Development of an automated two-dimensional gel electrophoresis device and an automated SDS-PAGE-blotting device

SHINICHI GOTO
SHARP CORPORATION

This product was developed under the program of Japan Science and Technology Agency, Development of Systems and Technology for Advanced Measurement and Analysis.

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Background

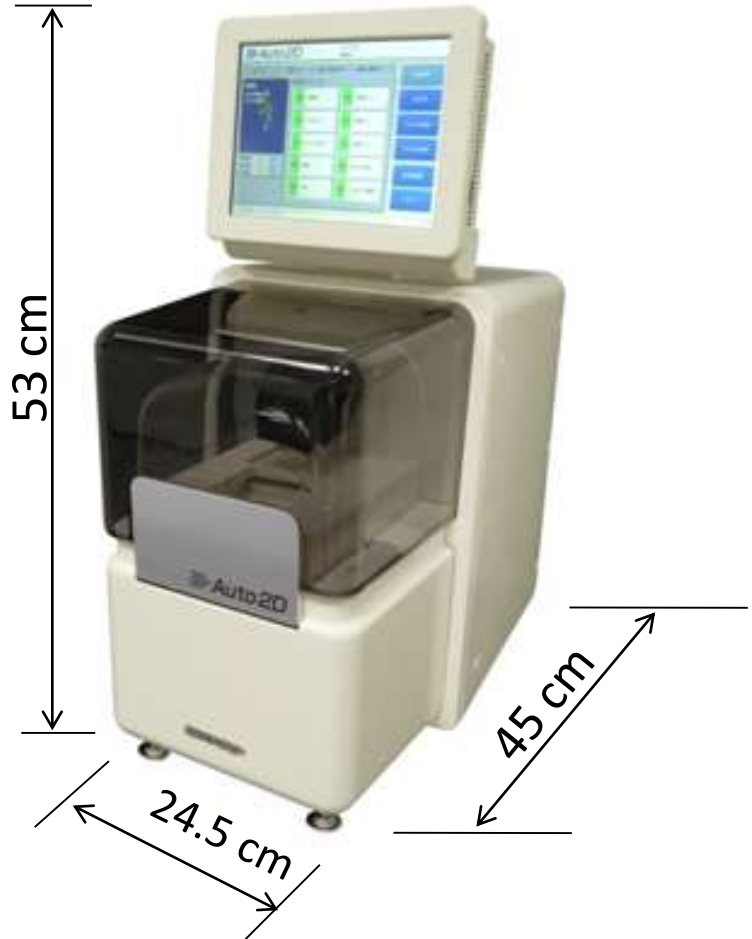
◆electrophoresis-based separation and characterization of proteins from complex biological samples has been predominately performed in life science.

◆Two-dimensional (2D) electrophoresis is a powerful method for the analysis of protein samples not only for laboratory use but also for use in pharmaceutical industries and medical institutions

◆ Gel electrophoresis and electro-blotting is one of the most fundamental approach also known as Western blot for protein expression analysis, biomarker discovery and diagnostics

Auto 2D

Automated 2 Dimensional Electrophoresis System for Protein Analysis with High Reproducibility , High Separation Ability and Quick Analysis



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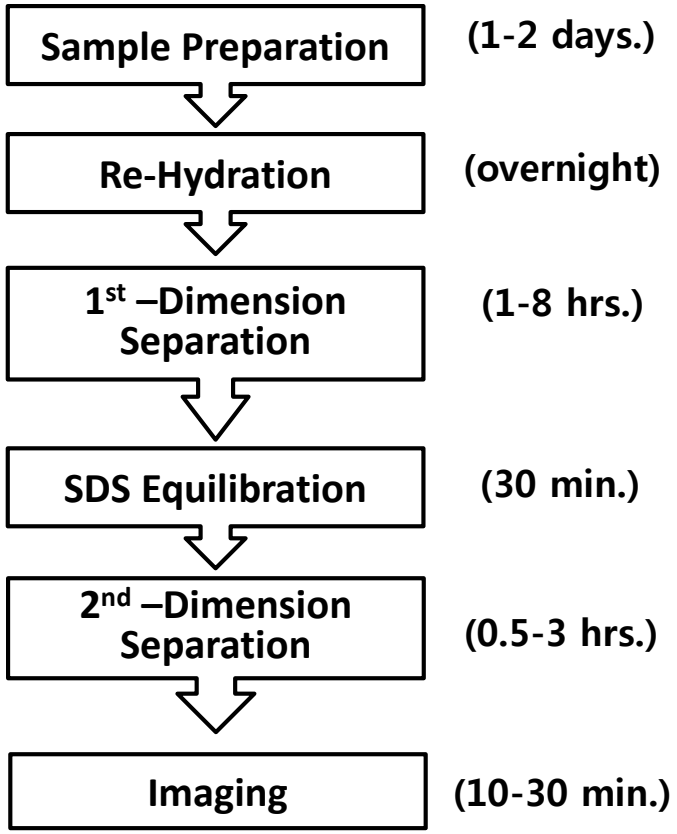
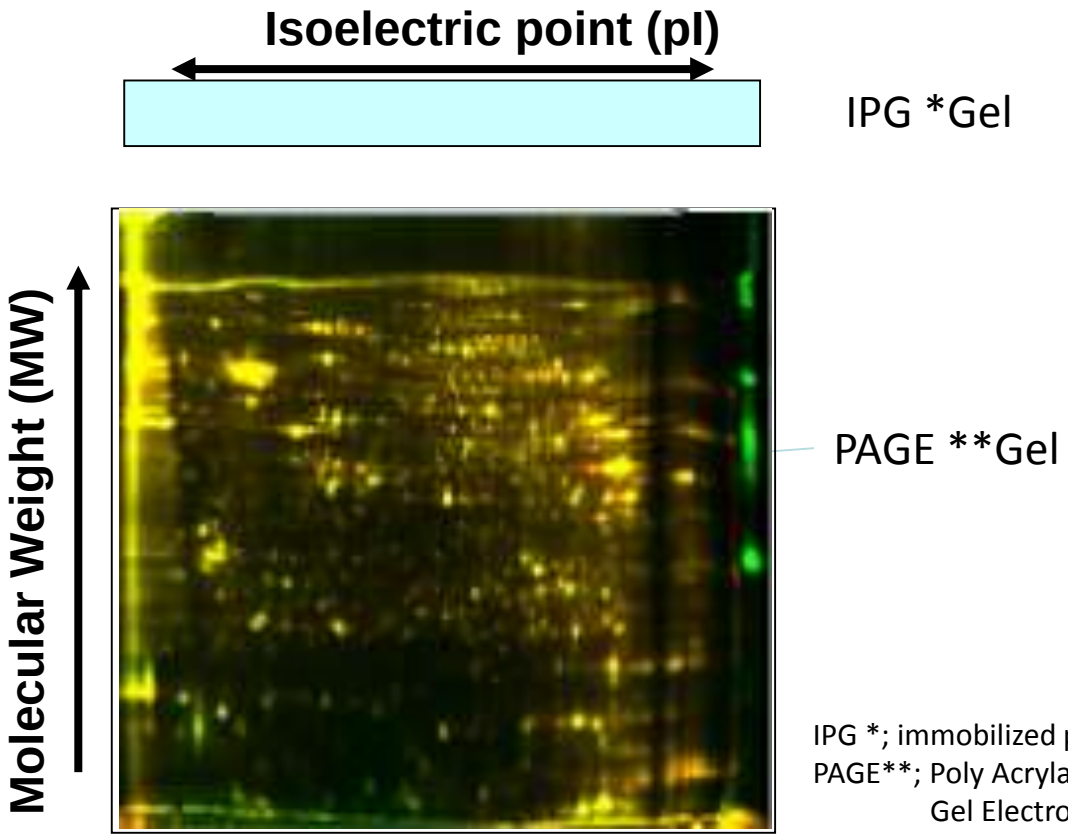
Principle of 2-Dimensional Electrophoresis

The two dimensions that proteins are separated by a isoelectric point (pI) and a molecular weight (MW) of proteins





IPG *; immobilized pH Gradient
PAGE**; Poly Acrylamide Gel Electrophoresis

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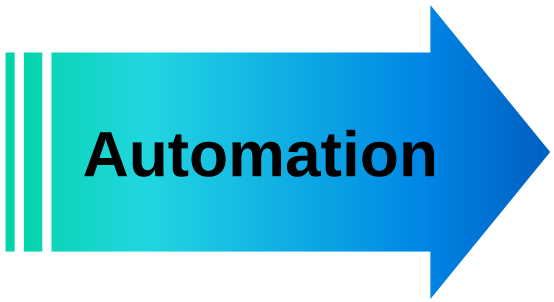
Problems of 2D Electrophoresis

Current methods

✓ Long time analysis (Total 2 days)

✓Poor Reproducibility (less 40%)

✓Need high Skill



Auto 2D

✓Quick analysis (Total 100 min.)

✓Good Reproducibility (CV；5%)

✓Consistent result can be got even not skilled personnel

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Feature 1: Rapid Separation

Reduced analysis time greatly by automated electrophoresis

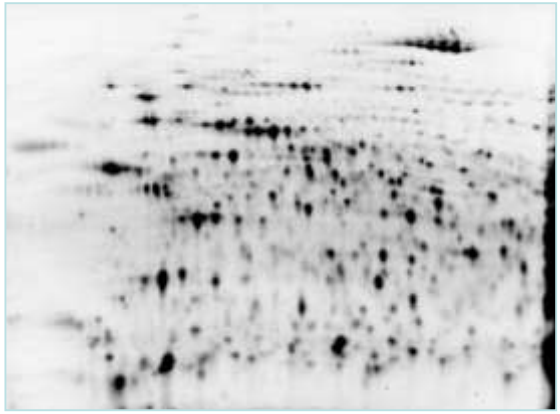
Comparison with Conventional Method

	Sample Absorption	First Electro-phoresis	Equili-bration	Second Electro-phoresis	Total Time
Conventional	Minimun 8 Hrs	2 Hrs	10 min,	40 min.	minimun 10 Hrs (2 days)
	Manual	Manual	Manual		
Auto 2D	35 min.	30 min.	5 min.	30 min.	About 100 min
	Automation				

Feature 2: High Reproducibility

High reproducibility with automated electrophoresis

No.1



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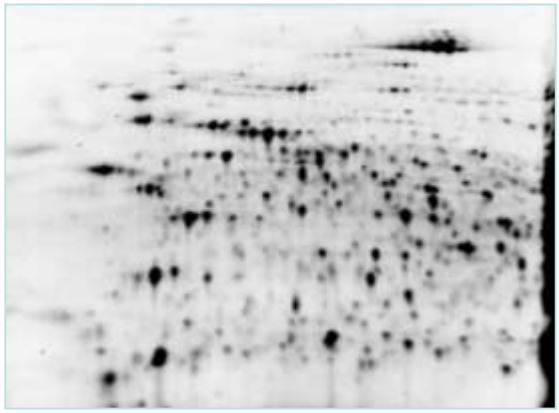

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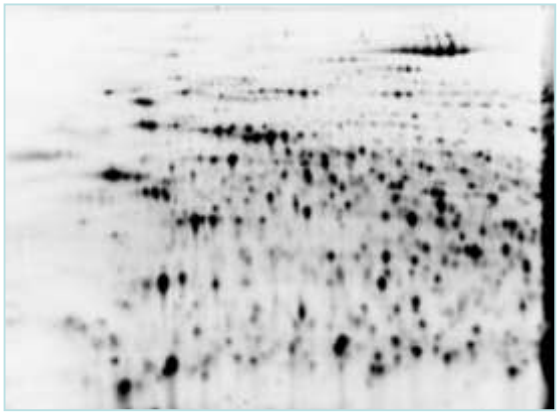

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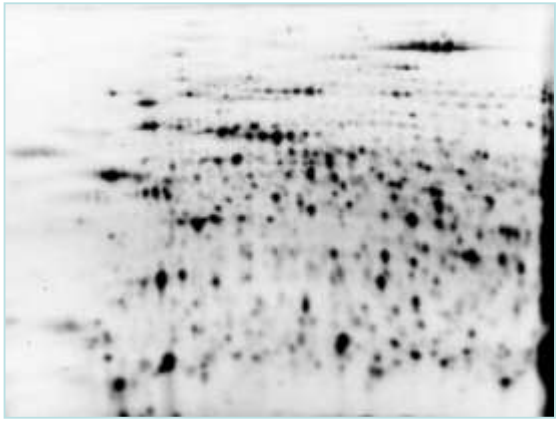
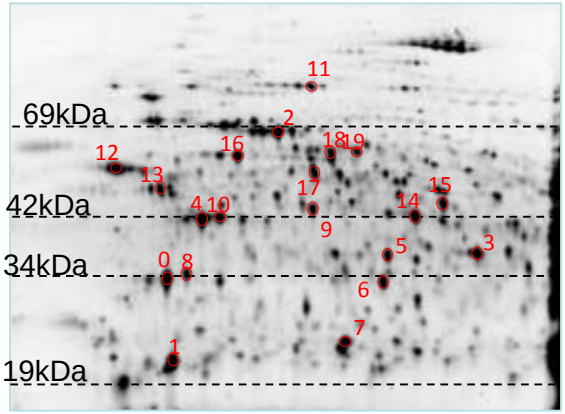


No.5

Evaluated Spots



Result of 5 times of Mouse liver soluble protein samples

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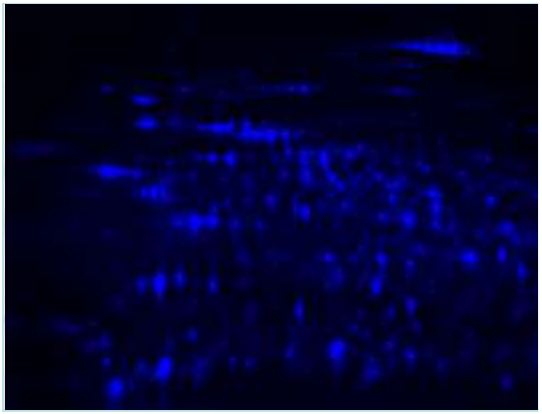
Feature 2: High Reproducibility

No.1



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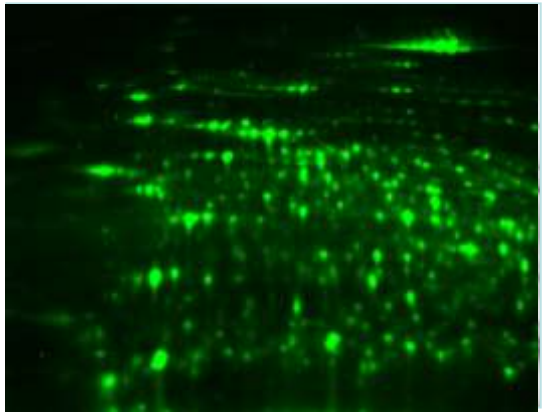

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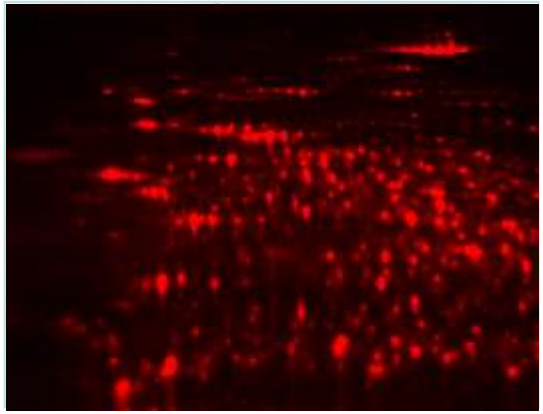

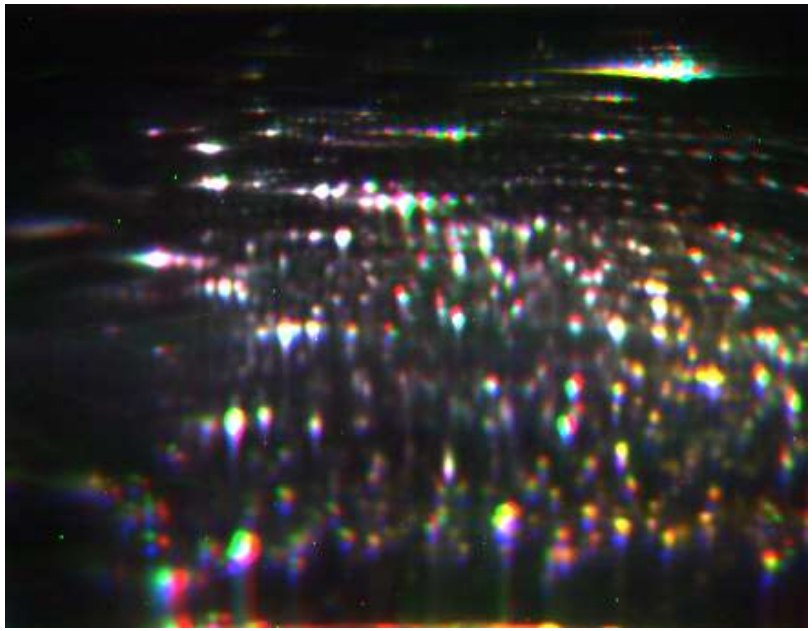

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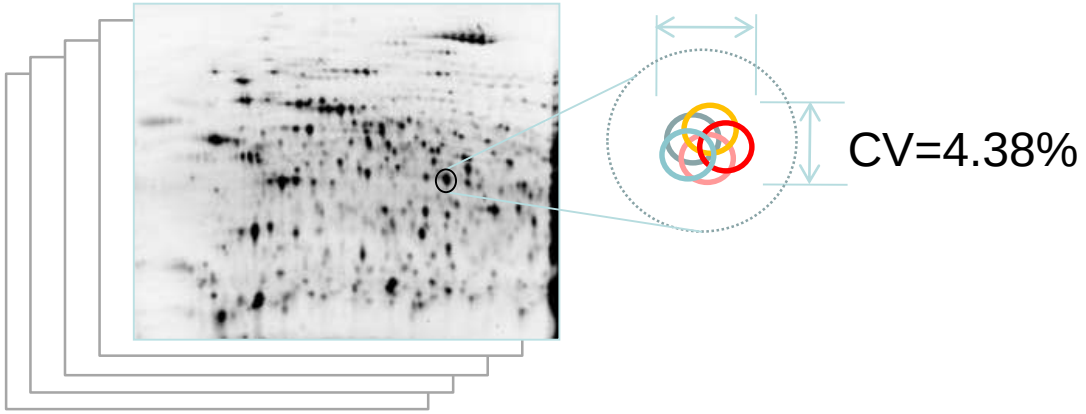
Feature 3: High Resolution

High reproducibility of spot position & intensity of each protein

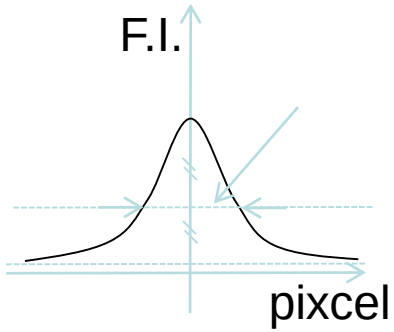
	Average Spot Fluorescence Intensity	Variations in Spot Positions	Spot position Fluctuation Difference CV%	Spot Resolution Molecular Weight	Spot Resolution Isoelectric Point
Evaluated Spots: 20 (Gel N=5)	11.3%	0.055 pH	4.38 %	M.W. ≤ 2kDa	pH ≤ 0.02

Overlap of 5 results

Variation
Ave. pH0.055



Width of Spot Half Value
Isoelectric Point = pH0.02
Molecular weight = 2kDa



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Feature 3: High Resolution

Protein Phosphorylation Detection that requires high resolution

Detection of Phosphorylation Pattern of Human Vimentin Protein

Auto2D result by anti-vimentin antibody after 2D Electrophoresis of brain tumor derived protein

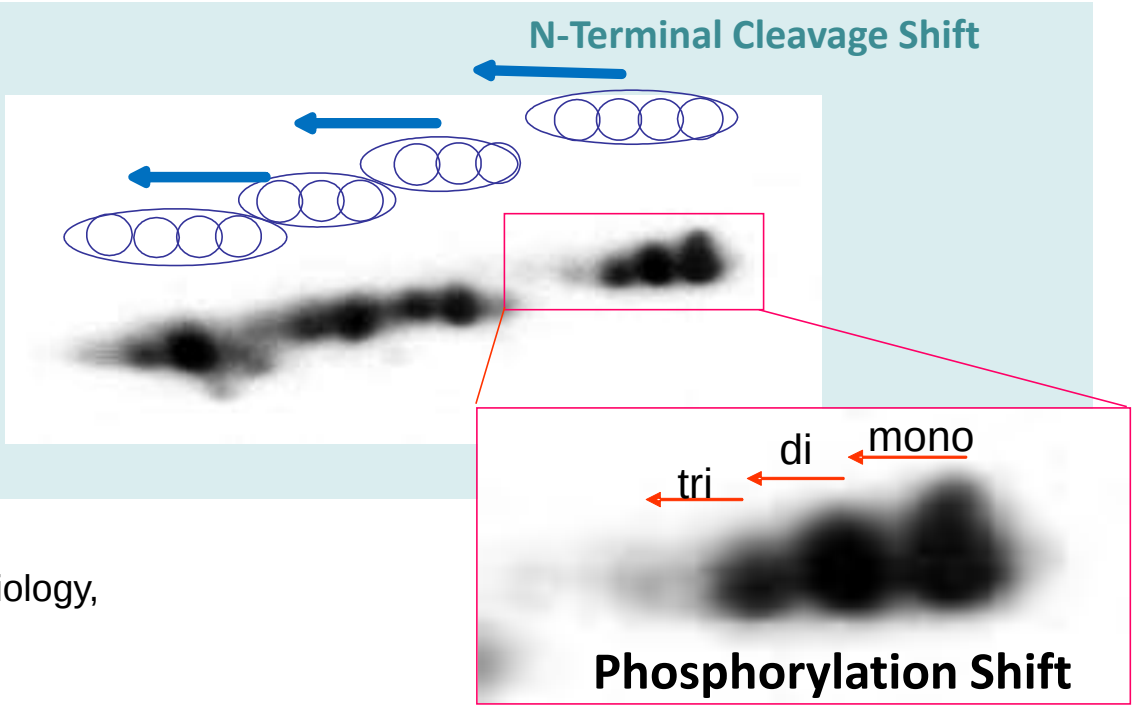
●IEF Chip: pH4-7 ●SDS-PAGE Chip: 10% Acrylamid Gel

Result

The N-terminal cleavage shift and phosphorylation shift of human brain tumor derived vimentin protein could be detected at the same time.

High resolution separation pattern could be obtained

IEF time: 45 min.



Data by: Prof. Norie Araki
Department of Tumor Genetics and Biology,
Kumamoto University Hospital

Feature 4: Easy to Use

Automated 2-D Electrophoresis

Step 1: Setting of chips
Stack PAGE Gel and Solution chips ser them to Auto2D.

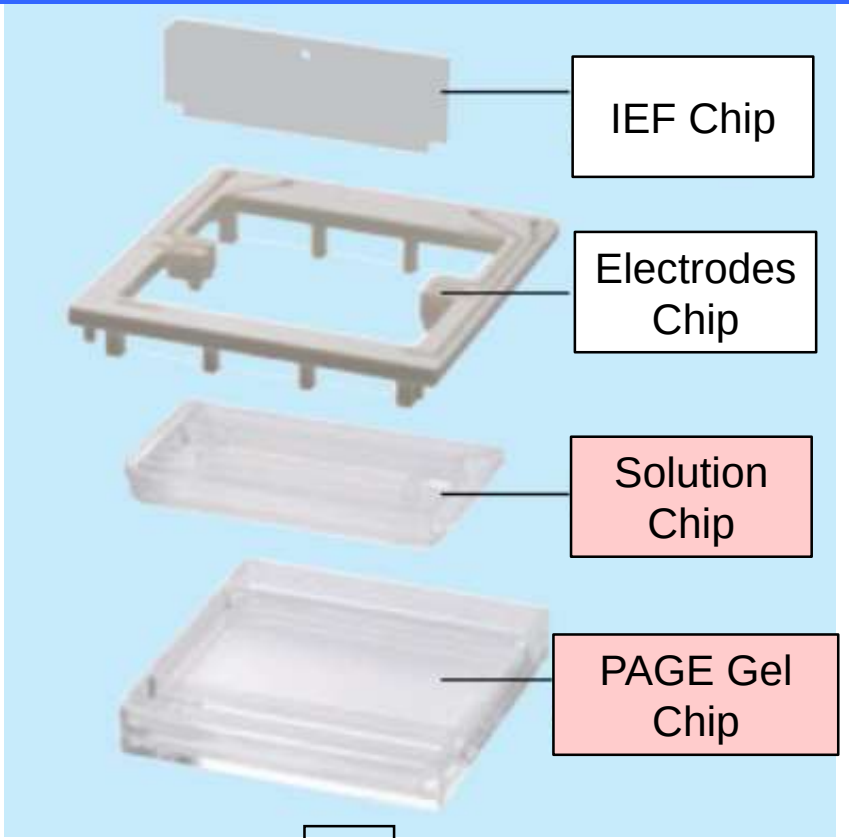
Step 2: Addition of Reagent
Dose reagent in the grooves.

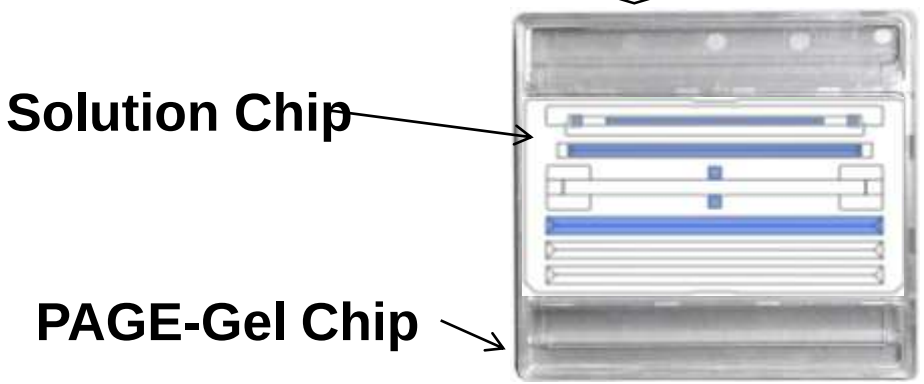
Step 3: Setting of Chips
Set Electrode and IEF chips.

Step 4: Select a Menu and Start

Step 5: Automated Processes
All the electrophoresis processes are done automatically

Analysis Time: 100 minutes





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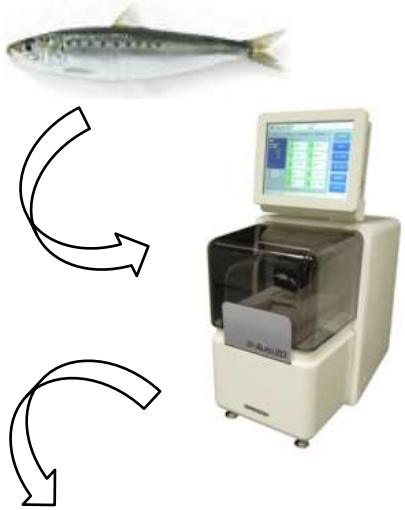
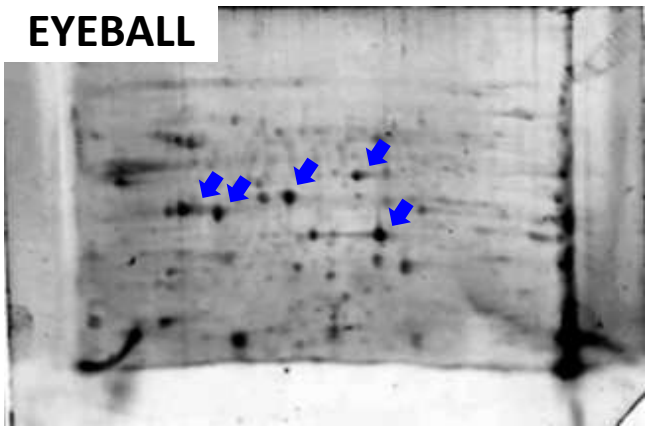
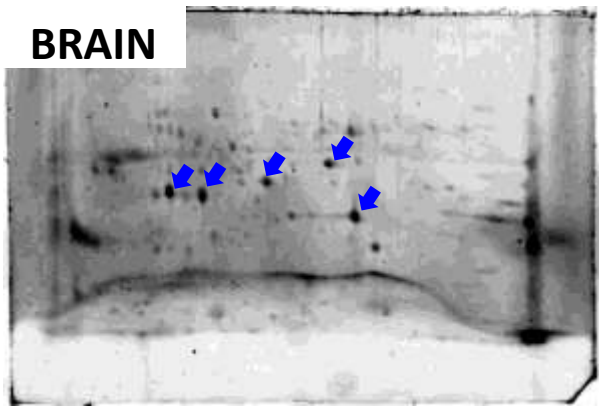
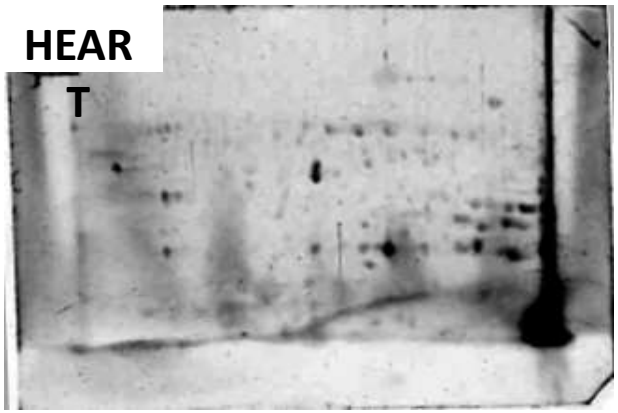
Feature 4: Easy to Use

Science seminar for high school students in SHARP





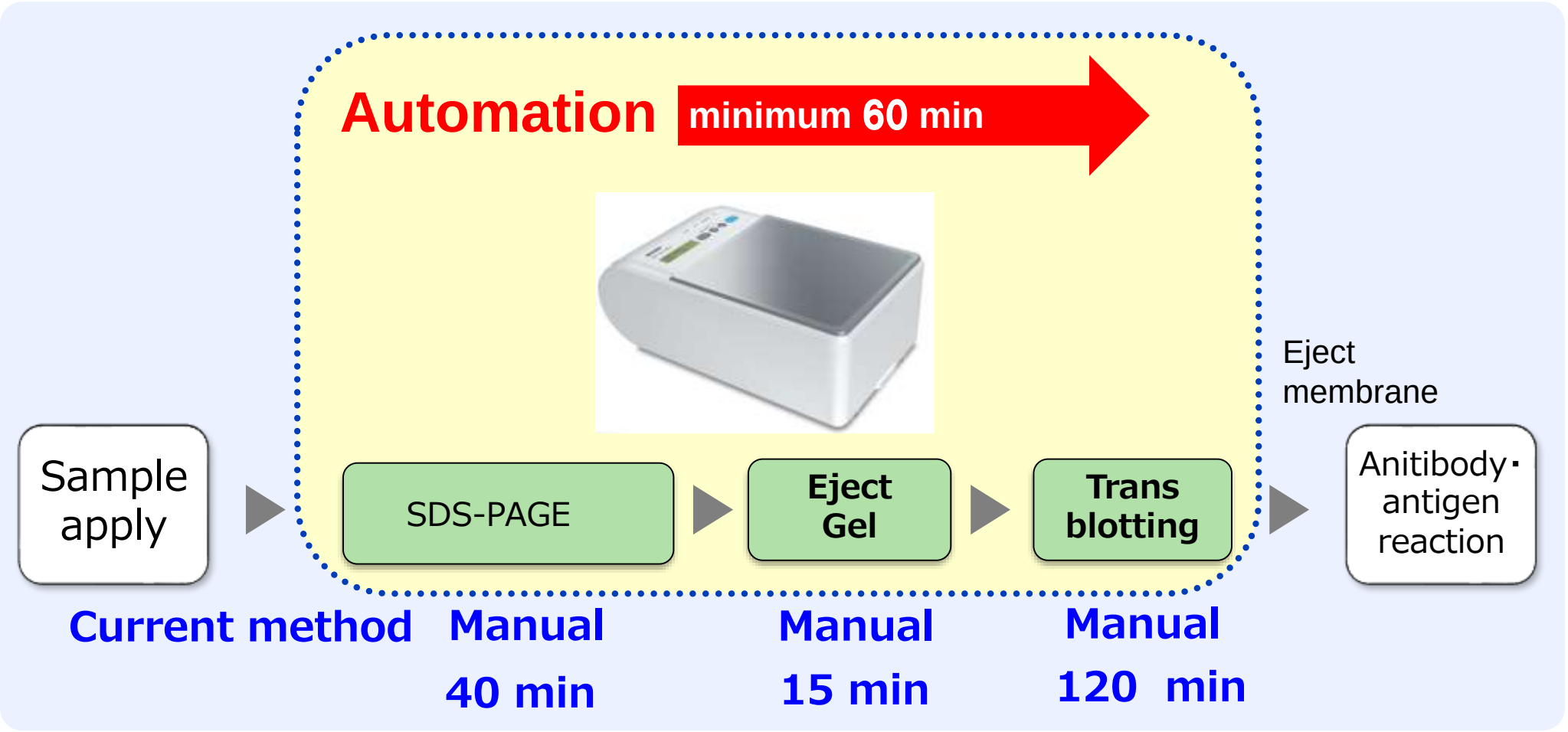




Automated SDS-PAGE-blotting system

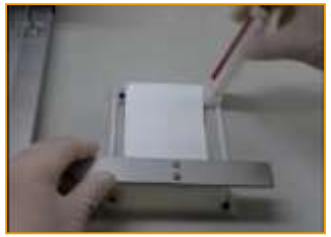
BM−80

procedure for western blotting



Realize automation system from SDS-PAGE to trans blotting process

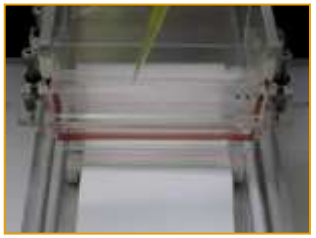
Easy to use



Setting of membrane in holder

Setting of SDS-PAGE chip

Setting of chamber in apparatus



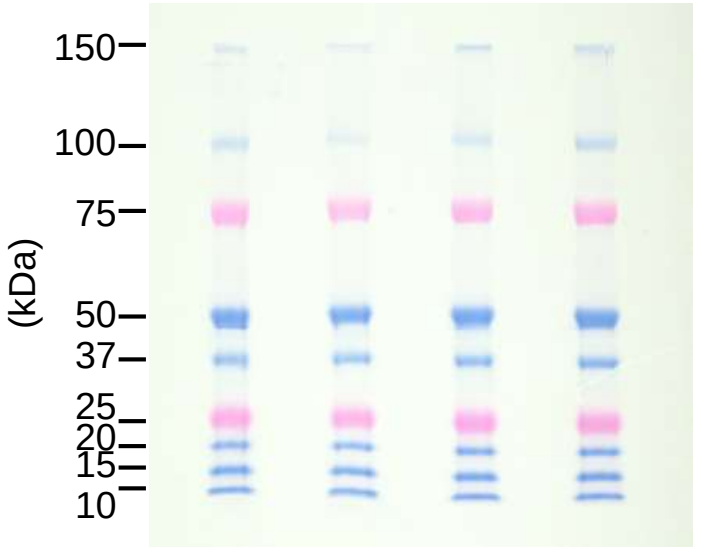
Protein sample Apply

High Reproducibility

Automation

minimum 60 min

■sample
colored protein marker



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Acknowledgement

“Auto2D” and “automated SDSPAGE-blotting system” were developed under the program of Japan Science and Technology Agency, Development of Systems and Technology for Advance Measurement and Analysis.

Automation of Auto2D™

Step 1; Chips-Setting

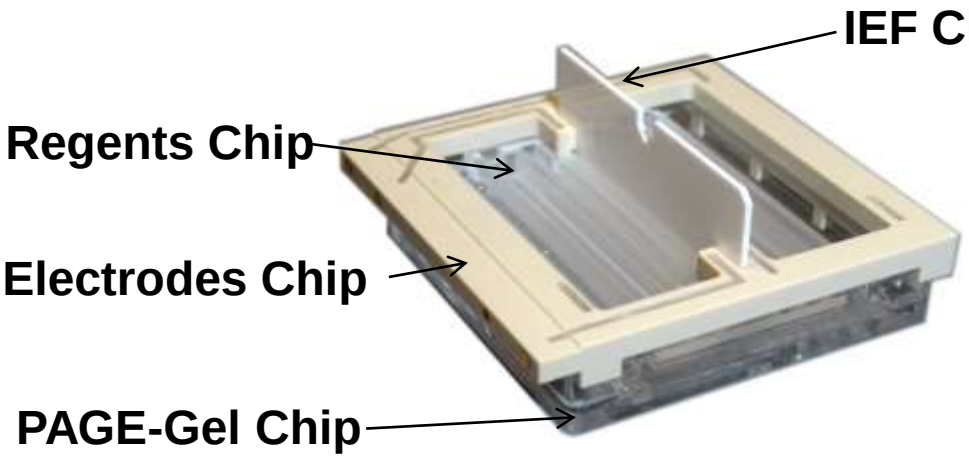
Setting the 3 kinds of Chips
On to the Auto2D

Step 2; Sample Apply

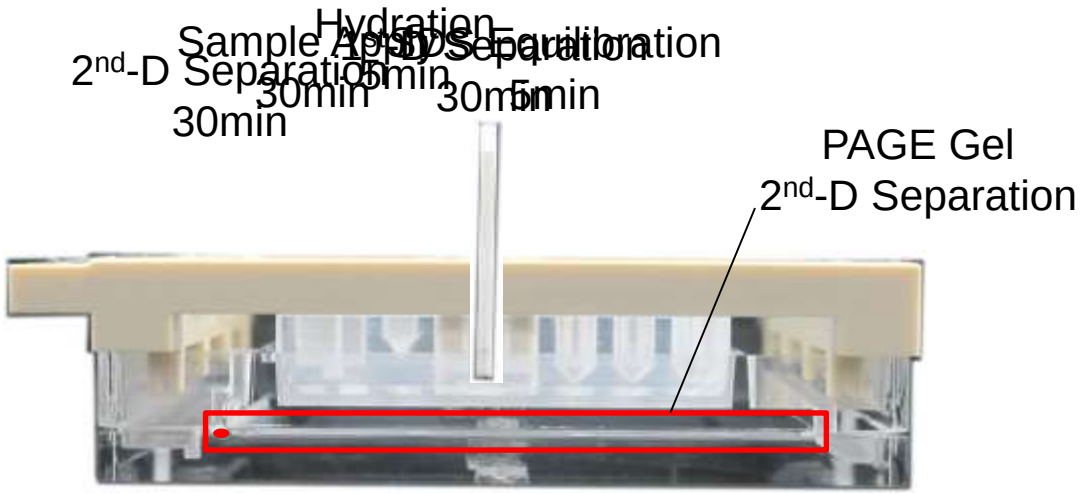
Apply the sample to
the shell

Step 3; Automatically Start the Operation

Moving the IEF with
IPG gel



Analysis Time: 100 min.



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