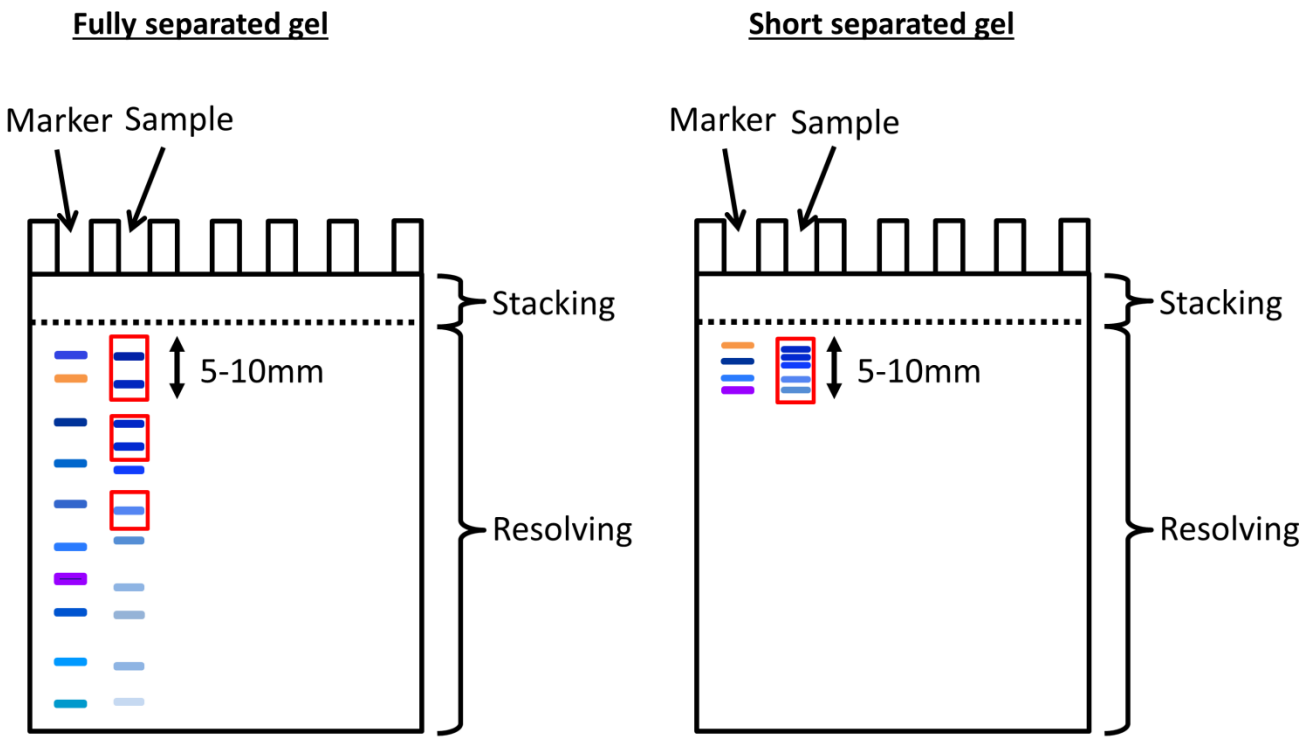
Short Gel Fractionation for Quick Assessment of Proteomic Samples:

During a typical mini-gel SDS-PAGE fractionation, proteins migrate 7–8 cm and the gel lane is divided into 10–24 fractions, each to be processed separately via GeLC-MS/MS. However, to gain a quick assessment of the major proteins present in complex samples short gel SDS-PAGE fractionation is commonly used, wherein proteins are migrated only 1 cm into the gel, after which a single 1 cm gel slice is processed per lane.

SDS-PAGE analysis:

Below is our recommended protocol for running short separated gels based on Invitrogen Minigel system. The actual voltage setting and run time may vary for different gel apparatus.

Obtain a pre-stained molecular weight ladder standard such as Invitrogen's SeeBlue Plus2 Pre-stained Protein Standard (ThermoFisher LC5925). Load 6µl of your pre-stained standard into one lane and your sample into another lane of a 4-12% Bis Tris SDS-PAGE gel (ThermoFisher NP0321BOX). Run gel at 150 V for about 10-12 minutes or until sample enters fully into gel. Wash the gel with two quick 5 minute washes and stain gel for 30 minutes using SimplyBlue Safe Stain (ThermoFisher LC6060). Destain gel with two 5 minute washes and then continue to destain overnight. Excise your sample from gel and place into a 1.5mL Eppendorf microcentrifuge tube. Add 200µl of ultrapure water or enough to cover the gel. Gel bands can be stored in 4˚C until ready to be delivered to BioMS Center.



Example publications using short separated gel

1. Raman D, Neel NF, Sai J, Mernaugh RL, Ham AJ, Richmond AJ. Characterization of chemokine receptor CXCR2 interacting proteins using a proteomics approach to define the CXCR2 "chemosynapse". Methods Enzymol. 2009; 460:315-30. PubMed PMID: 19446732; PubMed Central PMCID: PMC3140414.
2. Thakur D, Rejtar T, Wang D, Bones J, Cha S, Clodfelder-Miller B, Richardson E,Binns S, Dahiya S, Sgroi D, Karger BL. Microproteomic analysis of 10,000 laser captured microdissected breast tumor cells using short-range sodium dodecyl sulfate-polyacrylamide gel electrophoresis and porous layer open tubular liquid chromatography tandem mass spectrometry. J Chromatogr A. 2011 Nov11; 1218(45):8168-74. PubMed PMID: 21982995; PubMed Central PMCID: PMC3205921.
3. Paulo JA, Kadiyala V, Brizard S, Banks PA, Conwell DL, Steen H. Short Gel, Long Gradient Liquid Chromatography Tandem Mass Spectrometry to Discover Urinary Biomarkers of Chronic Pancreatitis. Open Proteomics J. 2013; 6:1-13. PubMed PMID: 25346780; PubMed Central PMCID: PMC4207084.