

EVALUATION OF PLASMA MARKERS IN BREAST CANCER *IN VITRO* MODELS

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INTRODUCTION

Breast Cancer (BC), the most frequent among women, is a complex and heterogeneous disease, which can be stratified in the subtypes Luminal A, Luminal B, Luminal HER2, HER2 and Triple Negative by the expression of hormone receptors. The importance of this classification is related with disease progression, prognosis and therapeutic intervention. Thus, early diagnosis enables better results in treatment and recovery. For this reason, the blood plasma has been broadly investigated for neoplastic markers. A proteomic study conducted in our laboratory identified circulating proteins in the plasma of patients with BC, suggesting that they are potential BC markers.

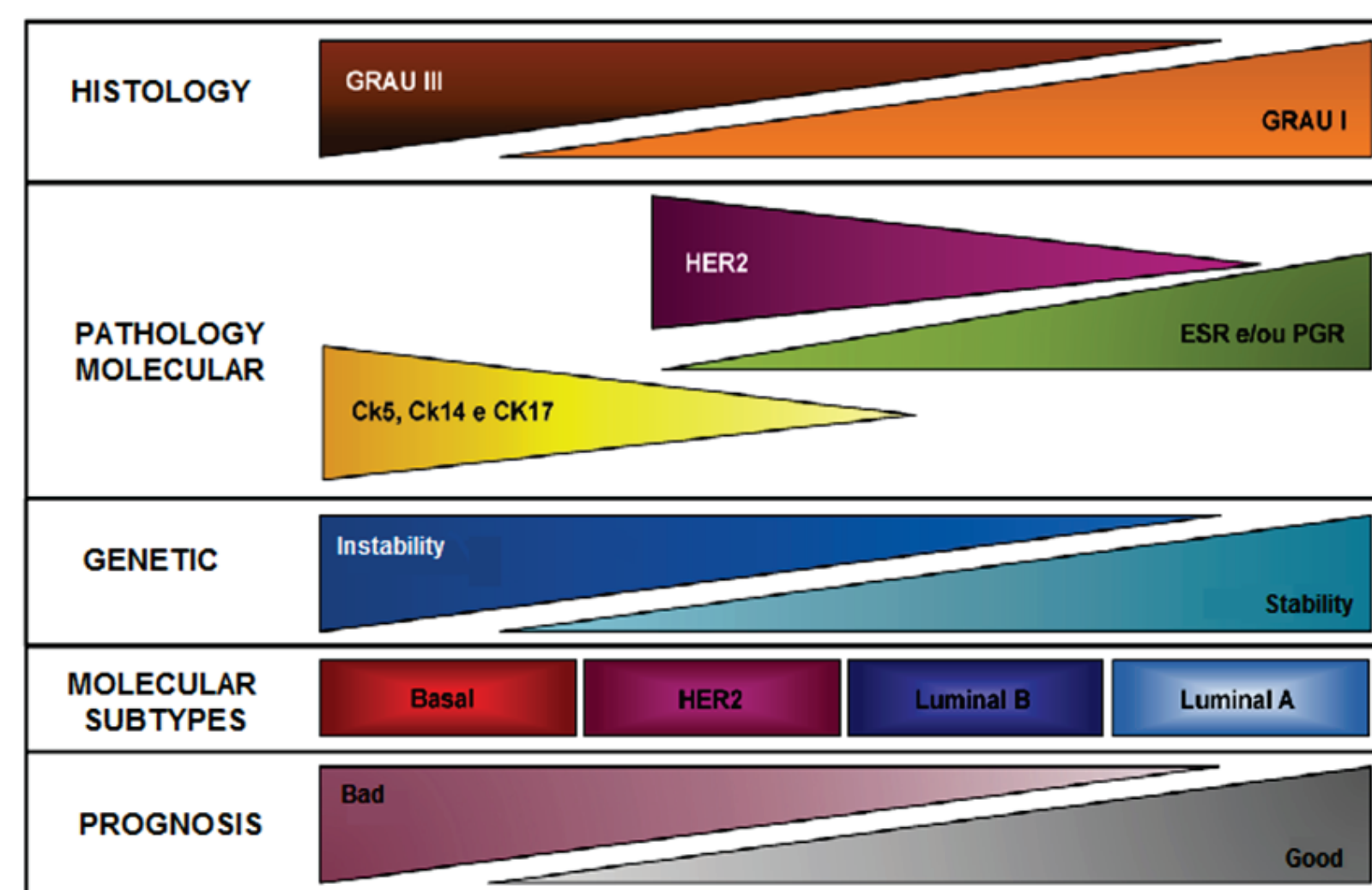


Figure 1: Classification of molecular subtypes of Breast Cancer. Adapted from Sims e cols, 2007.

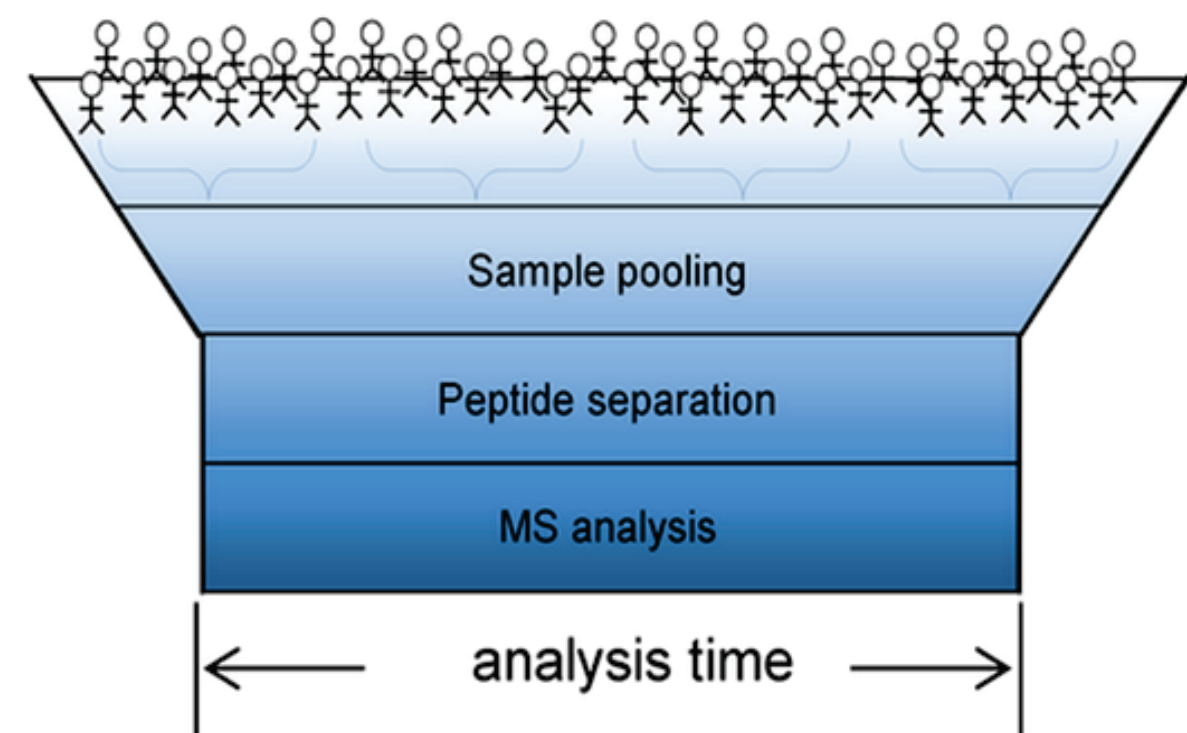


Figure 2: Representative analysis of samples in proteomic experiment for biomarkers. Orton e Doucette, Proteomes 2013.

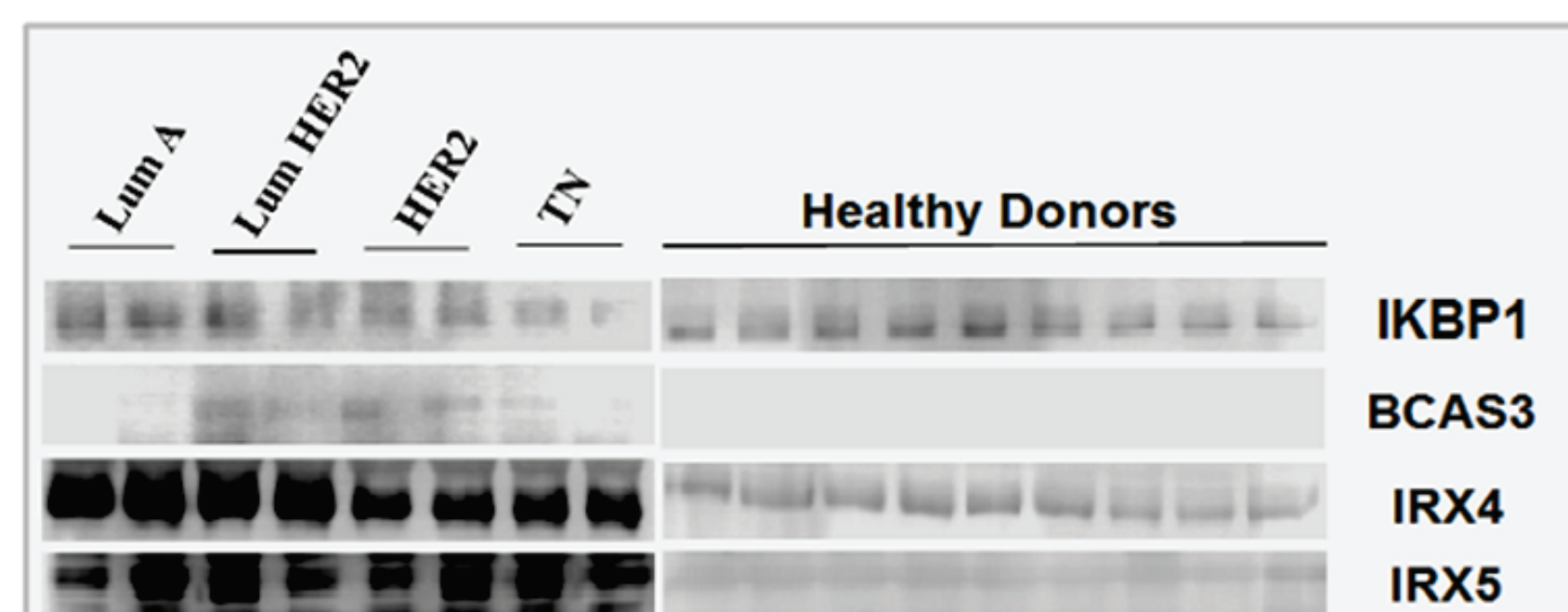
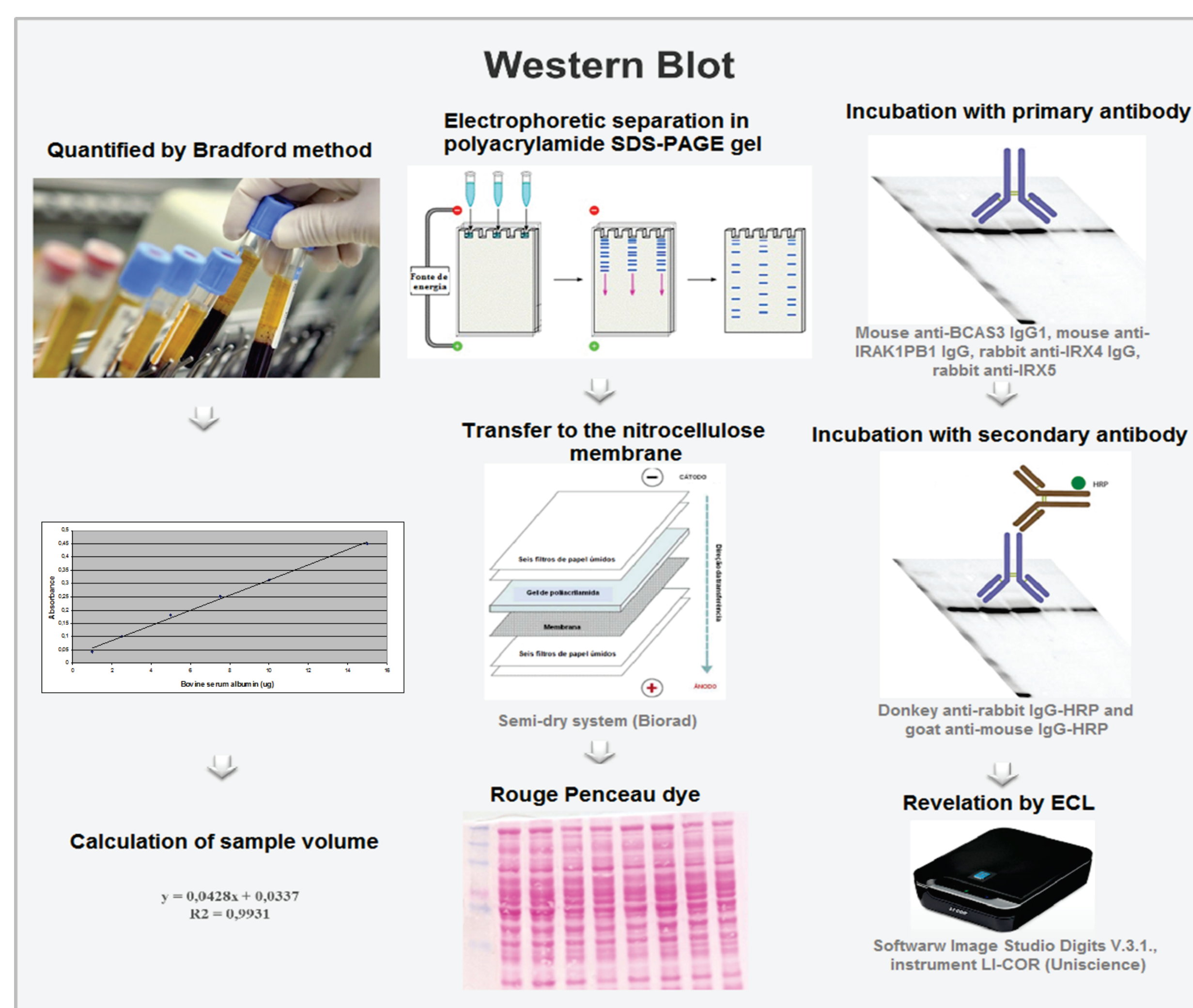


Figure 3: Detection by Western Blot of IRBP1, BCAS3, IRX4 and IRX5 proteins in blood plasma of BC patients and healthy donors for the comparison as biomarkes of BC disease.

GOAL

The goal of this study is to investigate the expression of IKBP1, BCAS3 IRX4 and IRX5 proteins in cell lines and their culture supernatant, in order to correlate the obtained data with patients results.

METHODOLOGY



RESULTS

Quality Control in BC cell lines

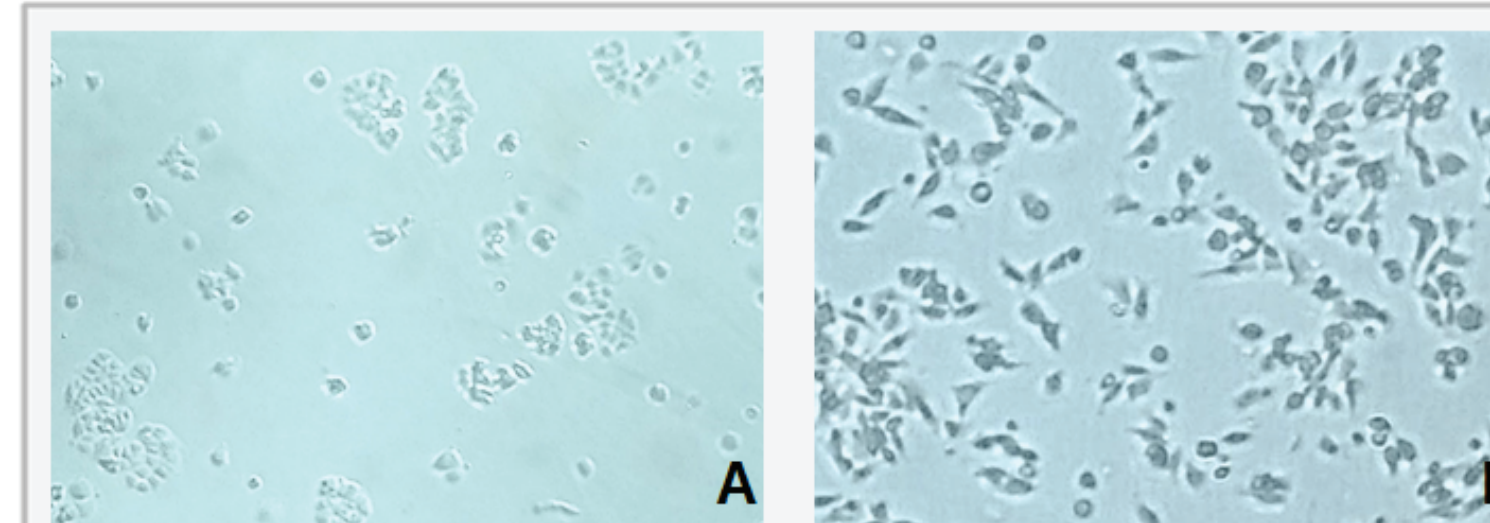


Figure 4: Representative BC cell lines culture. A) MCF-7. B) MDA-MB-231.

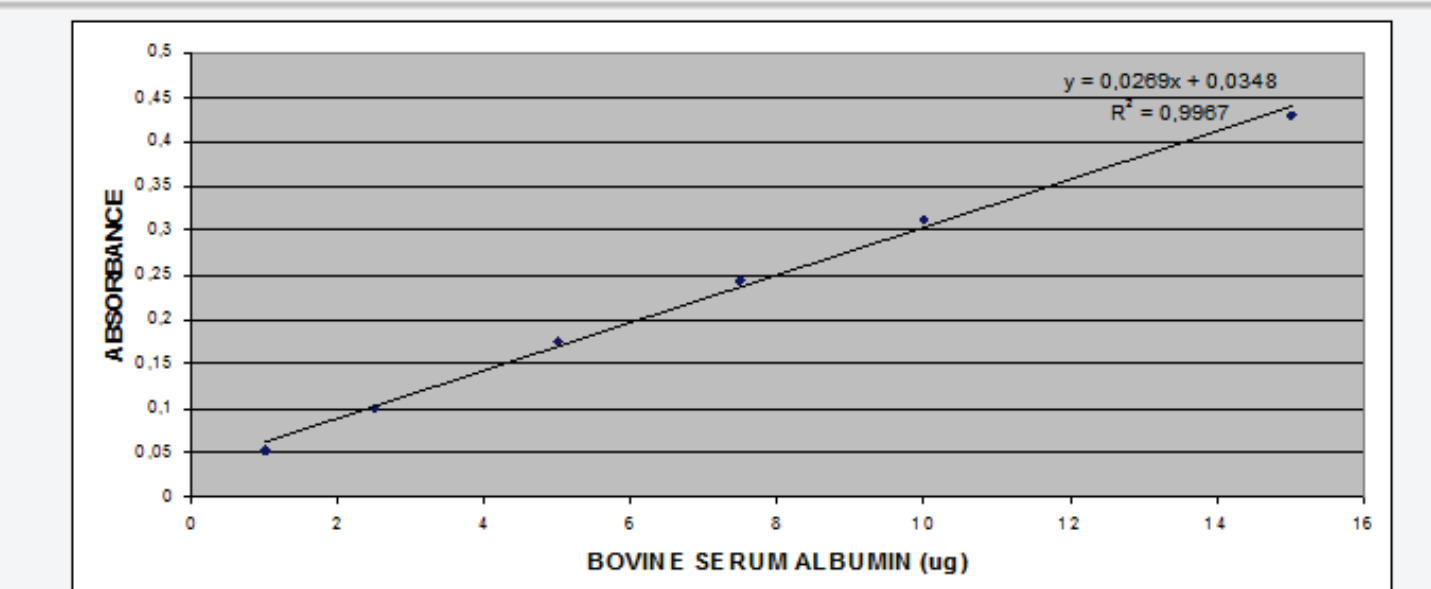


Figure 5: BSA standart curve for quantification of proteins by Bradford method with equation of straight.

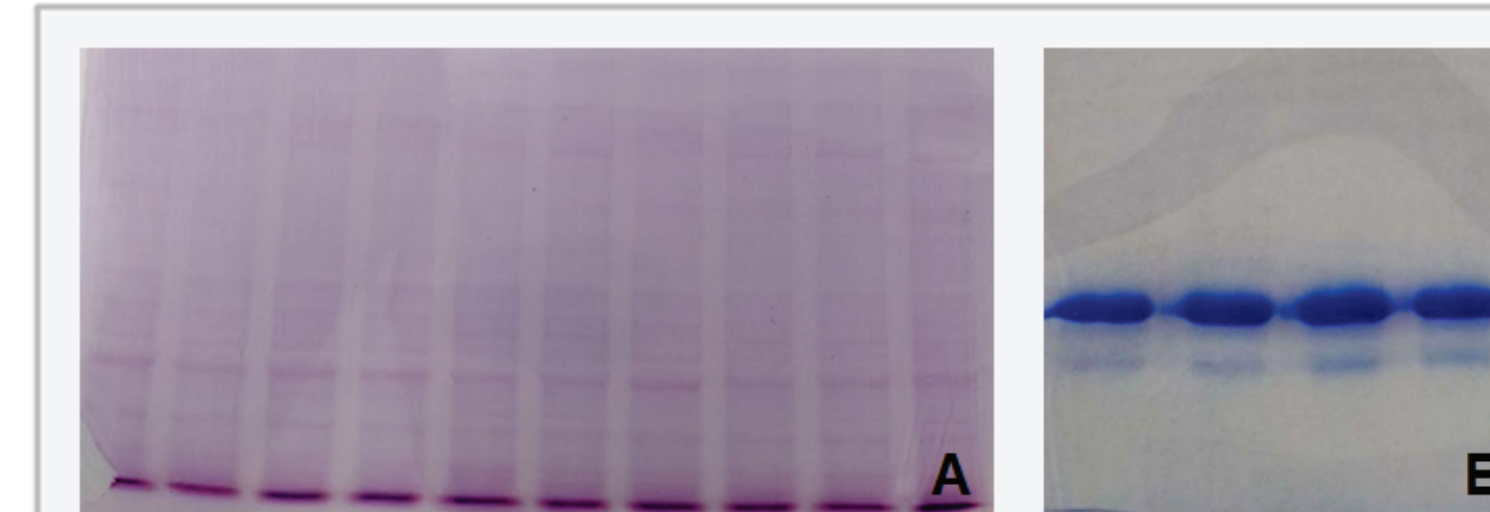


Figure 6: Representative SDS-PAGE gels 10% (20 µg) from A) protein extracts and B) sobrenatants stained with Coomassie Blue

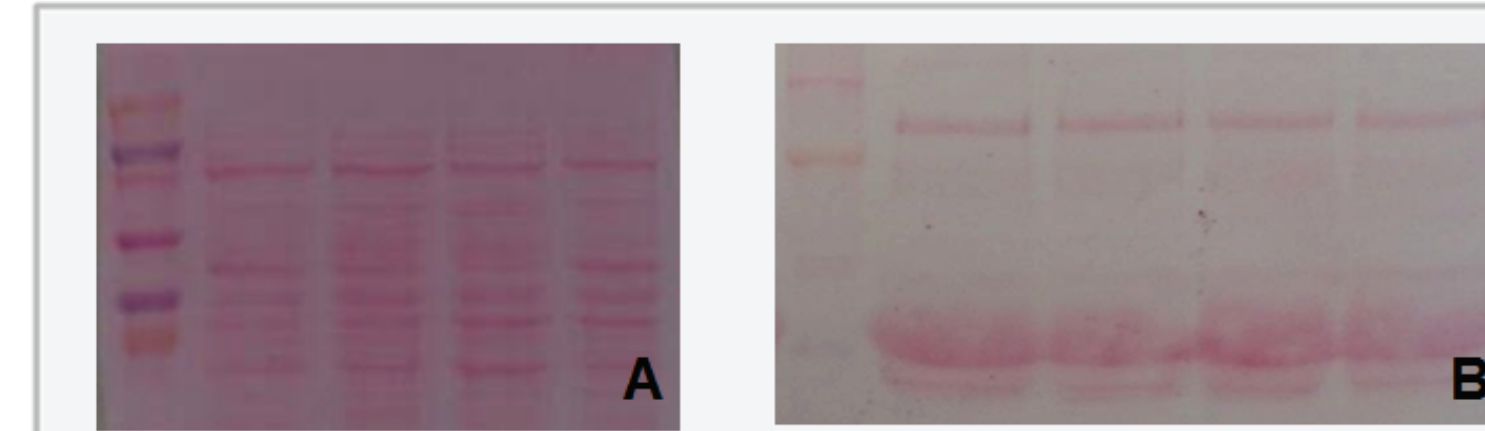


Figure 7: Rouge Ponceau stain after transfer to nitrocellulose membranes. A) Total protein extracts in representative SDS-PAGE gel 12,5% (40 µg). B) Sobrenatant samples from each cell line in gel SDS-PAGE 10% (30 µg).

Difference in protein expression *in vitro* models of BC subtypes

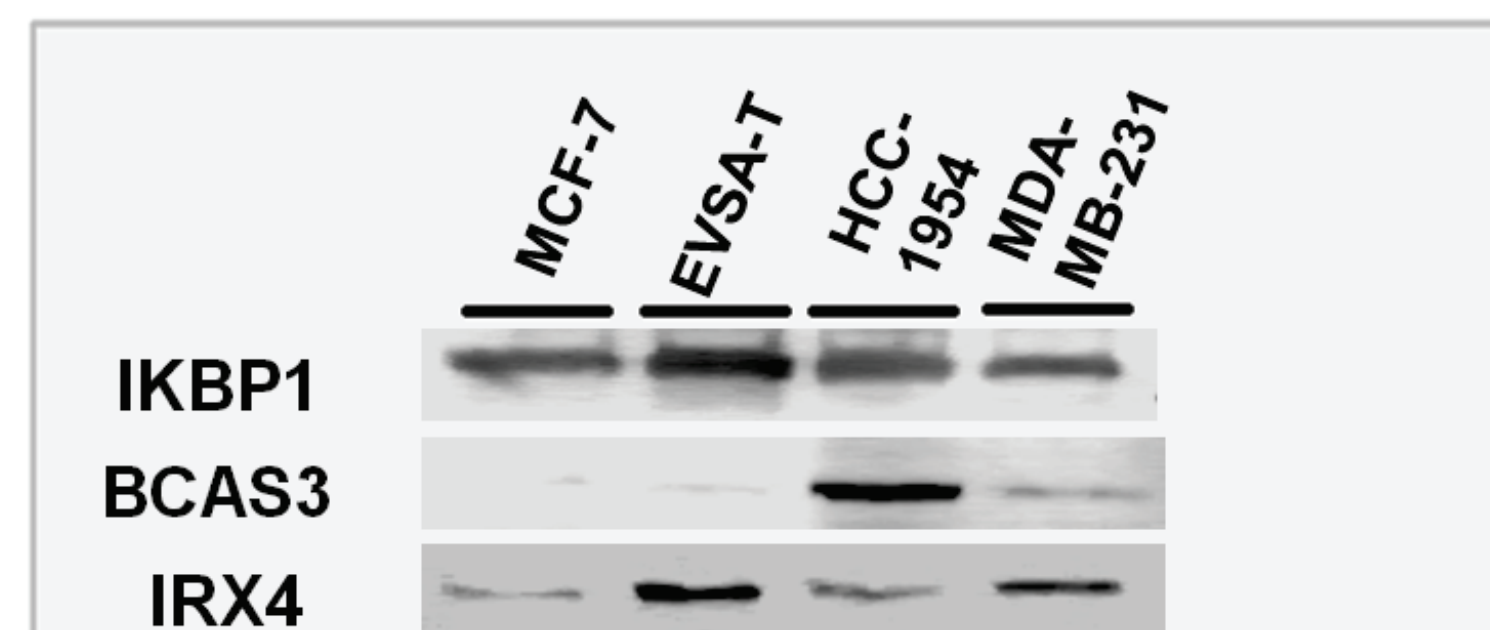


Figure 8: Western Blot analyzes of IKBP1, BCAS3 and IRX4 proteins in biological samples, evaluating different expression of this potential biomarkers between the cell lines' protein extracts.

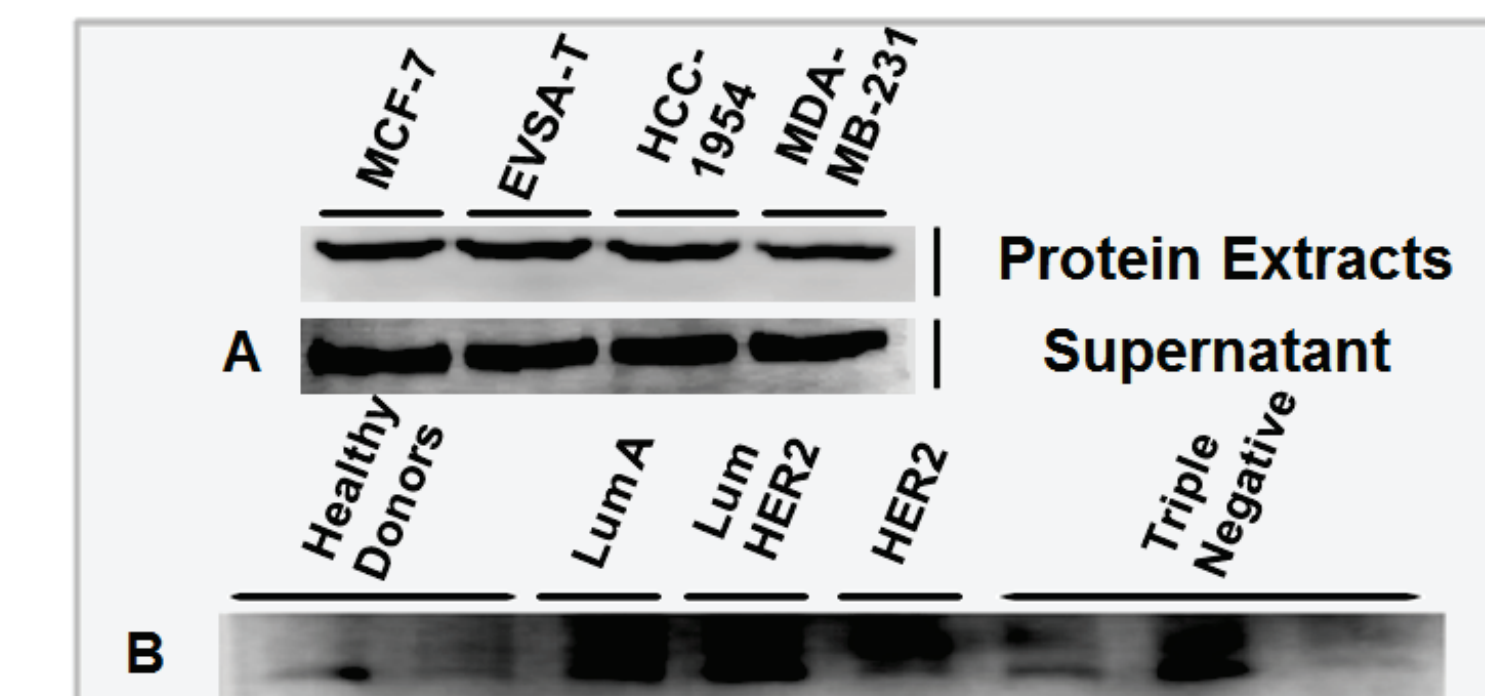


Figure 9: Analysis of IRX5 in A) protein extracts and supernatant samples of BC subtypes compared with B) previous results in patient's blood plasma.

Subcellular localization of evaluated proteins

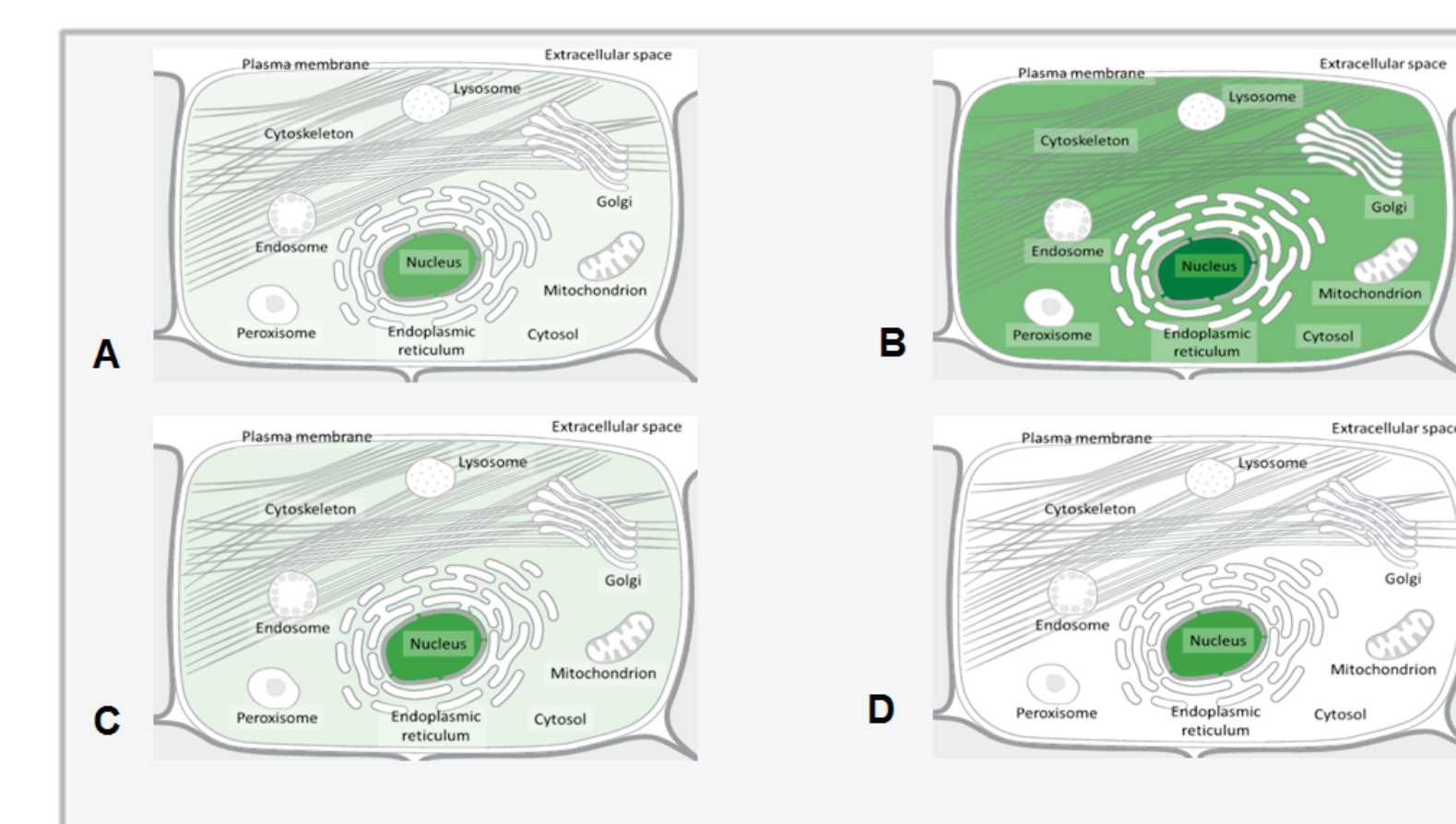
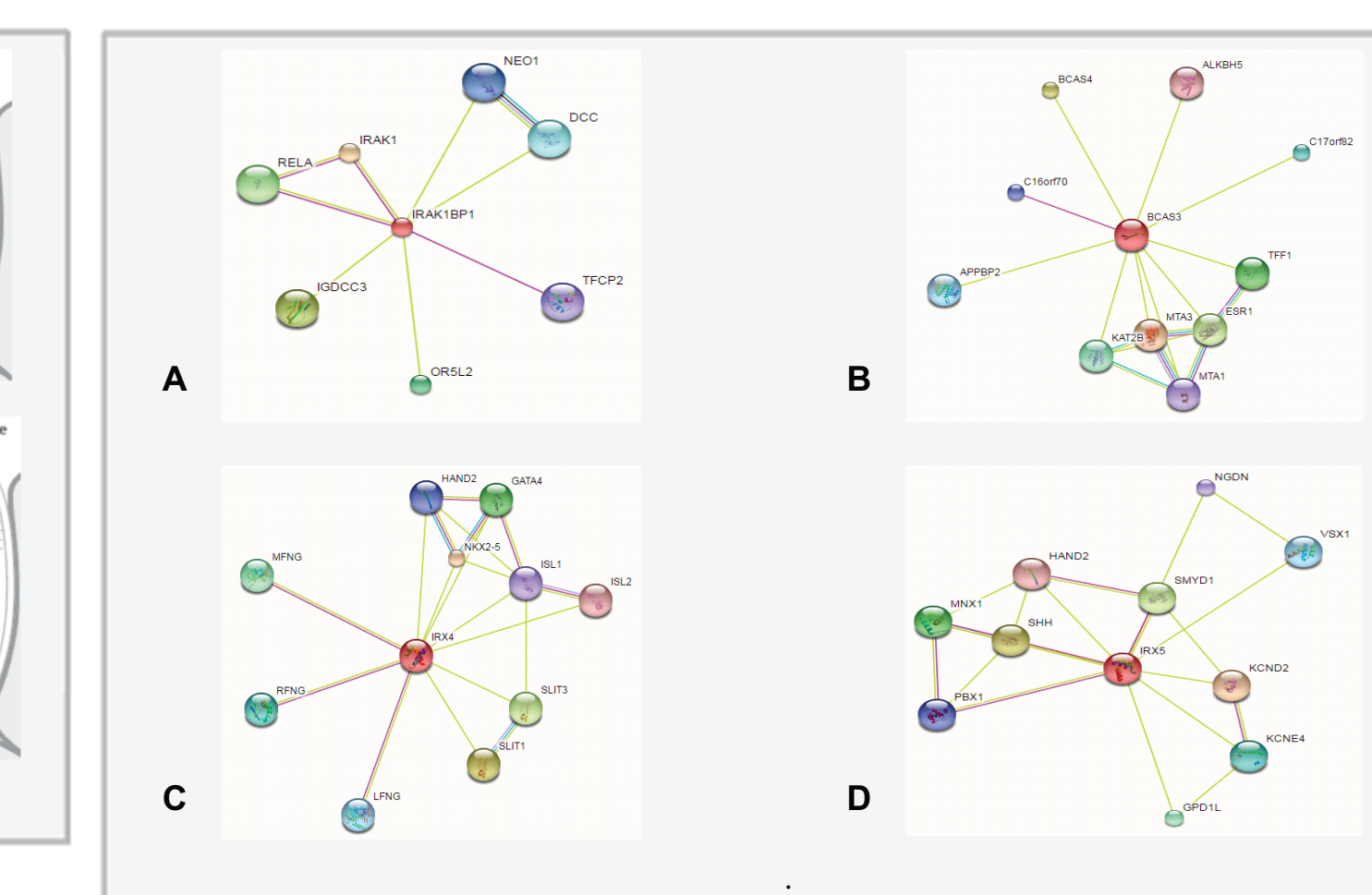


Figure 11: Localization of A) IKBP1, B) BCAS3, C) IRX4 and D) IRX5.

Interaction analysis of potential markers



CONCLUSION

In this work, we evaluated and confirmed the differential protein expression in cell lines and their supernatants, comparing these results with previous findings in BC patient's blood plasma. We expect further to validate the expression of other proteins in the BC cell lines in order to further clarify the biological processes in which they are involved.

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Projeto Gráfico: Serviço de Edição e Informação Técnico-Científica / INCA