Clontech antibody microarray 500: a powerful tool for high-throughput protein expression analysis

Hentrich K, PhD, Nikoloff CR.*

Clontech Laboratories - A Takara Bio Company. *Takara Bio Europe, F-78100 St.Germain-en-Laye; klaus.hentrich@clontech-europe.com

Introduction

Clontech's Antibody Microarray 500 is a powerful tool for proteomic analysis of differential expression patterns between cell, tissue, or body fluids samples. Antibody (Ab) Array analysis detects relative differences in protein abundance of over 500 target antigens simultaneously. Here we demonstrate the utility of the Ab Array approach by profiling protein expression patterns of matched normal vs. tumour cell lines. Differential protein expression was validated by Western blotting, and by comparison with data from the literature.

Material and methods

Clontech's Ab Array consists of over 500 distinct monoclonal antibodies covering five major functional categories: apoptosis, cancer, the cell cycle, protein kinases, and neurobiology. The carefully selected, well-characterized monoclonal antibodies offer the advantages of unlimited supply, reproducibility, sensitivity and specificity. Clontech´s proprietary process ensures that the covalently immobilized antibodies remain functionally active.

In this study, a human lymphoblastoid cell line (normal) and a human breast carcinoma cell line from the same individual (both from ATCC) were compared. Proteins were extracted with Clontech´s nondenaturing Extraction/Labelling Buffer and Cy3/Cy5 labelling was performed according to the Ab Microarray User Manual. Labelled protein extracts were analyzed on the Ab Microarray 500. Internal normalisation was achieved through a dye swapping procedure and data analysis with the internally normalized ratio (INR) algorithm (Andersson et al., 2005).

Results

Twenty-three proteins with elevated abundance levels in the tumor sample (*Table II*) and nine proteins with elevated abundance levels in the normal sample (*Table I*) were identified. Twenty-four proteins comprising targets from both groups were validated by Western blot analysis. Seventeen confirmed the array results, four were inconsistent and three did not show a signal (*Tables I, II*). The Ab Array results showed 81% correlation with the Western blot results, confirming the validity of the array screening approach.

Clontech's Ab Arrays have been used successfully in many other proteomics studies, e.g. on spinal muscular atrophy (Anderson et al., 2003), mantle-cell lymphoma (Ghobrial et al., 2005), cystic fibrosis (Srivastava et al., 2006), detection of autoantibodies (Ehrlich et al., 2007) and biomarker discovery (Caiazzo Jr. et al. (2007).

Conclusions

A fast-growing number of published studies describe the successful use of Clontech's Ab Array in research fields such as signal transduction, cancer and other diseases, underlining its potential for the discovery of disease markers and drug targets.

References

Anderson, K. et al. (2003) Brain 126(9):2052–2064.

Andersson, O., et al. (2005) J. Proteome Res. 4(3):758–767.

Caiazzo Jr., R. J. et al. (2007) Expert Rev. Proteomics

Ehrlich, J. R. et al. (2007) Proteomics Clin. Appl. 1(5):476–485.

Ghobrial, I. M. et al. (2005) Blood 105(9):3722–3730.

Srivastava, M. et al. (2006) Mol. Genet. Metab. 87(4):303–310.

Table I: Control Elevated

Ab-Ag	Ave. INR	Literature	Western	ap.	can.	cc	neuro	pk	Ab-Ag	Ave. INR	Literature	Western	ap.	can.	cc	neuro	pk
Rag-2	0.66	+	+			+			Dynamin I	0.76		-					
Perforin	0.67		+			+			hcKrox	0.77		+					+
SNX 1	0.70		+						Exportin-1/CRM1	0.78		+				+	
DNA Topoisomerase IIb	0.75		+			+			Myogenin	0.78	+	+					+
									MGMT	0.79	+				+	+	

ap: apoptosis; can: cancer; cc: cell cycle; neuro: neurobiology; pk: protein kinases

Table II: Tumor Elevated

Ab-Ag	Ave. INR	Literature	Western	ap.	can.	cc	neuro	pk	Ab-Ag	Ave. INR	Literature	Western	ap.	can.	cc	neuro	pk
HSF4	4.99	+	+					+	p45 (SUG1)	2.04					+		
Neurogenin 3	3.38							+	EGF Receptor	2.02	+	+		+		+	+
IL-13	3.17	+	NA					+	COMT	2.02	+						
Flotillin 2 (ESA)	3.04	+			+				Mcl-1	1.92	+	-					
Ref-1	3.00					+	+		APC	1.91		+		+		+	
IL-5	2.52	+	NA					+	CAS	1.91	+	+	+		+		
Annexin XI	2.16	+	-						D4-GDI	1.89					+		
SMRT	2.15	+	NA					+	cGB-PDE (PDES)	1.88		+					+
P54^{nrb}	2.12	+	+	+					Cdk4	1.87	+	+			+		+
Brm	2.11							+	NMDA Receptor (NR-2B)	1.87		+					
PARP	2.09		-	+		+			NF-ATc2	1.86		+					+
									MAP4	1.79	+	+		+			

INR < 0.79: Protein more abundant in control (normal)
INR > 1.79: Protein more abundant in breast cancer (tumor)
Ave. INR = 1.19
NA: no signal on the Western blot