

## **Image analysis**

- (1) CBB-stained gels were analyzed with Image Master 2D-Elite software (GE Healthcare). 2D-PAGE gels using IEF (around pI 3.5 to 8.0) and IPG (pI 6.0 to 10.0) for first dimension were overlapped around pI 5.6. The proteins in edges of each gels were carefully overlapped the corresponding proteins. 2D-PAGE images were formed and evaluated automatically, and the amount of protein in each spot was estimated with Image Master 2D Elite software. Specially, spot detection, spot measurement, background subtraction and spot matching were performed. Following automatic spot detection, gel images were carefully edited. Prior to performing spot matching between gel images, one gel image was selected as the reference gel.
- (2) After automatic matching, the unmatched spots of the member gels were added to the reference gel. The amount of a protein spot was expressed as the volume of the spot which was defined the sum of the intensities of all the pixels that make up the spot. In order to correct the variability due to CBB staining and reflect the quantitative variations in intensity of protein spots, the spot volumes were normalized as a percentage of the total volume in all of the spots present in gel.
- (3) The resulting data from image analysis were transferred to 2D Elite software or PDQuest software (Bio-Rad) for querying protein showing quantitative or qualitative variations.