ASSOCIATION OF BIOMOLECULAR RESOURCE FACILITIES STUDY

SUMMARY OF RESULTS

The samples A and B were dissolved in 50 mM ammonium bicarbonate to a final concentration of 2 ug/ul. After that they were transfer to two clean vials and they were added water up to 90 ul and 10 ul of 77%(w/v) tricloroacetic acid in cold acetone. Then it was stirred and this mixture is incubated to -20°C during 20 minutes. Then it was centrifuged to 15000g during 15 minutes to 4°C. The supernatant was discarded. The precipitate is washed with 40 ul of cold acetones and it was centrifuge by 5 minutes, three times. The precipitate is dried at room temperature and it was resuspended in DIGE buffer (7 M Urea, 2M Thiourea, 30 mM Tris, 4% CHAPS).

Sample B (5.5 ug) was added to 120 ul of rehydration buffer of the strip (8M Urea, 8M Thiourea, 2% CHAPS, 2% IPG buffer (GE Healthcare), 0.002% bromophenol blue). The 7cm strip was rehydrated for 16 hours. Isoelectric focusing was achieved in the IPGphor equipment (GE Healthcare). Fig 1 shows the graphic obtained when we run isoelectric focusing.

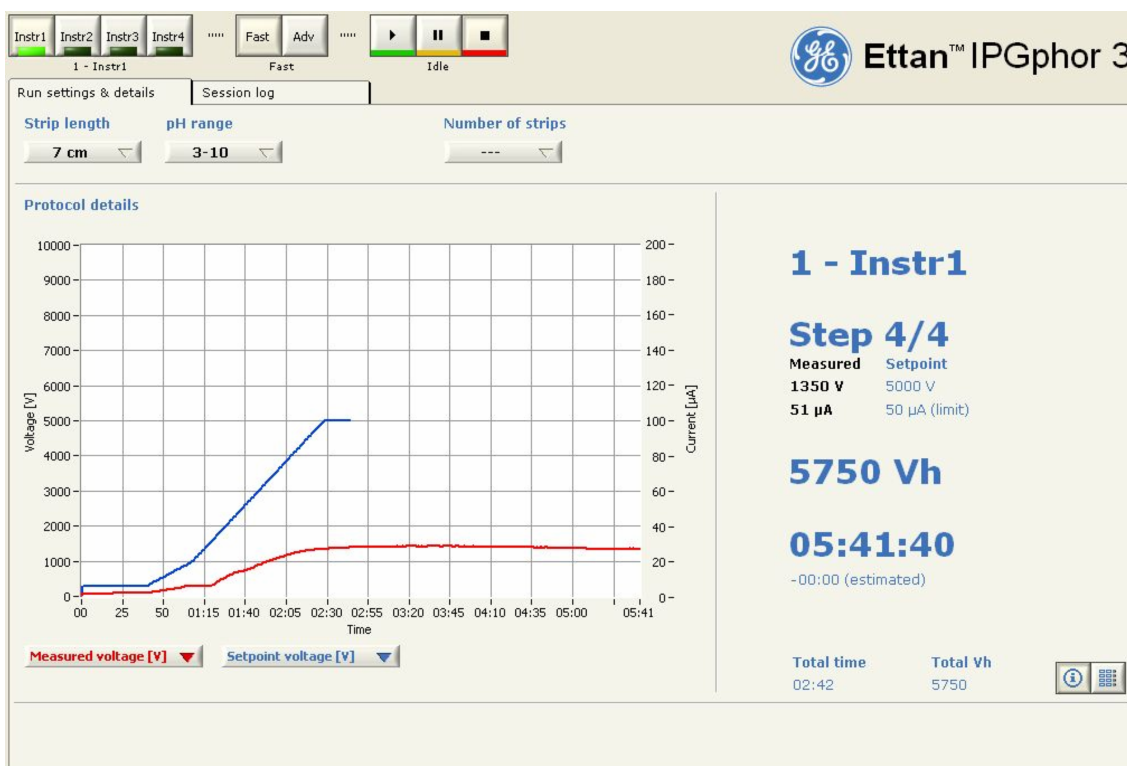


Fig. 1. Isoelectric focusing of sample B (5.5 ug).

The SDS- PAGE (12% polyacrilamide and buffer Laemli) was run to 60V-70V, 3A, 300W during 3 hours.

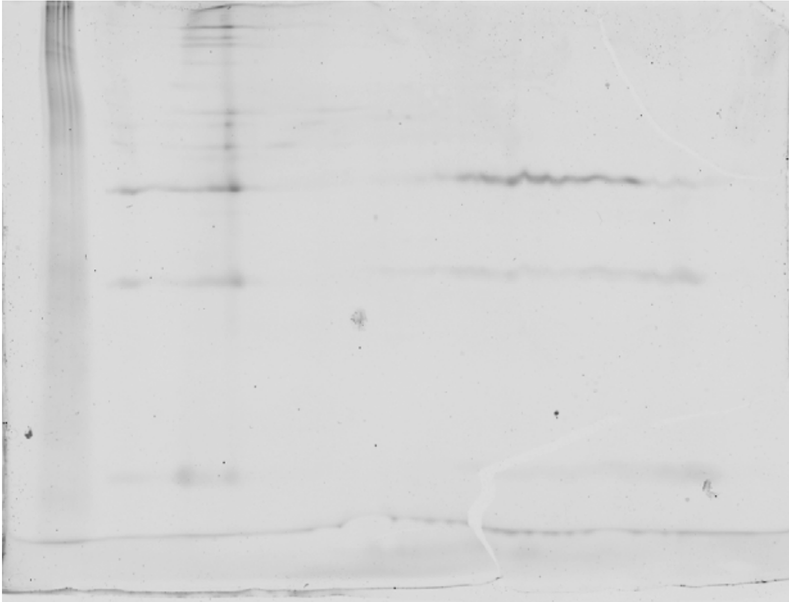


Fig. 2. 2D-Gel of Sample B stained with Fluorescent Deep purple (GE Healthcare).

As it can be observed in figures 1 and 2 even though the set of samples was precipitated initially in order to eliminate any ionic contaminant there is resistance to isoelectric focusing.

Then samples A and B (3 ug of each one) were label with Cy3 and Cy5 respectively, and internal standard (1.5 ug of A and B respectively) with Cy2. The chemical reaction was performed with 3 ug of each sample and 100 pmol of each CyDye. Figure 3, shows the result of the isoelectric focusing of the mixture of the three samples labeled with CyDyes and charged in a 13 cm strip with a pH range of 4-7.

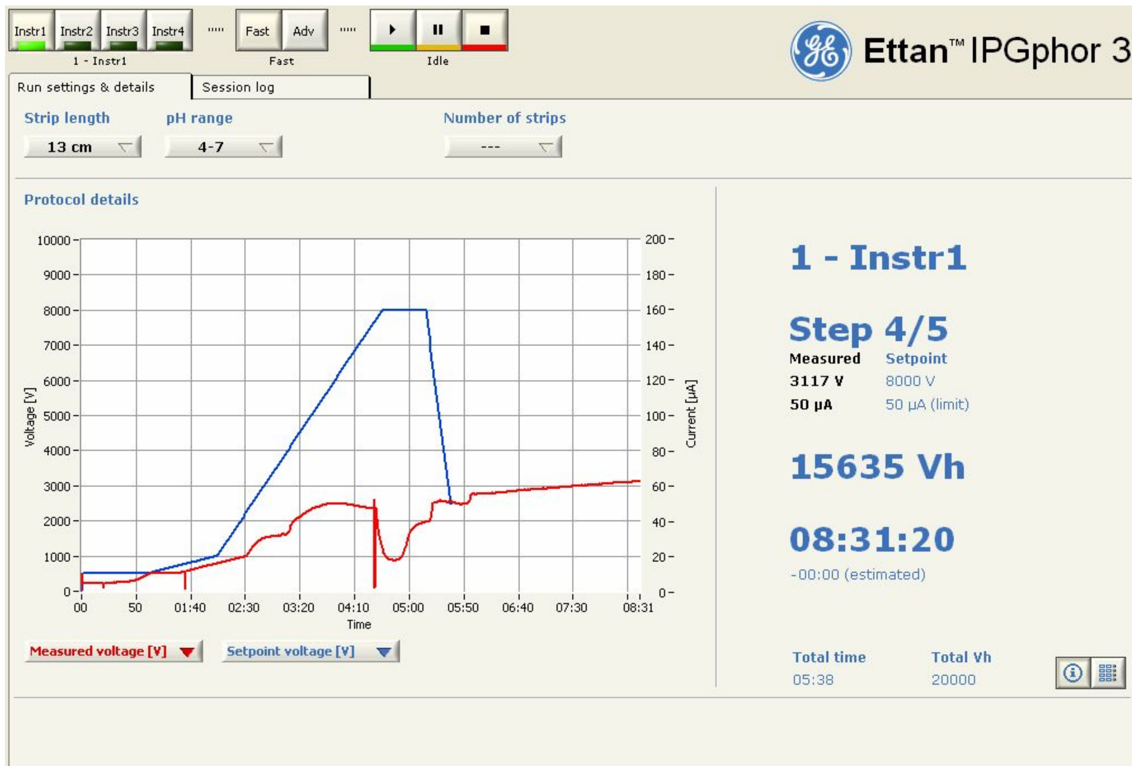
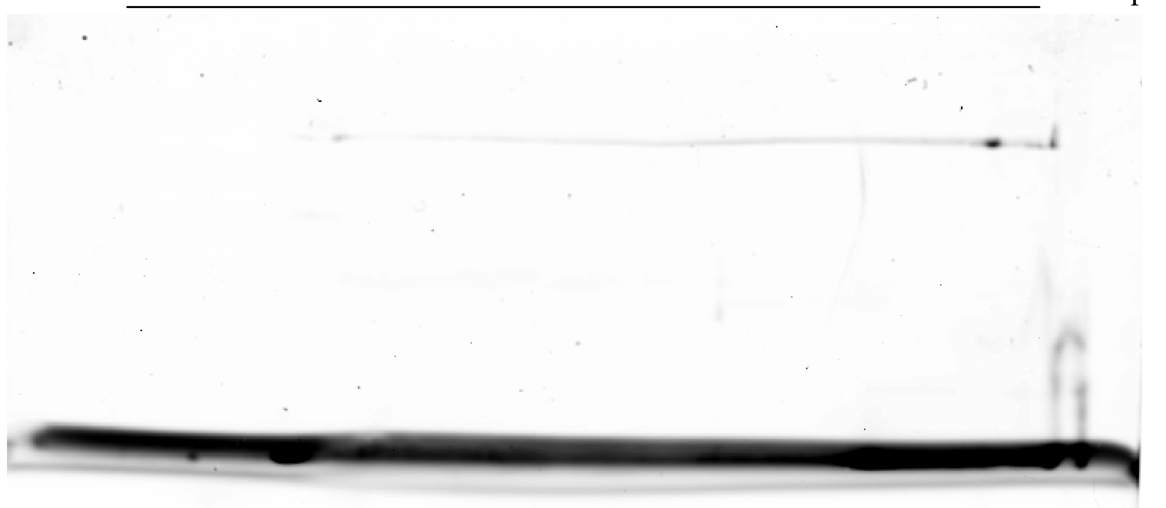


Fig 3. Isoelectric focusing of samples A and B labeled with Cy3 and Cy5 and an internal standard (A+B) labeled with Cy2.

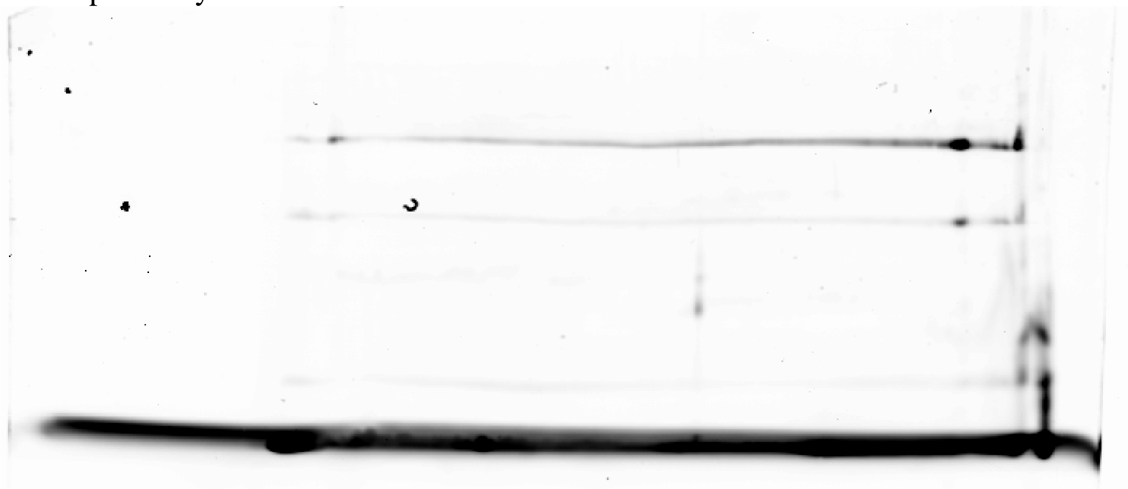
Then second dimension was run at 70V, 3A, 300W during 6 hours. Figure 4 a-d shows the images obtained for Cy2, Cy3 and Cy5, and also post-staining with Fluorescent Deep Purple (GE Healthcare).

a. Sample A. Cy3

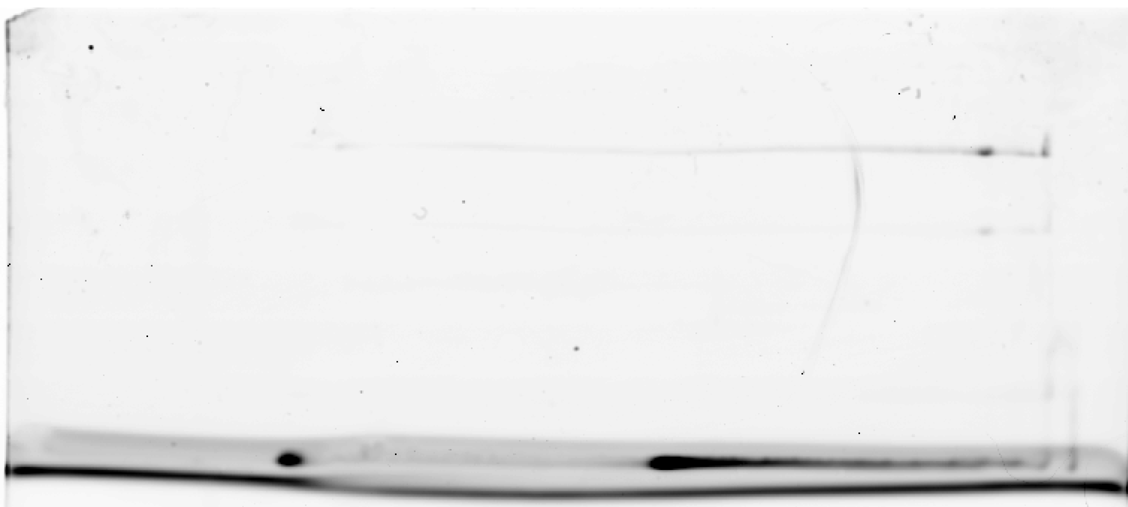
4 7 pI



b. Sample B. Cy5



c. Internal standard. Cy2



d. Deep purple staining

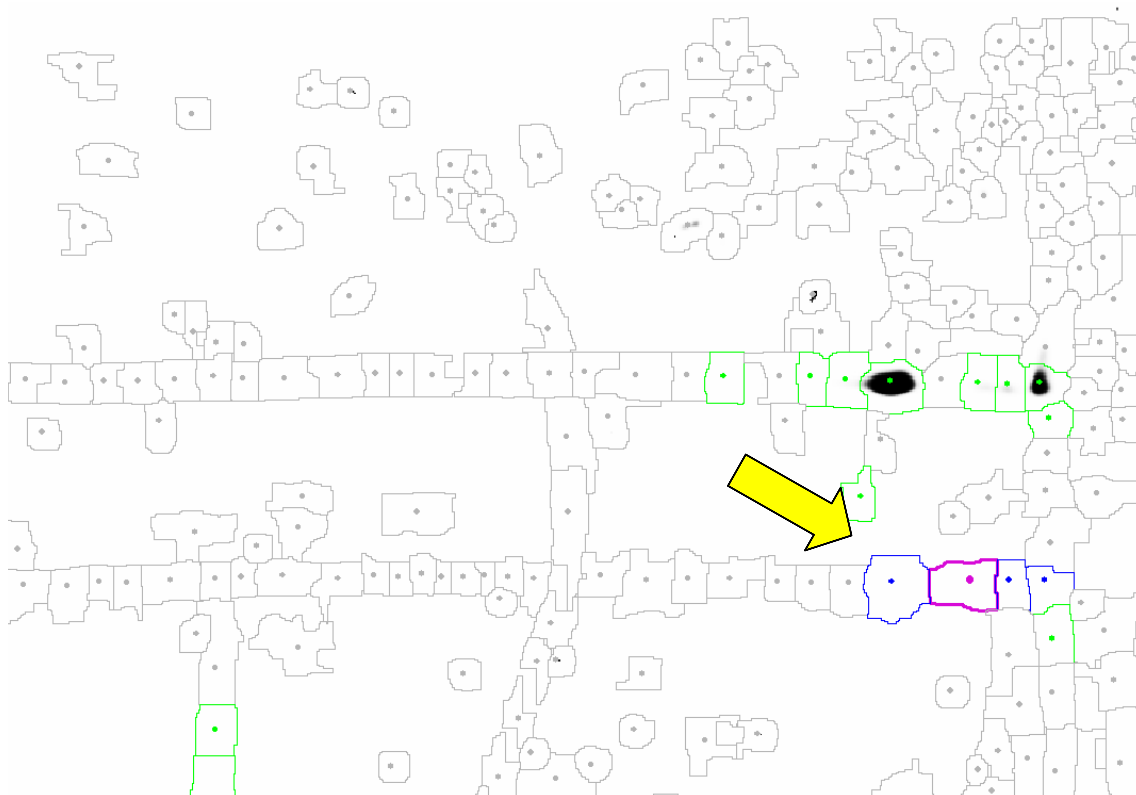
Fig 4. a-d. Differential in gel electrophoresis (DIGE) of Sample A and B

The analysis of spots using Decyder 6.0 (GE Healthcare) showed the next data:

Total spots of protein: 47
Increased: 4 (4 fold in sample B), 16 (2 fold), 2 (3 fold)
Decreased: 0 (4 fold in sample B), 1 (2 fold), 1 (3 fold)
Similar spots: 23
2 SD: 4.28736
Threshold: 4.0

CONCLUSION.

Accordingly the group of proteins increased are located at neutral-basic pH and approximately 14000-30000 Da, only in sample B. (Fig 5)



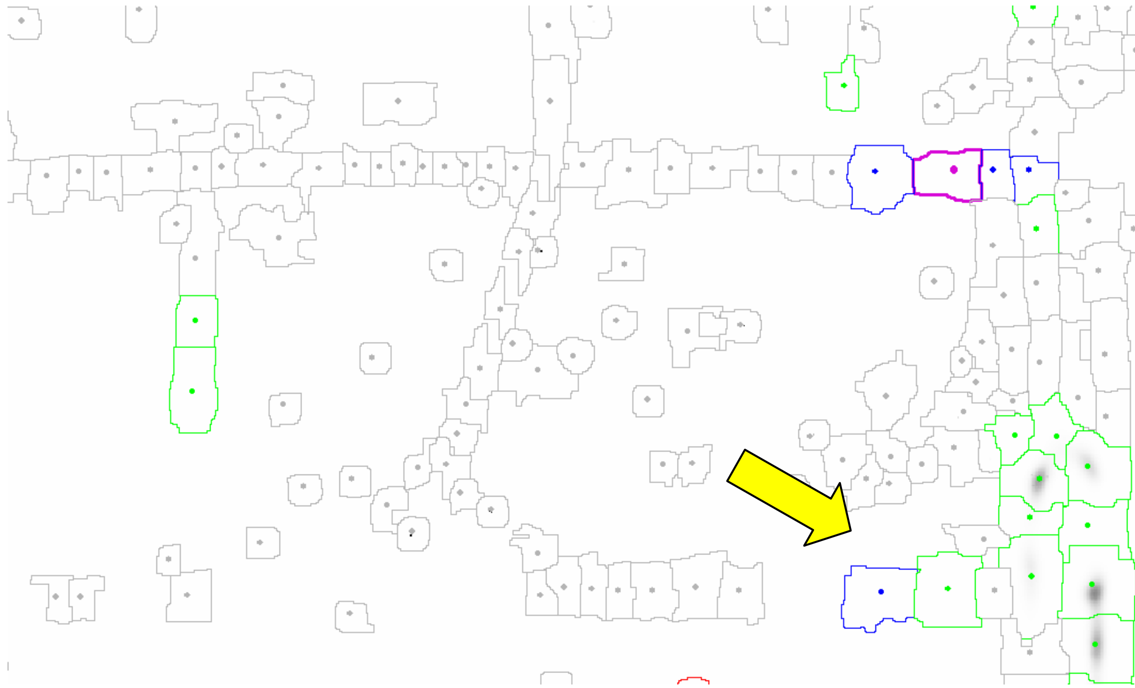


Fig 5 a – b. Analysis of 2Dgel (DIGE) with Decyder 6.0 (GE Healthcare).

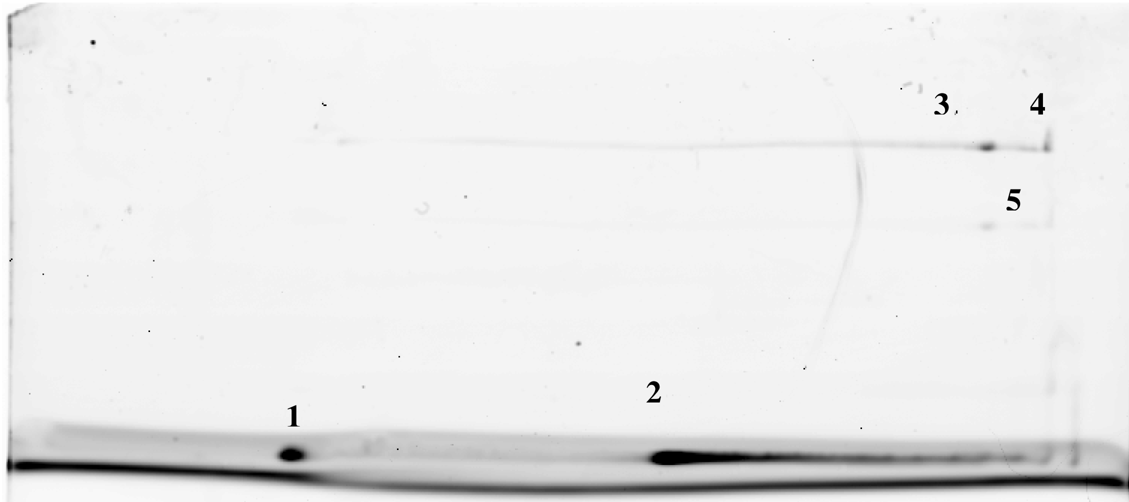


Fig. 6. Spots selected to identify by mass spectrometry.

Preparation of the sample for mass spectrometry.

Pieces of gel were cut from the 2D-gel and were added a solution containing 50% acetonitrile in 50mM NH_4CO_3. Then samples were incubated 5 minutes to 50°C. Then liquid was removed. This step was repeated until dye was removed completely. Then pieces of gel were dried by adding 100 µl of acentronitrile during 5 minutes at room temperature.

Trypsin (25 ng/ml) was added to the pieces of gel and they were incubated at 37°C overnight. Peptides obtained by trysin digestion were cleaned with Zip-tip C18 clean-up (Millipore)

The identities of the proteins by mass spectrometry was delivered in the web site by ABRF survey.