Avoiding acidic region streaking in two-dimensional gel electrophoresis: Case study with two bacterial whole cell protein extracts

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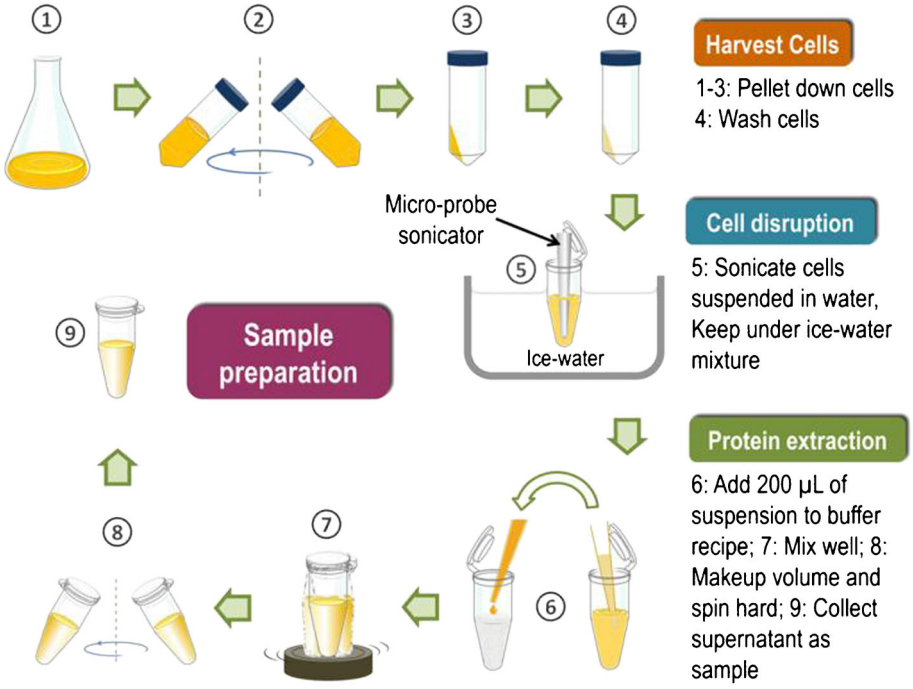
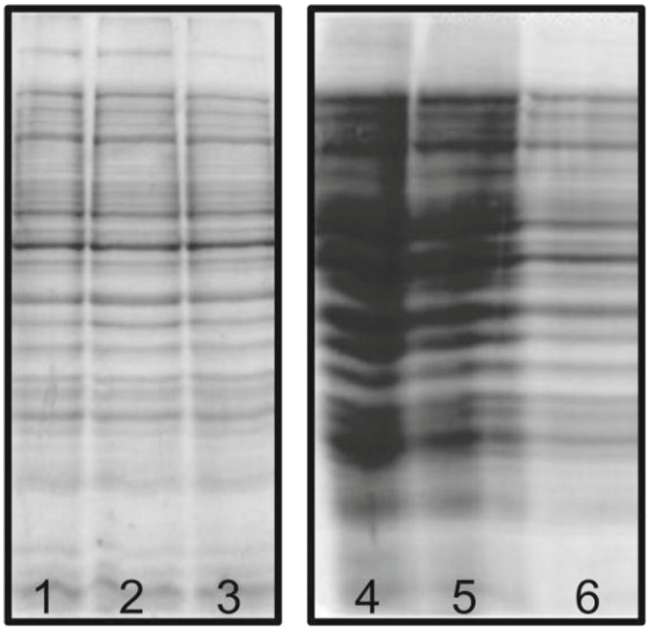
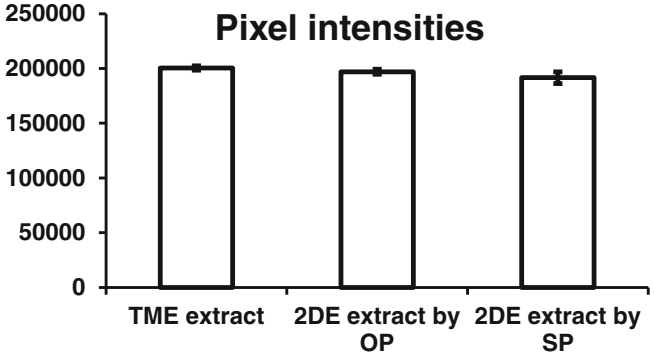
Supplementary material



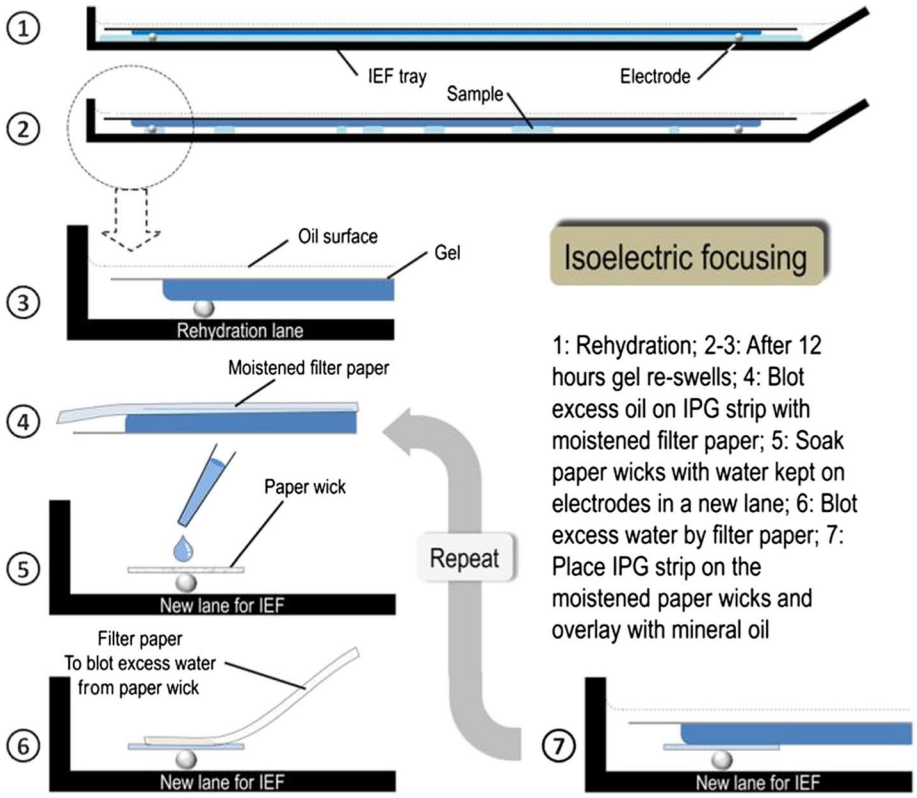
Supplementary figure 1. WCPE sample preparation from *E. coli* for 2DE by our protocol. Note that cells are sonicated in pure water rather than in viscous rehydration buffer. After cell lysis urea, thiourea, CHAPS, DTT and ampholyte are added.



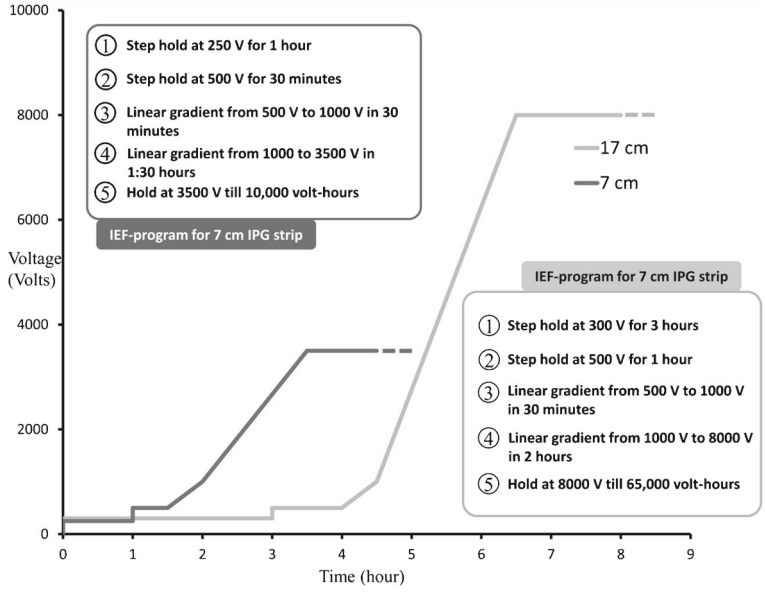
Supplementary figure 2. Comparison of protein content in *E. coli* WCPE by SDS-PAGE. Lanes marked represent (1) TME extract; (2) 2DE extract by OP; (3) 2DE extract by SP; (4) 2DE extract by SP (5) 2DE extract by SP and then DNase/RNase treated; (6) 2DE extract by SP and then one round of phenol/chloroform/isoamyl alcohol treatment. Equal volumes of sample were loaded in lanes 1, 2 and 3; and equal volumes of sample were loaded in lanes 4, 5 and 6. There is a notable amount of protein loss in lane 6.



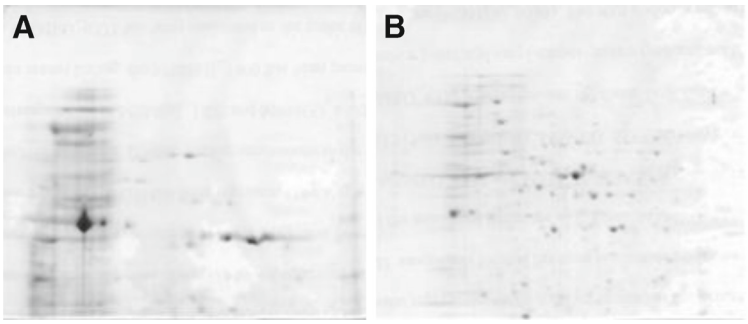
Supplementary figure 3. Pixel intensities of lane 1, 2 and 3 from figure S2 above. Pixel intensity was calculated for the same area and each lane was sampled thrice. Error bars indicate standard deviation.



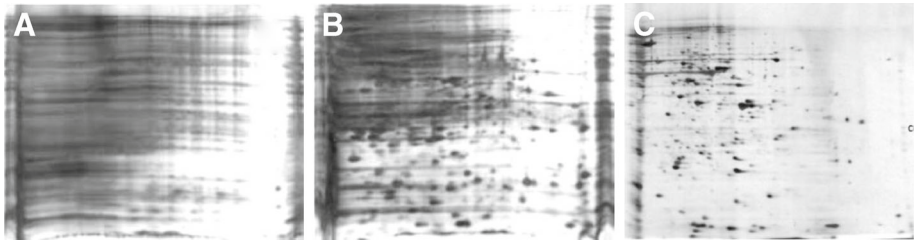
Supplementary figure 4. Schematic representation of paper wick position and changing strategy.



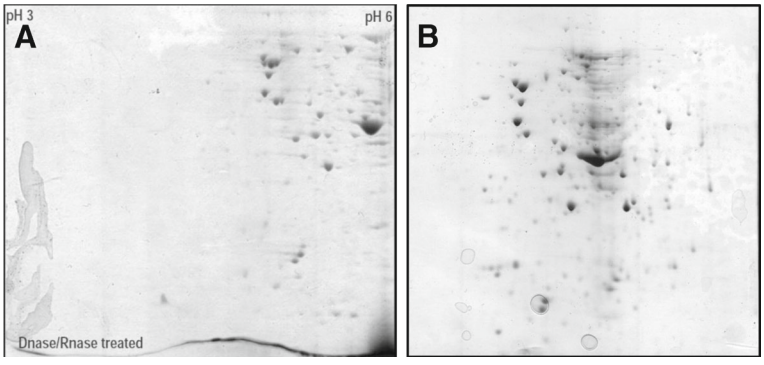
Supplementary figure 5. Schematic representation of IEF-program for 7 cm and 17 cm IPG strips.



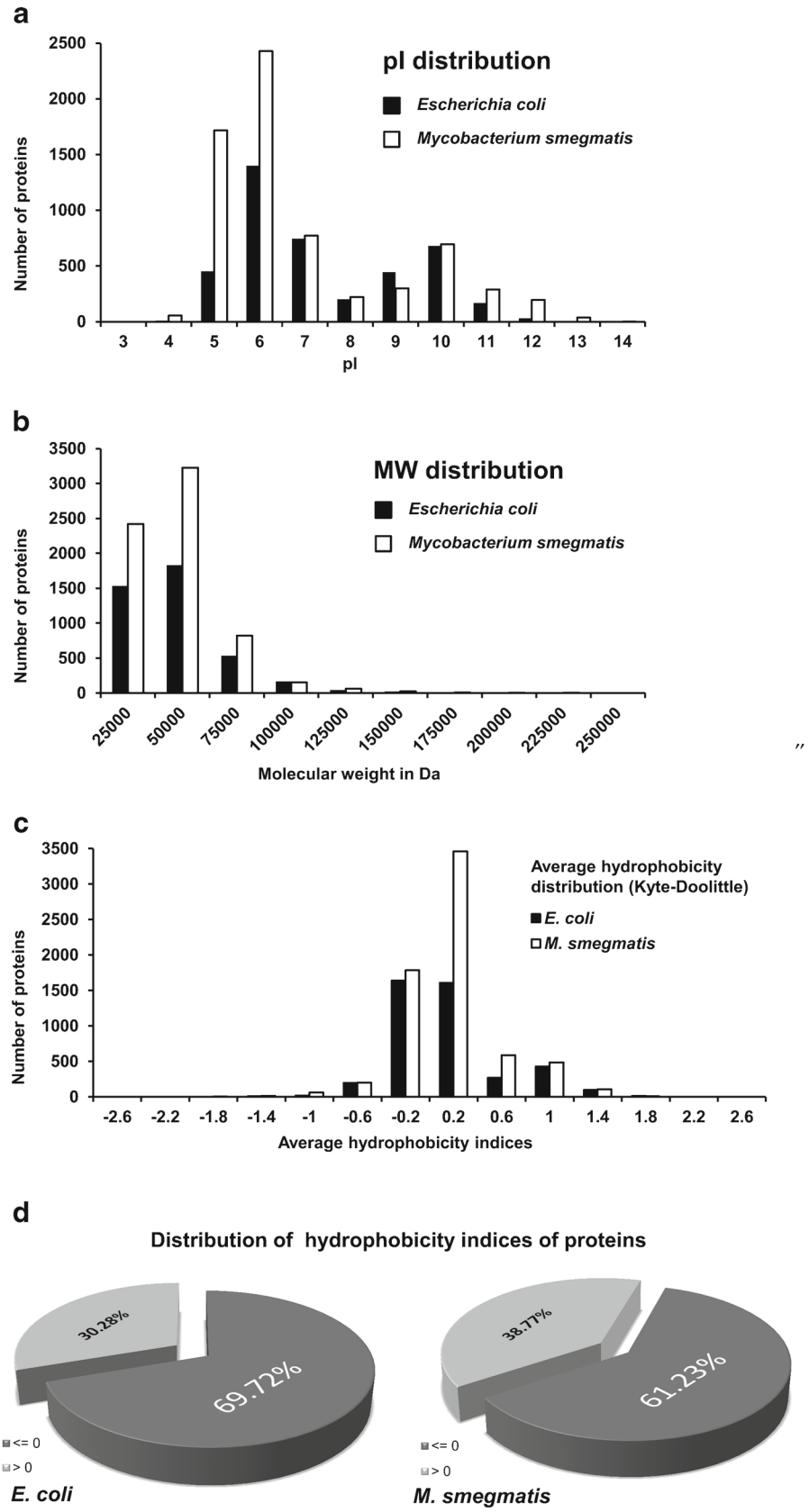
Supplementary figure 6. 2DE gel images of *E. coli* WCPE extracted by SP on pH 4-7, 7 cm IPG strip. Buffer recipe were **(A)** 8 M urea, 2% CHAPS, 50 mM DTT and 0.2% ampholyte and **(B)** 7 M urea, 2 M thiourea, 2% CHAPS, 50 mM DTT and 0.2% ampholyte, respectively.



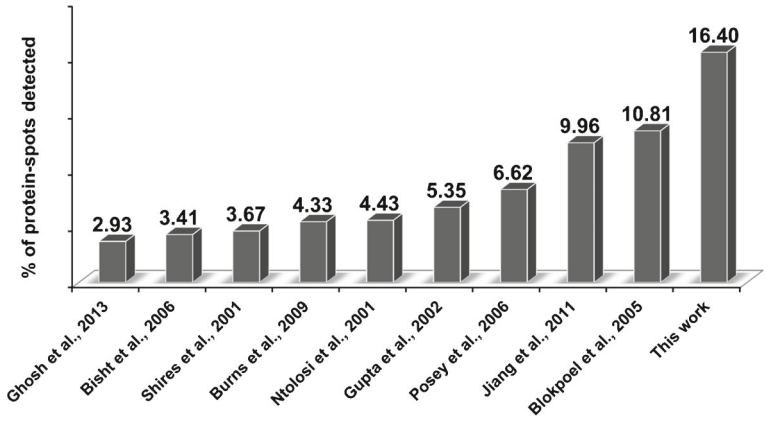
Supplementary figure 7. 2DE gel images of *E. coli* WCPE extracted by SP run on pH 5-8 7 cm IPG. (**A**) 50 µg protein before cleanup (**B**) 50 µg protein after cleanup and (**C**) 10 µg protein after cleanup.



Supplementary figure 8. 2DE images of *E. coli* WCPE **(A)** prepared by O and then DNase/RNase treated run on pH 3-6 7 cm IPG strip **(B)** prepared by O run on pH 4-7 7 cm IPG strip with excess water on paper wicks.



Supplementary figure 9. Differences in expressed proteins of *E. coli* and *M. smegmatis*.



Supplementary figure 10. Comparison of protein recovery in terms of detected spots between all published 2DE gels with *Mycobacterium smegmatis* and this work.

Supplementary table 1. Comparative analysis of ARS in 2DE images produced by O and in few recently published 2DE gel images of *E. coli* WCPE

Article detail	Length of longest streak in pH unit	Number of acidic streaks
Fedyunin *et al*. 2012	4.02	6
Zuo *et al*. 2000	2.54	9
Valenete *et al*. 2012; Fig. 5E	2.43	3
Nandnakumar *et al*. 2003	2.355	10
Cheung *et al*. 2012	2.35	10
Choe *et al*. 2000	2.34	6
Valenete et al. 2012; Fig. 5D	2.15	10
Qi *et al*. 2013; Fig. 1B	2.13	10
Kim *et al*. 2012; Fig. 5A	1.74	8
Kim *et al*. 2012; Fig. 5B	1.64	10
Smejkal *et al*. 2006	1.38	10
Herbert *et al*. 2006	1.29	10
Qi *et al*. 2013; Fig. 1A	1.19	8
Han *et al*. 2005	1.18	10
Riley *et al*. 2012; Fig. 2C	1.1	10
Ramachandran *et al*. 2012; Fig. 5A	1.06	4
Piras *et al*. 2012	1.06	4
Aich *et al*. 2012	1.03	9
Ramachandran *et al*. 2012; Fig. 5C	1.01	8
Schliep *et al*. 2012	0.76	8
Riley *et al*. 2012; Fig. 2A	0.68	10
Aich *et al*. 2012	0.65	6
This work	0.43	1

Supplementary table 2. Comparative analysis of ARS and spots detected in 2DE images produced by O and in few recently published 2DE gel images of *M. smegmatis* WCPE

Article details	% of proteins detected on average	Length of longest streak in pH unit	Number of acidic streaks
Bisht *et al*. 2006	3.413940256	0	0
Shires *et al*. 2001	3.66542871	0	0
Ntolosi *et al*. 2001	4.432423704	0	0
Gupta *et al*. 2002	5.351976856	0	0
Blokpoel *et al*. 2005	10.81081081	0.61	10
Jiang *et al*. 2011	9.957325747	1.55	10
Ghosh *et al*. 2013	2.926234505	2.43	10
Posey *et al*. 2006	6.624669783	3	8
Burns *et al*. 2009	4.327466494	3.89	10
This work	16.39910587	0.212	3

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