GeLC Checklist

Optimize Your Results

- Load the greatest quantity possible
- Determine the number of gel sections (**S**)
    - The more sections, the greater the depth and sensitivity of the result. (Greater fractionation.)
- Optimal gel thickness is 0.75 – 1.0mm; avoid 0.5mm or 1.5mm gels when possible.
- Excise within the edge of each lane – avoid excising an excess area
- In multiple lane experiments, run a blank lane with buffer between samples. Be cautious of spillover in the wells when loading.

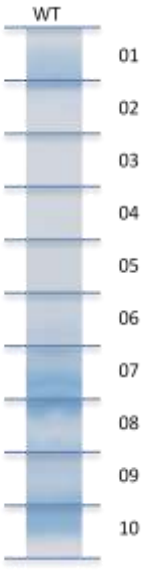
Determine How Far to Run the Sample into the Gel (L)

- The distance that you will run the sample into your gel (**L**) is determined by three parameters:
    - The number of sections (**S**), (minimum =7): *Your choice.* **S =** _____
    - The width of the lane in mm (**W**): *Determined by your apparatus:* **W =** _____*mm*
    - The optimal area for a gel section is 25 to 35 sq mm (**A**): *Fixed range.*
- Thus the maximum and minimum distance (**L**) you will run your proteins is:
    - Maximum (in mm) = **S** x (35 / **W**)
    - Minimum (in mm) = **S** x (25 / **W**) or 25mm (2.5cm), whichever is greater.
    - For example:
        - 10 sections on a gel with 8 mm wide lanes should be run between 31 and 44 mm, e.g. (10 x 25 / 8) and (10 x 35 / 8).
        - 7 sections on a gel with 10mm wide lanes should be run the minimum of 25mm because the max and min calculations are both less than 25mm.

# Sections (S)	Width of Lane in mm (W)			
	4	5	8	10
6	38-53	30-42	25	25
7	44-61	35-49	25-31	25
8	50-70	40-56	25-35	25-28
9	56-79	45-63	28-39	25-32
10	63-88	50-70	31-44	25-35
11	69-96	55-77	34-48	28-39
12	75-105	60-84	38-53	30-42
13	81-114	65-91	41-57	33-46
14	88-123	70-98	44-61	35-49
15	94-131	75-105	47-66	38-53
16	100-140	80-112	50-70	40-56

Labeled Photo of the Gel

- Take a photo of the stained gel – minimize handling
    - This can be done however you like but avoid handling that might reintroduce dust/epidermal protein contamination. A photo in the staining tray is sufficient as long as the density of staining is clear.
    - Drop this image into PowerPoint. Avoid using uncompressed TIFF or BMP files due to their large file size.
    - Using the drawing tools, draw horizontal lines where the gel sections are taken
    - Label the gel image as follows:
        - Sections should be numbered top to bottom with the highest MW = 1 and the lowest MW = S, where S equals the number of sections.
        - The top of each lane should be labeled with a short but relevant to the experiment name. (Try to avoid using just your initials.) Add text that briefly describes what each lane is.
        - The sample names are constructed by the lane names appended with each section number, e.g. WT1, WT2, WT3… or KO1, KO2, KO3, etc.
        - If the experiment is a comparison, clearly define which lanes are being compared, which are controls, whether you are interested in increases or decreases (or both) in one over the other.



Delivery

- The labels on the gel image, on the tubes and on the forms must agree.
- Labeled image should be emailed as a PowerPoint or PDF at the same time as the sample shipment or the queuing of the samples will be delayed.
- Forms should accompany the samples, one form per tube; please do not email sample submission forms.

HARVARD UNIVERSITY

Mass Spectrometry and Proteomics Resource Laboratory

HPLC MS/MS and PROTEOMICS for a Series Submission (GeLC)

Your Name: _____ Date: _____

Sample Name: _____ Phone: _____

Email: _____

Sample Information:

SAMPLE SET #X (use 1 form per sample set)						
Protein Name						
Number of Gel slices						
Organism source protein of interest						
Organism of any other species present in sample provided including vectors, baits etc.						
Name of Lane						
Stain (type and source)						
Notes:						
Protein ID	Gel slice ID#	Lane Name	MW (kDa)	Weight (ug)	Amount (pmol)	Wash with 50% ACN/water?
	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
	9					
	10					
	11					
	12					

Billing Information:

Principal Investigator: _____

Institution: _____

Billing Address: _____

Purchasing Agent Phone: _____

PO#: _____

or Harvard 33-digit Account: _____

NOTE: *We cannot begin work until we have a confirming copy of the P.O. (faxed or emailed to us prior to work start)*

For Lab Use Only: observed staining___ surface area___ est. weight___ est. pmole___ Dig vol____Buffer_____ Enzyme____ E/S ratio____
Dig time_____ Ready Vol_____ Ready date_____ % original sample used_____

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