In 2-D DIGE, protein samples are labeled with size and charge-matched CyDye™ fluoros. Three fluorescently labeled protein samples can be combined and resolved using 2-D gel electrophoresis: isoelectric focusing (IEF) in the first dimension and SDS-PAGE in the second dimension. Then, the gel is scanned using the highly sensitive Typhoon scanner, and DeCyder software is used to automatically locate and analyze the protein spots. Protein spots are excised from the gel using a robot picker, and the protein ID is analyzed using either MALDI-TOF/TOF MS or ESI-QTOF MS/MS.

Advantages of 2D-DIGE:
- Separation of samples in a single gel eliminates gel-to-gel variation.
- Isoforms can be directly visualized.
- Provide greater statistical confidence and level of reliability with its internal control.
- 2-D DIGE is highly sensitive and quantitative. Detect as low as 2 ng of a single protein.