

A Comprehensive Survey of Staining Methods in 2-D Gel Electrophoresis on Their Mass Spectrometry



Compatibility and Protein Detection by Peptide Mass Fingerprinting

Chau-Wen Chou and Ryan Dow, Proteomics Core Facility and Department of Physiology

Louisiana State University HSC-New Orleans, New Orleans, LA 70112

Overview

- To investigate differences in staining patterns of 4 popular stains and their correlations with the quality of MALDI MS results.
- Identical gels from dog heart proteins stained with Bio-Safe, Sypro Ruby, Silver Stain Plus, and Copper.
- Matched spots were subjected to MALDI MS analysis, and Mascot database search and the quantity analysis.
- Coomassie is shown to be the most compatible with MALDI MS analysis.

Introduction

2D gel electrophoresis and MALDI mass spectrometry have been the two major tools for protein identification (ID) by Peptide Mass Fingerprinting (PMF). Among the staining methods for the visualization of protein spots, Sypro Ruby, Coomassie Blue and copper are said to be most compatible with MALDI analysis. Silver staining is the traditional stain for sensitive protein detection, but not very compatible with MS analysis. In this study, we examine the sensitivity and relative quantity difference of protein detection among 2D gel images stained with these methods. Most spots that show significant differences are subjected to PMF and database search for MS compatibility study.

Method

- Protein Extraction:** Sliced tissue from dog heart was homogenized using 40mM Tris HCl, 2mM EDTA, 1% protease inhibitor and 1% Triton X-100.
- 2D Gel Electrophoresis:** Bio-Rad Protean IEF/Criterion PAGE.
- Staining Methods:** Sypro Ruby, Bio-Safe, Silver Stain Plus (a "MS-friendly" silver stain) and copper (a negative stain).
- Gel Visualization:** Bio-Rad GS-800 (all except Ruby stained gels) and GE Typhoon (Ruby) and analyzed by Bio-Rad PDQuest.
- Gel Spot Comparison:** Intensities of all spots were normalized according to the intensity sum of all matched spots in each gel.
- In-gel Digestion:** a PE MultiProbe II liquid handling system with a revised ZipPlate protocol from Millipore.
- Silver Destaining:** the Ferricyanide/thiosulfate method.
- MALDI MS Analyses:** α -Cyano-4-hydroxycinnamic acid is used as the matrix. All MALDI analyses were performed on an Applied Biosystems 4700 Proteome Analyzer and GPS (w/ Mascot) for database search.

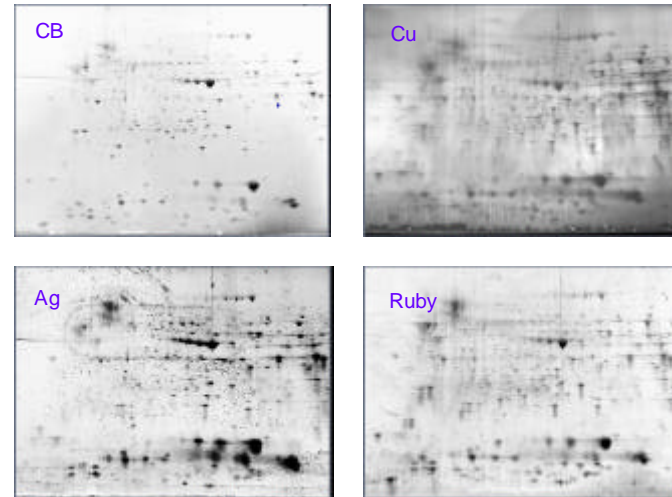


Figure 1 It illustrates the variation in staining pattern and spot intensity among four different gel stains. CB: Bio-Safe Coomassie Blue, Cu: copper (inverted negative image), Ag: Silver Stain Plus, Ruby: Sypro-Ruby.

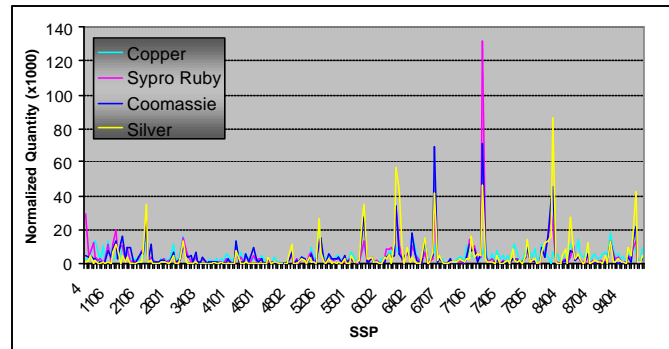


Figure 2 The normalized quantities of all matched spots in the different stains. Normalized quantity = (an individual spot) x1000 / (sum of all spots) in this gel.

Table 1 Comparative Spot Analysis and Match Rates

All match rates are determined by comparison to the master gel (highlighted in red).

Gel Name	Spots	Matched	Match Rate 1	Match Rate 2	Corr Coeff
*Coomassie	389	386	99%	100%	1
Copper	555	331	59%	85%	0.636
Silver	319	259	81%	67%	0.712
Sypro Ruby	579	348	60%	90%	0.877

Note: Match Rate 1 = (# of Matched)/(# of Spots) in this set.
Match Rate 2 = (# of Matched of this set)/(# of Matched in the Match set).

Results

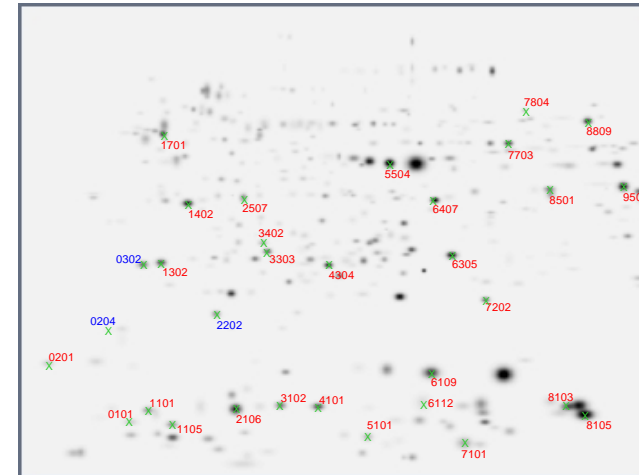


Figure 3 The locations of selected spots of interest. The spots marked in red SSP are spots that show significant differences in staining intensity among these stains. The spots in blue SSP are not detected in all 4 staining methods.

Table 2 The identifications of spots that do not show in all stains and their corresponding intensities (Figure 3 in blue).

SSP	Protein ID	Copper	Sypro Ruby	Bio-Safe	Silver-Plus
204	myosin light chain 2v, MLC2v-myosin light chain 2 isoform (mice_heart_Peptide_166.aal)	0.00	4.25	1.34	0.00
302	PREDICTED: similar to apolipoprotein A-I precursor - dog [Canis familiaris]	17.02	10.08	21.22	0.00
2202	PREDICTED: similar to Myosin light polypeptide 3 (Myosin light chain 1, slow-twitch muscle B/ventricular isoform) (MLC1SB) (Ventricular/slow twitch myosin alkali light chain) (Cardiac myosin light chain-1) (CMLC1) [Canis familiaris]	0.00	13.80	12.61	0.00

Summary

- Not all proteins respond to these 4 stains uniformly. Sypro Ruby and copper show similar sensitivity indicated by # of detected spots.
- Coomassie and Sypro Ruby show the highest degree of quantity correlation and are still the best options for total protein staining by yielding similar Match Rate 2 and correlation coefficient.
- Copper also demonstrates similar protein staining characteristics as Coomassie and Sypro Ruby. However, it has a higher background and is not compatible with the PDQuest integrated excision tool at this time.
- Coomassie blue is the most compatible with MS analysis yielding the highest protein scores. Sypro Ruby shows a lower compatibility with MS analysis. Silver yields the lowest protein scores of all stains subjected to MS analysis.

Table 3. Selected spots that show some significant variation in staining intensity (Figure 3 in red) and their corresponding protein scores and identifications.

SSP	Staining Method	BioSafe		Copper		Silver Plus		SyproRuby	
		Protein Score	Quantity (x10 normalized)	Protein Score	Quantity (x10 normalized)	Protein Score	Quantity (x10 normalized)	Protein Score	Quantity (x10 normalized)
101	ventricular myosin light chain 2 [Canis familiaris]	152	3.44	N/A	2.43	74	2.02	148	4.82
201	Chain H, Bovine F1-AtPase Inhibited By Dccid [Dicyclohexylcarbodiimide]	82	2.90	N/A	12.06	-	0.65	-	11.84
1101	Chain R, Bovine Heart Cytochrome C Oxidase At The Fully Reduced State	379	7.25	N/A	13.41	-	0.72	-	11.40
1105	ventricular myosin light chain 2 [Canis familiaris]	289	7.97	N/A	1.92	-	6.60	-	11.40
1302	similar to 17,000 dalton myosin light chain [Rattus norvegicus]	54	11.84	N/A	1.16	-	0.21	64	9.86
1402	haptoglobin heavy chain, HpH chain [dog_Peptide_245.aal]	253	15.64	N/A	9.83	253	4.40	50	10.08
1701	Chain F, Beryllium Fluoride Inhibited Bovine F1-AtPase	707	9.78	N/A	8.74	222	4.04	316	9.21
2106	fatty acid binding protein 3 [Sus scrofa]	380	26.03	N/A	18.66	170	34.66	188	24.33
2507	lactate dehydrogenase B [Bos taurus]	287	6.04	N/A	11.07	67	4.12	258	4.60
3102	heart fatty acid-binding protein [Sus scrofa]	424	9.91	N/A	16.37	97	12.83	184	14.69
3303	anti-oxidant protein 2 (non-selenium glutathione peroxidase, acidic calcium-independent phospholipase A2) [Bos taurus]	256	6.70	N/A	3.44	60	1.50	-	3.51
3402	anti-oxidant protein 2 (non-selenium glutathione peroxidase, acidic calcium-independent phospholipase A2) [Bos taurus]	462	3.20	N/A	2.98	-	0.38	-	2.41
	albumin [Canis familiaris]	318	-	-	-	49	-	90	-
4101	Cytochrome c oxidase VI [Bos taurus]	270	12.74	N/A	13.54	85	7.15	166	11.18
4304	creatine kinase (EC 2.7.3.2) chain M - dog	494	9.04	N/A	2.81	55	0.14	203	4.16
5101	hemoglobin beta chain - coyote (tentative sequence)	632	5.13	N/A	9.64	116	1.40	-	6.80
5504	creatine kinase (EC 2.7.3.2) (tentative sequence)	559	27.12	N/A	12.45	509	34.91	512	12.93
6109	myoglobin - African hunting dog (tentative sequence)	862	34.49	N/A	9.60	-	56.78	169	33.54
6112	hemoglobin alpha-I chain - dog	299	5.13	N/A	5.16	221	44.16	241	11.18
6305	Triosephosphate isomerase (TIM) (Triose-phosphate isomerase)	668	17.94	N/A	16.08	510	3.79	174	10.30
6407	creatine kinase (EC 2.7.3.2) chain M - dog	734	14.01	N/A	10.81	241	14.76	401	6.58
7101	hemoglobin alpha chain - coyote (tentative sequence)	397	11.54	N/A	4.10	90	2.50	150	14.47
7202	Phosphatidylethanolamine Binding Protein From Calf Brain	83	6.28	N/A	12.21	70	2.24	-	4.38
7703	beta beta enolase [Oryctolagus cuniculus]	260	9.24	N/A	11.69	161	14.22	-	7.01
7804	pyruvate kinase (EC 2.7.1.40), muscle splice form M1 [invalidated] - cat	434	1.27	N/A	8.34	-	2.66	139	1.75
8103	hemoglobin beta chain - coyote (tentative sequence)	625	16.13	N/A	3.86	409	15.95	83	16.22
8105	hemoglobin alpha chain - coyote (tentative sequence)	516	45.72	N/A	6.46	421	86.68	344	22.80
8501	malate dehydrogenase precursor (EC 1.1.1.37)	309	6.95	N/A	11.64	112	27.14	115	3.51
8809	ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit [Bos taurus]	682	0.42	N/A	2.75	124	0.69	137	0.44

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<http://www.medschool.lsuhs.edu/physiology/proteomics/>