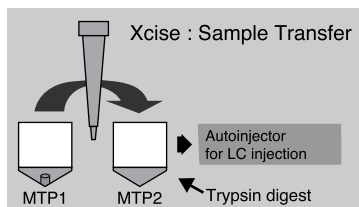
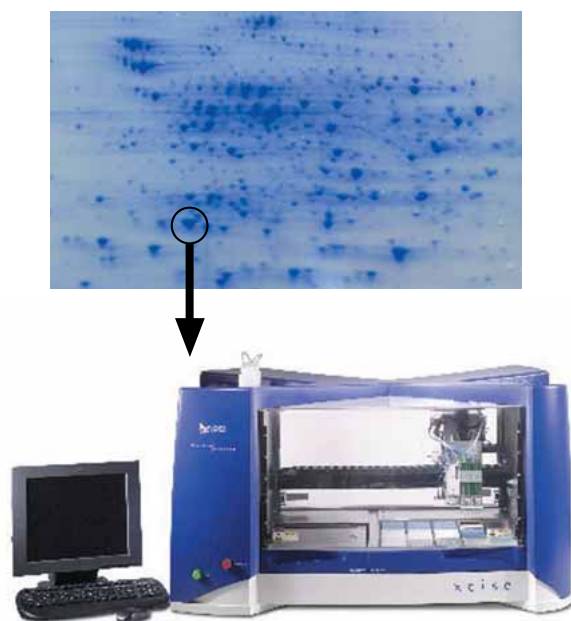


High Throughput Gel Excision Processor

Sample Preparation Using Xcise for nanoLC/MS/MS System

The nanoLC/MS/MS system is effective for identifying proteins in protein mixtures, and is widely used as an auxiliary way to MALDI-TOFMS. Shimadzu gel processor, Xcise, incorporates a "Sample Transfer" function, which transfers the trypsin digest to a separate MTP well after in-gel digestion. By using this function, an LC-MS/MS sample that doesn't contain a gel piece can be prepared. Introduced here is an example in which an in-gel digest prepared from a 2-dimensional electrophoresis gel using the Xcise is analyzed by the nanoLC-LCQ Deca XP from Thermo Electron.



Only digest is transferred to MTP2 from MTP1 that contains the digest with a gel piece.



MS/MS System (LCQ Deca XP, Thermo Electron)

nanoLC Analytical Conditions (Proteome Systems Ltd.)

System	: Surveyer System (Thermo Electron)
Column	: ProteCol™ SGE International Pty. Ltd. (www.sge.com) 10cm (L) X0.15mm (id), 3µm, C18, 300Å
Solvent A	: 0.1% formic acid
Solvent B	: 0.1% formic acid, 90% acetonitrile
Gradient	: A/B = 0-60% (30min), 60-100% (10min)
Flow rate Pump	: 120µL/min
Column	: 600nL/min (using splitter)

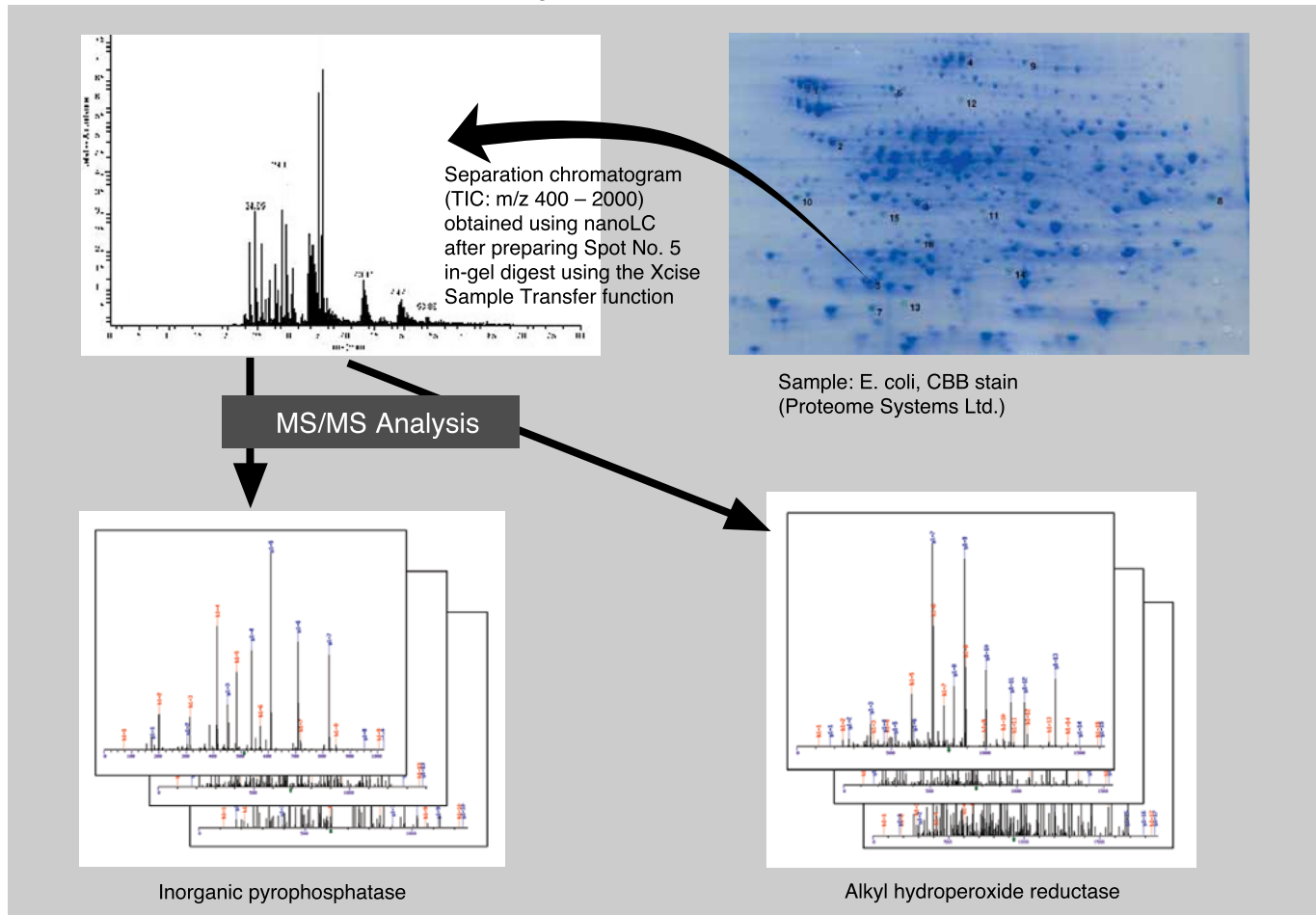
20µL of the trypsin digest was injected into a trap column packed with polymer-type reverse-phase resins for sample enrichment and desalting. This condition used Solvent A at a flow rate of 10µL/min.



ProteCol™
column

For the emitter, the New Objective Inc. PicoTip™ EMITTER was used.

Xcise – nanoLC/MS/MS Analysis



The table below shows nanoLC-MS/MS analysis results for all spots that were excised. Good amino acid coverage was obtained for each spot, and further, multiple proteins were identified for Spot No. 5, 6 and 8.

Spot Number	Protein Identified	Accession Number	Number of Peptides	Coverage (by AA Count)	MW
1	Chaperone protein dnaK	p04475	63	77.7%	68983
2	ATP synthase beta chain	p00824	31	78.6	50194
3	Elongation factor Ts (EF-Ts)	p02997	32	79.4	30291
4	ClpB protein (Heat shock protein F84.1)	p03815	59	68.1	95585
5	Alkyl hydroperoxide reductase C22 protein	p26427	19	80.1	20630
	Inorganic pyrophosphatase	p17288	14	60.6	19572
6	Chaperone protein htpG (Heat shock protein htpG)	p10413	36	54.3	71422
	Oligopeptidase A	p27298	10	16.6	77167
7	DnaK suppressor protein	p18274	9	37.7	17527
8	Glyceraldehyde 3-phosphate dehydrogenase A	p06977	25	84.8	35401
	Phosphate-binding periplasmic protein precursor (PBP)	p06128	6	23.4	37023
9	Catalase HP11	p21179	34	45.6	84162
10	Arginyl-tRNA synthetase	p11875	16	30.7	64683
11	Cytidine deaminase	p13652	10	31.6	31539
12	Spermidine/putrescine-binding periplasmic protein precursor	p23861	20	52	38867
13	Hypothetical protein ybeL	p46129	12	60.6	18797
14	KHG/KDPG aldolase	p10177	12	47.9	22284
15	3-oxoacyl-[acyl-carrier-protein] synthase III	p24249	13	46.7	33515
16	3-methyl-2-oxobutanoate hydroxymethyltransferase	p31057	6	34.1	28237

The data was obtained at Proteome Systems Ltd. in Sydney, Australia.

