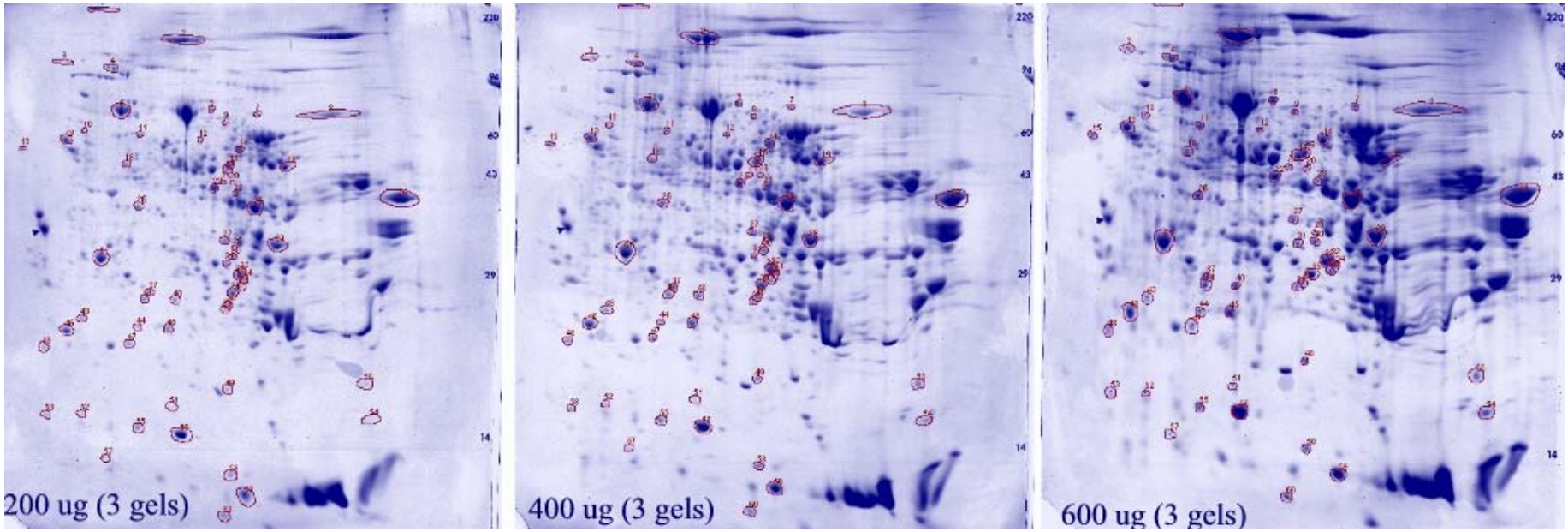
Evidence that our large format 2D system is robust for samples in SDS

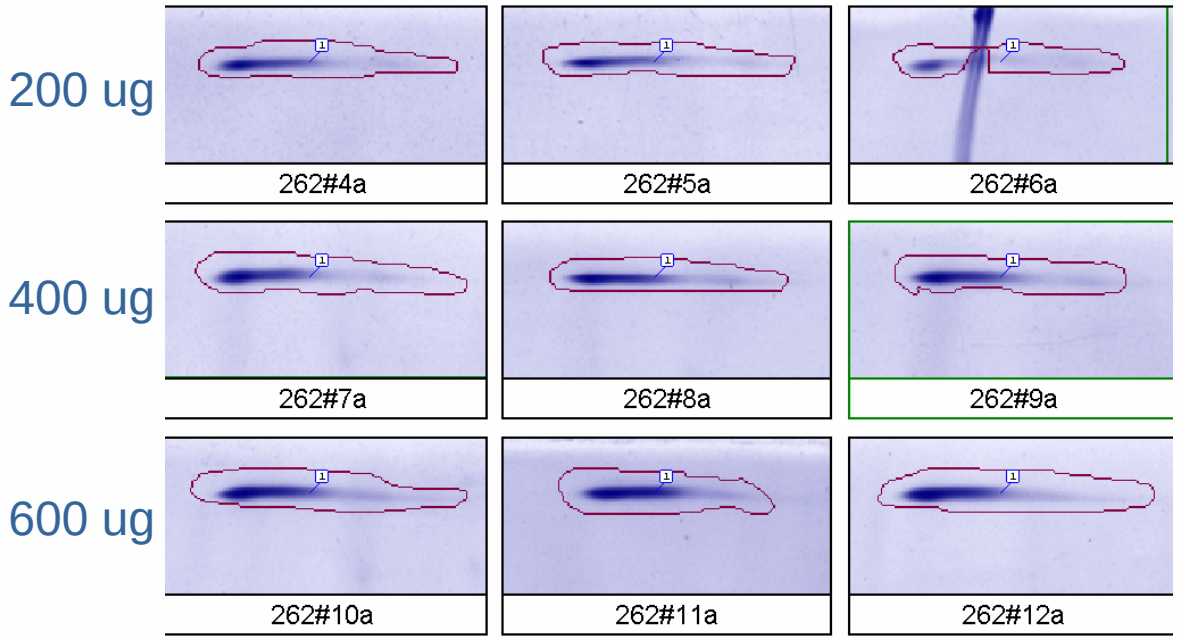
- Rat liver cytosol was run on large format 2D gels in triplicate at loads of 200, 400 and 600 µg according to Kendrick Labs SOPs.
- The gels were Coomassie blue stained and scanned with a laser densitometer linear over 0-3 OD.
- Sixty polypeptide spots were quantified with Progenesis software from Nonlinear Dynamics.

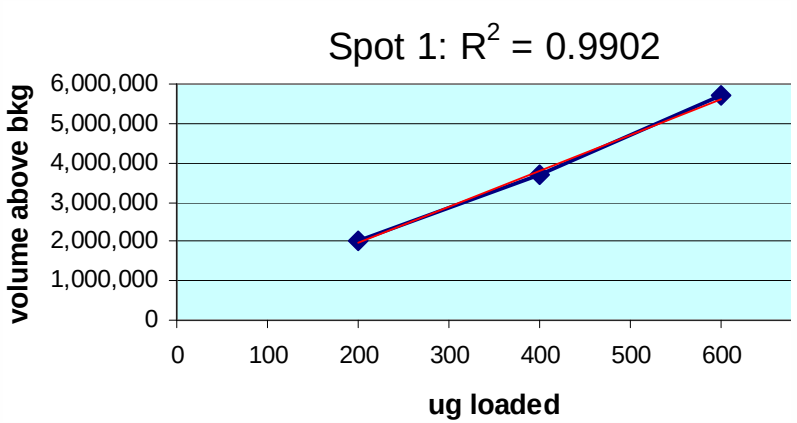
CA method of IEF allows SDS during sample preparation



Rat liver cytosol was diluted with buffer containing 2.5% SDS + 4.5 M urea before loading. Sixty analyzed spots are outlined in red.

Spot 1 montage: MW ~300,000, pI 5.7





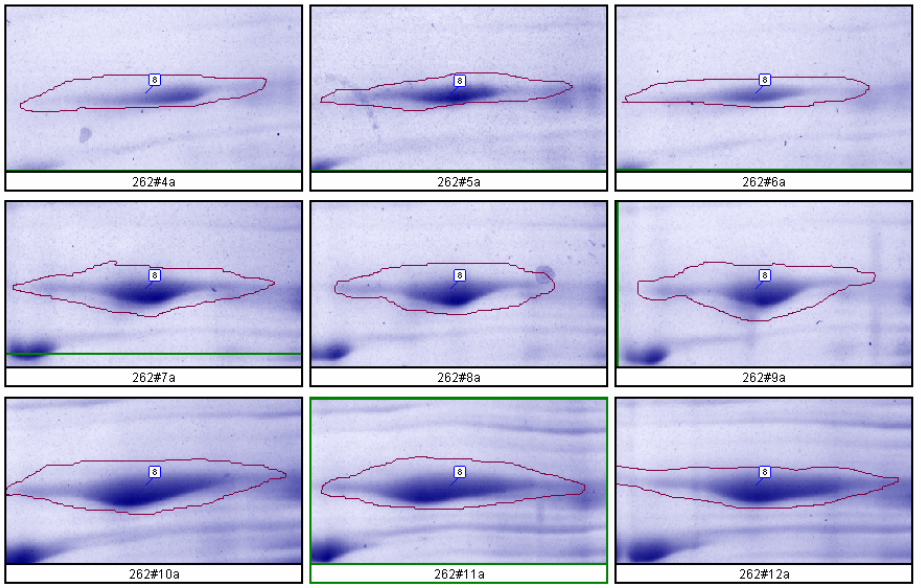
Plot of spot volume - bkg vs µg protein loaded

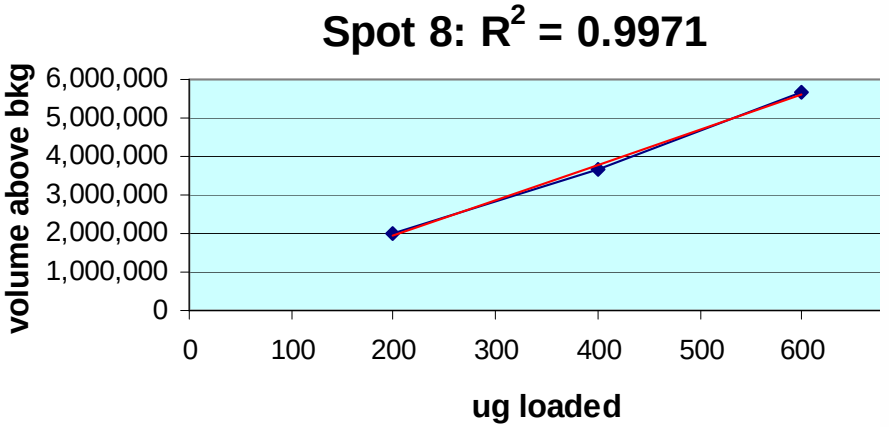
Spot 8 montage: MW 76,100, pI 8.1

200 ug

400 ug

600 ug





Plot of spot volume - bkg vs µg protein loaded

In reality, spot volume – bkg is not used for 2D gel quantification. Measurements are always normalized to correct for gel-to-gel staining differences by using spot %.

Spot % = (spot volume-bkg/all spot volumes -bkg combined) ×100

Results of spot volume and spot % measurements of 60 polypeptide spots on 9 gels

			CV (n=3)	CV (n=3)	CV (n=3)	linearity	CV (n=9)
Spot	pI	MW	200 ug	400 ug	600 ug	R^2	Spot %
1	~5.7	~300,000	16%	9%	7%	0.9902	12
2	6.3	176,653	24%	21%	9%	0.9985	16
3	5.4	131,000	8%	10%	13%	0.9737	13
4	5.7	118,500	2%	7%	10%	0.7858	20
5	6.7	79,852	11%	8%	9%	0.9977	12
6	5.8	78,369	4%	8%	12%	0.9889	9
7	7.4	78,255	6%	8%	15%	0.9981	14
8	8.1	76,087	9%	2%	2%	0.9971	4
9	6.9	73,919	18%	14%	16%	0.9837	16
10	5.5	67,872	4%	10%	4%	0.9822	8
11	6.0	65,477	4%	13%	11%	0.9940	8
12	6.5	63,993	7%	16%	13%	0.9982	15
13	5.4	60,799	3%	1%	7%	0.9902	4
14	7.1	60,342	2%	14%	5%	0.9852	10
15	4.9	58,369	8%	7%	5%	0.9999	14
16	7.0	54,134	11%	8%	11%	0.9836	16
17	6.9	52,775	8%	17%	5%	0.9778	24
18	5.9	52,616	5%	9%	9%	0.9689	11
19	7.6	52,010	6%	6%	3%	0.9794	8
20	7.0	48,186	37%	10%	13%	0.9582	34
21	6.8	47,676	17%	6%	7%	0.9986	17
22	6.7	44,361	7%	8%	9%	0.9905	15
23	7.1	43,597	36%	27%	8%	0.9706	24
24	9.4	41,012	10%	4%	9%	0.9887	8
25	7.4	40,857	8%	3%	8%	0.9999	4
26	6.0	40,547	5%	6%	14%	0.9940	8
27	6.8	37,614	10%	17%	4%	0.9999	13
28	7.1	36,279	32%	9%	16%	0.9837	23
29	7.5	35,927	1%	13%	16%	0.9850	12
30	7.1	35,046	1%	1%	7%	0.9921	8

			CV (n=3)	CV (n=3)	CV (n=3)	linearity	CV (n=9)
Spot	pI	MW	200 ug	400 ug	600 ug	R^2	Spot%
31	6.9	34,314	13%	18%	6%	0.9983	18
32	7.2	32,845	13%	20%	26%	0.9961	15
33	7.1	32,581	9%	4%	7%	0.9765	11
34	7.2	31,906	7%	5%	18%	0.9999	9
35	7.2	31,084	3%	12%	12%	0.9942	37
36	7.0	30,497	4%	5%	6%	0.9977	7
37	6.1	29,480	5%	31%	9%	0.9722	16
38	6.9	29,365	10%	3%	11%	0.9810	10
39	6.9	28,793	10%	8%	8%	0.9998	22
40	6.3	28,735	5%	6%	12%	0.9990	5
41	6.0	28,676	7%	18%	7%	0.9934	14
61	5.7	33,773	2%	1%	6%	0.9970	6
43	5.6	27,013	9%	8%	2%	0.9994	10
44	6.0	26,191	19%	1%	7%	0.9950	26
45	6.2	26,191	5%	1%	16%	0.9909	17
46	5.4	25,722	3%	2%	5%	0.9972	5
47	5.9	24,782	7%	7%	7%	0.9992	9
48	5.3	24,352	2%	3%	14%	0.9982	12
49	6.9	22,023	6%	7%	18%	0.9960	16
50	8.9	21,160	12%	10%	7%	0.9916	24
51	6.3	19,460	12%	5%	8%	0.9994	28
52	5.6	18,619	17%	2%	10%	0.9994	22
53	5.3	18,286	20%	11%	15%	0.9943	15
54	9.0	17,939	9%	8%	5%	0.9665	60
55	6.0	17,464	18%	6%	20%	0.9999	12
56	6.3	17,034	7%	0%	5%	0.9999	7
57	5.7	14,823	62%	4%	24%	0.9999	36
58	6.9	14,490	2%	1%	15%	0.9975	6
59	7.2	10,136	3%	2%	6%	0.9972	13
60	6.7	4,075	13%	5%	18%	0.9846	13
All Spots:		Ave	11%	8%	10%	0.9874	15

Spots with high error (colored cells) can be explained with one exception (pink): orange = splitting problem; green = faint spot on low load gel

Summary

- Average CV* for spot volume measurements =10%; average CV* for spot % = 15%
- Average R^2 value** for “spot volume vs µg loaded” plots = 0.9874
- Spots with high error can be explained (low abundance proteins, splitting problems) with one exception
- Our carrier ampholine 2DE system resolves protein mixtures reproducibly and quantitatively for samples prepared with 2.5% SDS.

*540 polypeptide spots, **60 plots