H69 cells pellet
Lysis with 1% SDS, 3 mM MgCl₂, 10 mM CHAPS
Protease inhibitor cocktail, 100 mM TEAB.

DNA and RNA degradation
Add Benzonase Nuclease prepared in 50 mM Tris, 20 mM NaCl, 2mM MgCl₂
30 min incubation at 4°C under agitation.

BCA protein quantitation and 50 μg
Protein aliquot separation

pFASP 10 kDa
50 μg x 3

pFASP 30 kDa
50 μg x 3

FASP
50 μg x 3

eFASP
50 μg x 3

MSTern-Blot
50 μg x 3

In-solution
50 μg x 3

Protein reduction with 20 mM DTT 5 min at 95°C then Alkylation of cysteine with 40 mM IAA at room temperature in dark for 45 min.

Mix 8 Volume of 8M urea, 10% isopropanol in 100 mM TEAB with protein mixture. Pre-wet the 10 kDa plate filter with 60% isopropanol

Mix 8 Volume of 8M urea, 10% isopropanol in 100 mM TEAB with protein mixture. Pre-wet the 30 kDa plate filter with 60% isopropanol

Mix 8 Volume of 8M urea, 100 mM Tris-HCl pH 8 with protein mixture. Pre-wet the Microcon filter unit with 60% Methanol

Mix 8 Volume of 8M urea, 0.2% DCA in 100 mM Tris-HCl pH 8 with protein mixture. Rinse the Microcon filter unit 3x with Ultrapure H₂O

Mix 8 Volume of 8M urea in 100 mM ABC with protein mixture. Wet the PVDF membrane with 70% ethanol and equilibrated with 8 M urea, 100 mM ABC

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Pellet Protein by centrifugation at 12,000 g for 10 min. Acetone was removed and protein resuspended in 100 mM TEAB

96-well plate
Transfer to plate filter
• spin 30 min at 3,100 x g
• 2x wash with 8M urea, 10% isopropanol, 100 mM TEAB
• spin 30 min at 3,100 x g

96-well plate
Transfer to plate filter
• spin 30 min at 3,100 x g
• 2 wash with 8M urea, 10% isopropanol, 100 mM TEAB
• spin 30 min at 3,100 x g each

96-well plate
Transfer to plate filter
• spin 15 min at 14,000 x g
• 2 wash with 8M urea in 100 mM Tris-HCl pH 8
• spin 15 min at 14,000 x g each

96-well plate
Transfer to plate filter
• spin 15 min at 14,000 x g
• 2 wash with 8M urea, 0.2% DCA in 100 mM Tris-HCl pH 8
• spin 15 min at 14,000 x g each

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96-well plate
Transfer to plate filter
• spin 15 min at 14,000 x g
• 2 wash with 8M urea, 100 mM Tris-HCl pH 8
• spin 15 min at 14,000 x g each

Urea removed by
• 2 washes with 50 mM TEAB, 10% isopropanol
• 1 wash with 50 mM TEAB
• All spins 30 min at 3,100 x g
• add 1 µg Trypsin in 50 mM TEAB. Digestion overnight at 37°C

Urea removed by
• 2 washes with 50 mM TEAB, 10% isopropanol
• 1 wash with 50 mM TEAB
• All spins 30 min at 3,100 x g
• add 1 µg Trypsin in 50 mM TEAB. Digestion overnight at 37°C

Urea removed by
• 3 washes with 100 mM Tris-HCl pH 8, spin 15 min at 14,000 x g each
• add 1 µg Trypsin in 50 mM ABC. Digestion overnight at 37°C

Urea removed by
• 3 washes with 50 mM ABC, 0.2% DCA
• spin 15 min at 14,000 x g each
• add 1 µg Trypsin in 50 mM ABC. Digestion overnight at 37°C

Urea removed by
• 1 wash with 8M Urea in 50 mM ABC
• 2 washes with 50 mM ABC
• liquid transfers done with vacuum manifold.
• add 1 µg Trypsin in 50 mM ABC. Digestion overnight at 37°C

Peptide recovered in 2 washes with 50 mM TEAB 10 min at 3,100 x g each. Peptide desalted by Zip-Tip

Peptide recovered in 2 washes with 50 mM TEAB 10 min at 3,100 x g each. Peptide desalted by Zip-Tip

Peptide recovered in 2 washes with 50 mM ABC 5 min at 14,000 x g each. Peptide desalted by Zip-Tip

Peptide recovered in 2 washes with 50 mM ABC 5 min at 14,000 x g each. Peptide desalted by Zip-Tip

Peptide recovered by 2 washes with 50 mM ABC 5 min at 14,000 x g each. Peptide desalted by Zip-Tip

Peptide recovered by 2 washes with 40% ACN, 0.1% TFA with vacuum manifold. Dried then desalted by Zip-Tip

26 hours

26 hours

24 hours

24 hours

22 hours

40 hours

LC-MS Analysis