

Direct PMF Analysis on Membrane using Chemical Inkjet Printer (CHIP-1000)

Protein spots on 2-dimensional electrophoresis-separated gel (2-DE gel) of *Escherichia coli* extract were transferred to a PVDF membrane. Microscale trypsin digestion on membrane for these protein spots was conducted using the chemical inkjet printer. Direct peptide mass fingerprinting (PMF) analysis on membrane with AXIMA-CFR was performed for the resulting peptide fragments, and each protein spot was identified based on a database search.

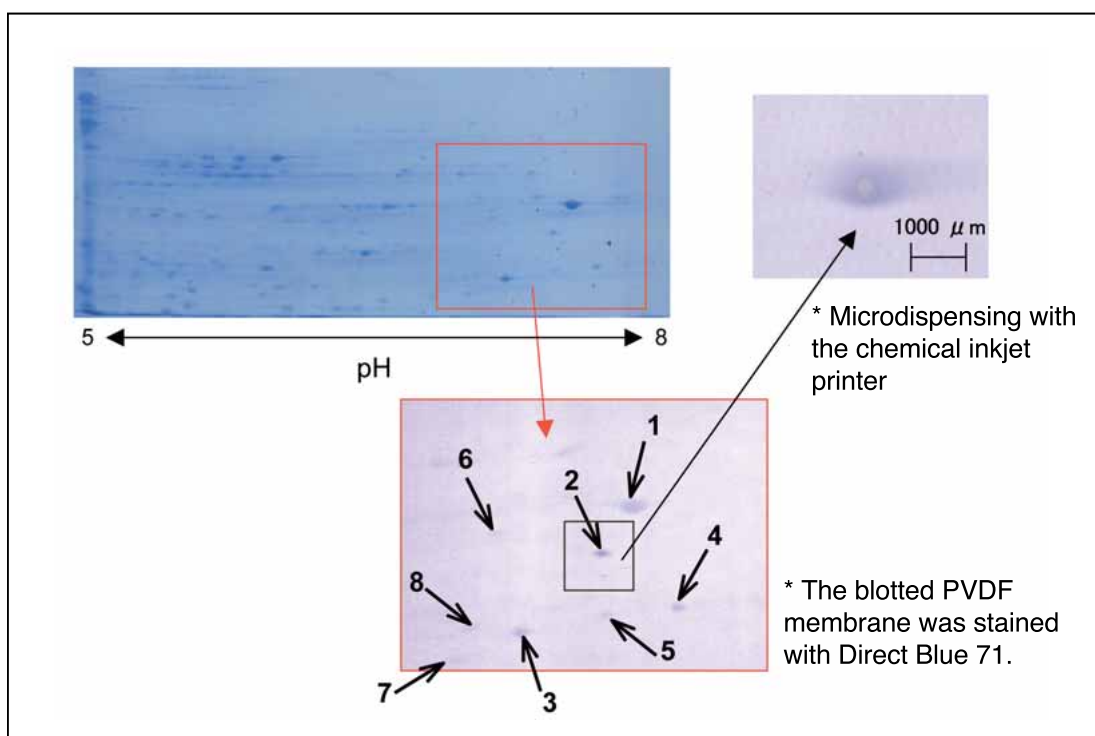


Figure 1 Image of 2-DE gel of *Escherichia coli* extract and the blotted PVDF membrane

Protein spots were electroblotted from the 2-DE gel of *Escherichia coli* extract to the PVDF membrane according to the standard semi-dry blotting method. After staining the blotted membrane with Direct Blue 71, the membrane was attached to the MS target plate using conductive double-sided adhesive tape. Subsequently, an image of the stained membrane was captured by using the scanner feature of the chemical inkjet printer. Target protein spots were selected on the resulting image, and reagents for trypsin digestion on membrane were microdispensed to the target spots using the chemical inkjet printer.

The chemical inkjet printer was used to microdispense PVP solution (7 nL) and trypsin solution (50 nL) to the selected print positions. After digestion in an external incubator, the matrix solution (100 nL) was microdispensed on the digested positions, and direct PMF analysis on membrane was performed using AXIMA-CFR.

Figure 2 shows the spectra obtained from direct PMF analyses on membrane using AXIMA-CFR. Each protein spot was identified using a database search with Mascot as the search engine (see table below).

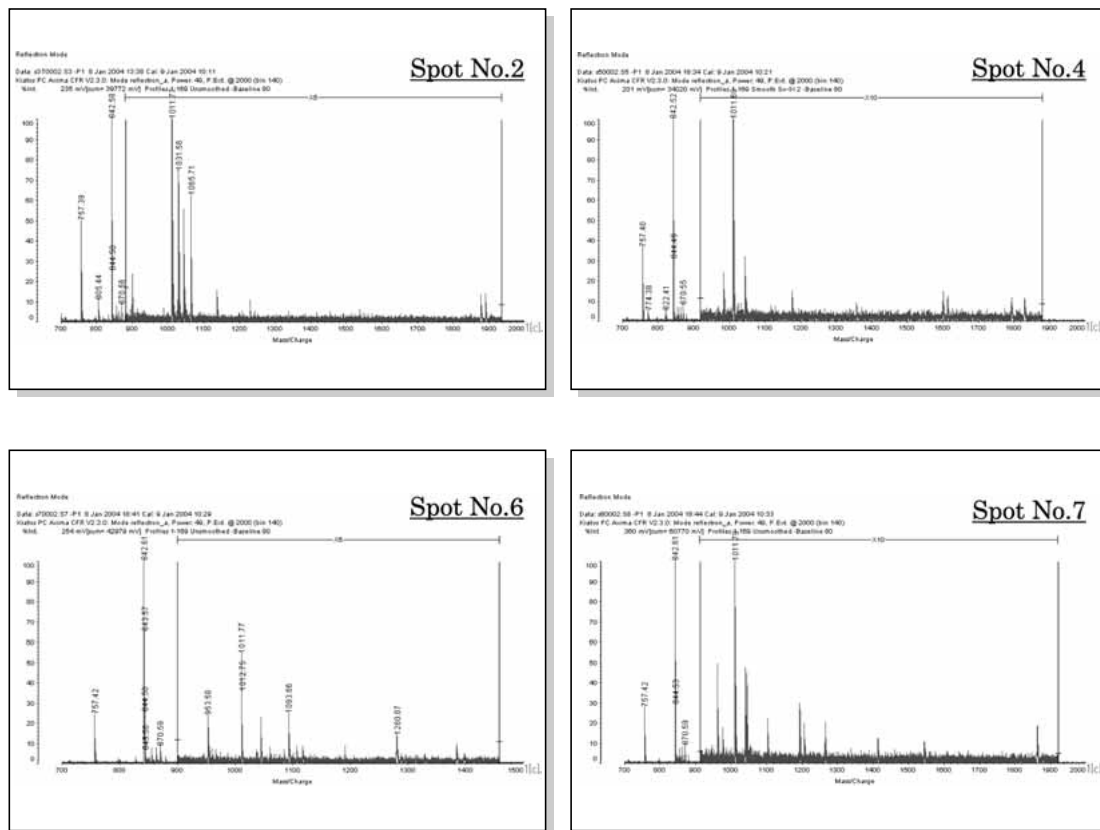


Figure 2 MS spectrum of on-membrane direct PMF analysis for each protein spot

Table 1 Results of database search for PMF analysis of each protein spot

spot No.	protein name	peptides matched	sequence coverage
1	Phosphate-binding protein	7	23%
2	FkbP-type peptidyl-prolyl cis-trans isomerase fkpA	8	28%
3	Superoxide dismutase	6	23%
4	Glutamine binding protein	7	37%
5	3-oxoacyl-[acyl-carrier-protein] reductase	5	17%
6	Thiosulfate-binding protein cysP precursor	8	21%
7	Transcription antitermination protein nusG	6	38%
8	Ribosome releasing factor	5	21%