background (volume) expressed as a percentage of total density above background of all spots measured. Difference is defined as fold-change of spot percentages. For example, if corresponding protein spots from different samples (e.g. mutant versus wild type) have the same spot %, the difference field will show 1.0; if the spot % from the mutant is twice as large as wild type, the difference field will display 2.0 indicating 2-fold up regulation. If the spot % from the mutant has a value half as large, the difference field will display – 2.0 indicating a 2-fold down regulation.

Note that the montage panels cannot be individually contrasted and sometimes appear overly dark. However, image contrasting at any level does not affect the spot percentage data or any calculations.

MW and pI Measurements

Note that the isoelectric point (pI) measurements are approximate being based on the pH gradient plot included on the next page for this batch of ampholines for conditions of 9M urea and room temperature of 22°C. 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 4-8mix

Name: Jacqueline Pickel **Date:** 2/5/2013

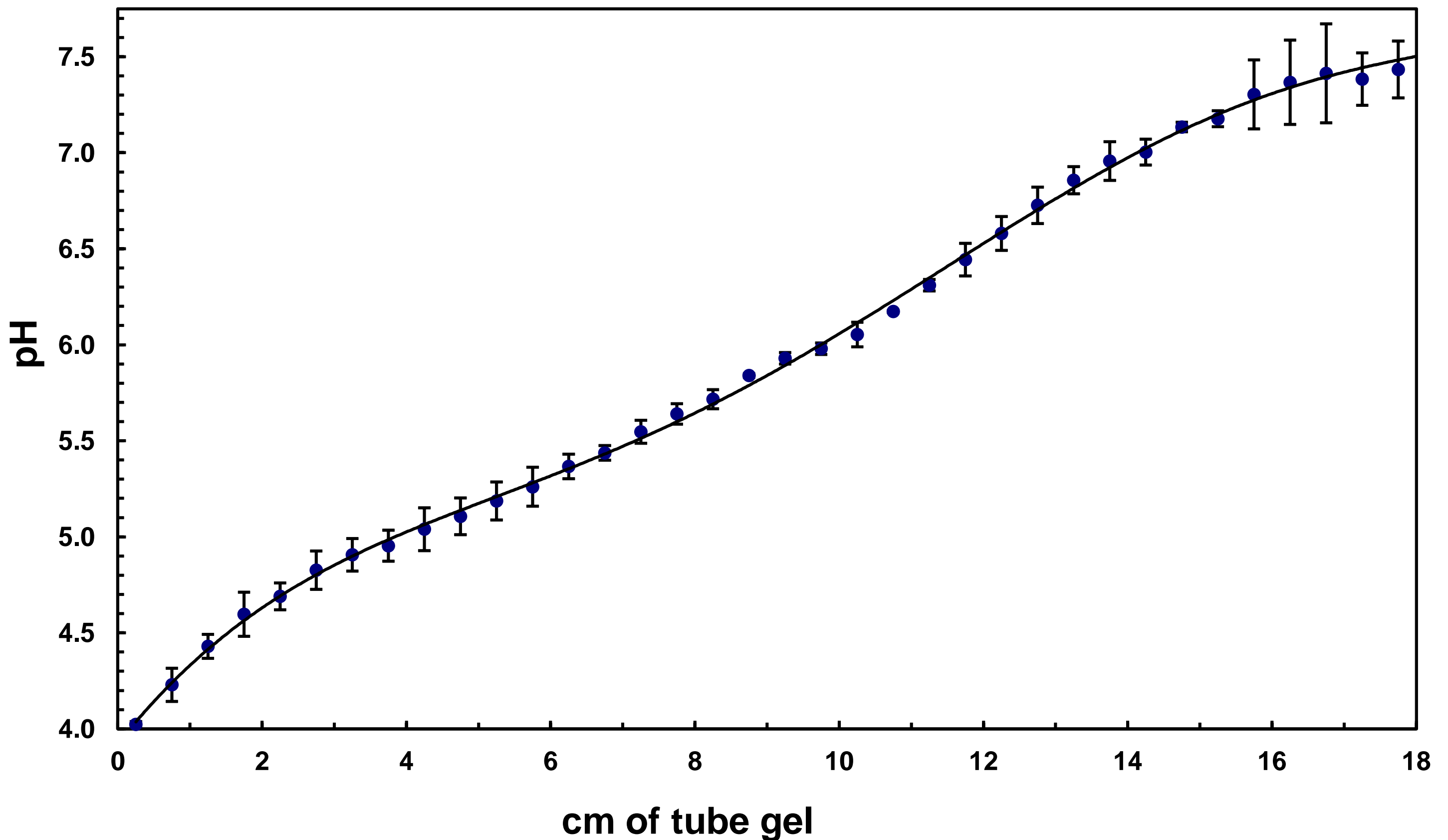
Sample(s): Pellets of *Neisseria gonorrhoeae* : BB22 FA1090 A25 PB(s) strain and BB22R1 FA1090 A25 PB(r) revertant

Ampholines: 1% pH 4-6 Serva & 1% 5-8 GE Healthcare

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

pH Gradient

p LF 496 1% pH 4-6 Serva & 1% pH 5-8 GE Healthcare + SDS



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.