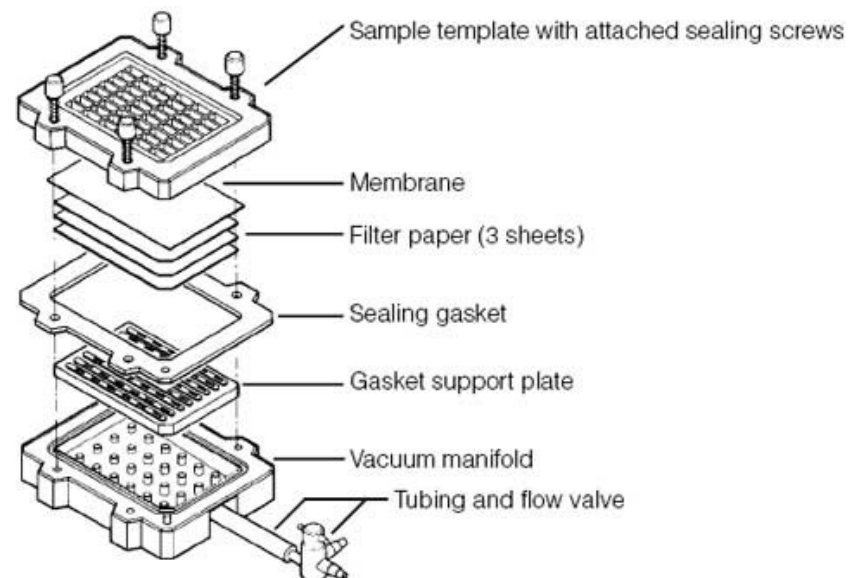
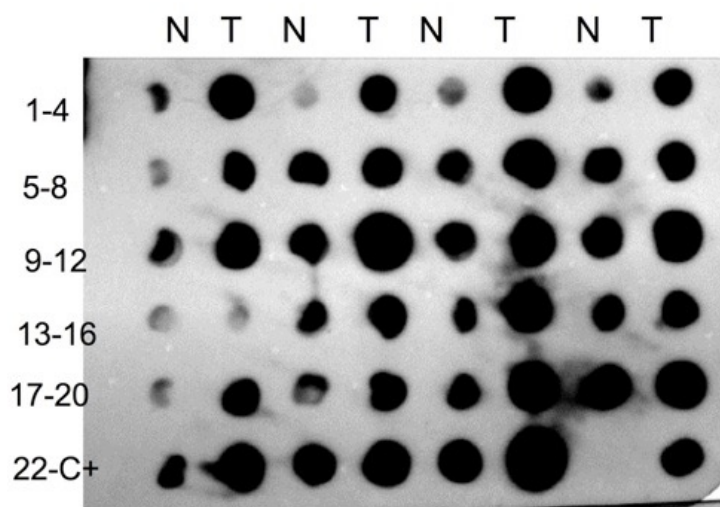


PROTEIN DOT BLOT

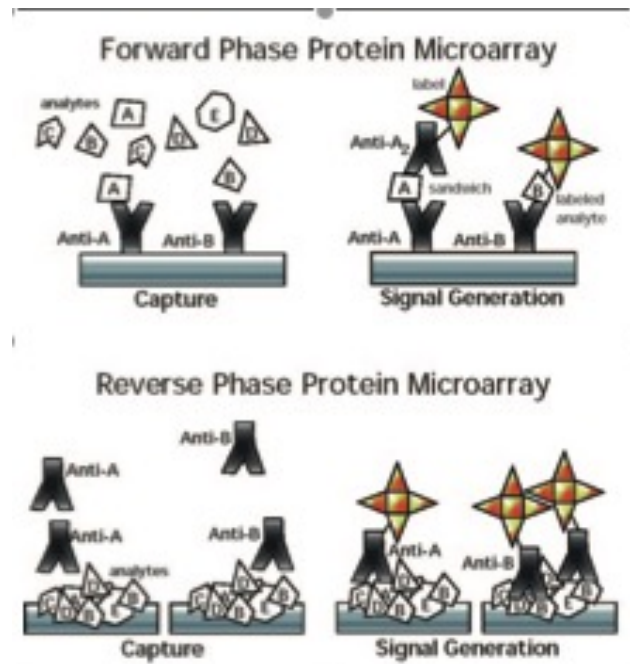
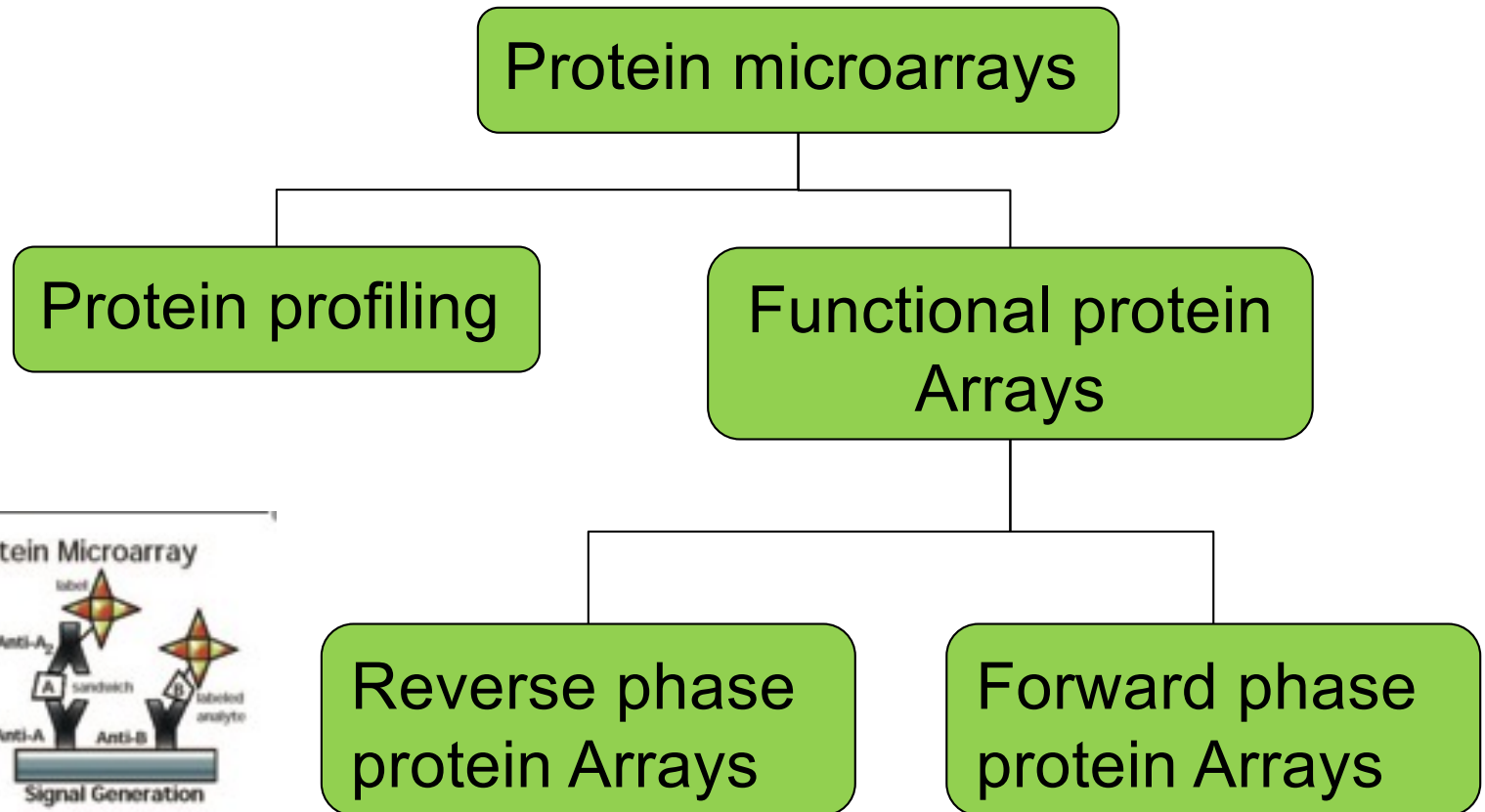
1. PVDF membrane activation
2. Membrane in PBS
3. Assembly of the apparatus
4. Spot the protein using the extraction buffer
5. Protein block as for WB

Cdk2 1:2500



2 μ g di estratto proteico per ogni campione; 5' di esposizione

Protein microarrays



Protein microarrays

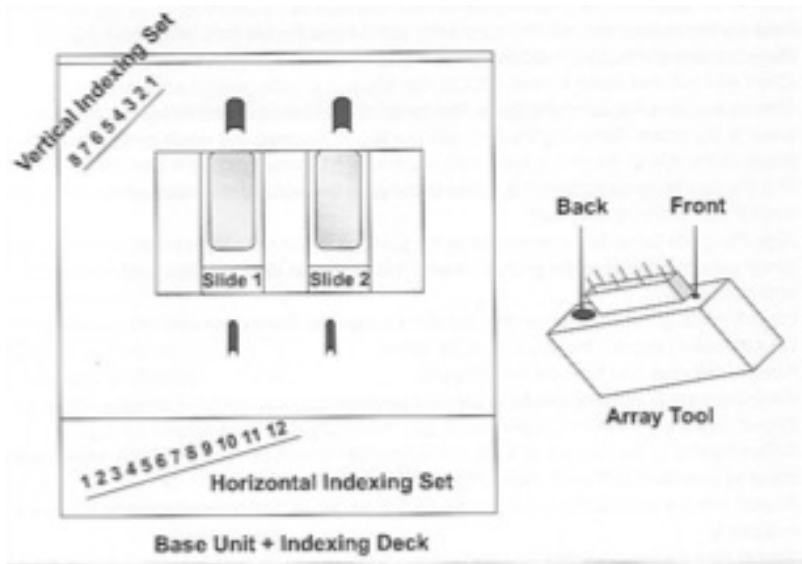
Forward protein microarrays

- In a forward-phase array format, a large number of immobilized capture molecules enable the analysis of many parameters from a single sample
- Each array, is incubated using a proteins extract (Patients' lysates or serum).
- More analytes are detected

Reverse phase protein microarrays

- Micro dot-blot
- In a reversed-phase array, many samples are immobilized.
- highly specific antibody used to analyze the expression of a single parameter in the immobilized samples.
- Specificity Ab \Rightarrow cross-reaction and Ab availability

MicroCaster System



Orientation of slides and pins

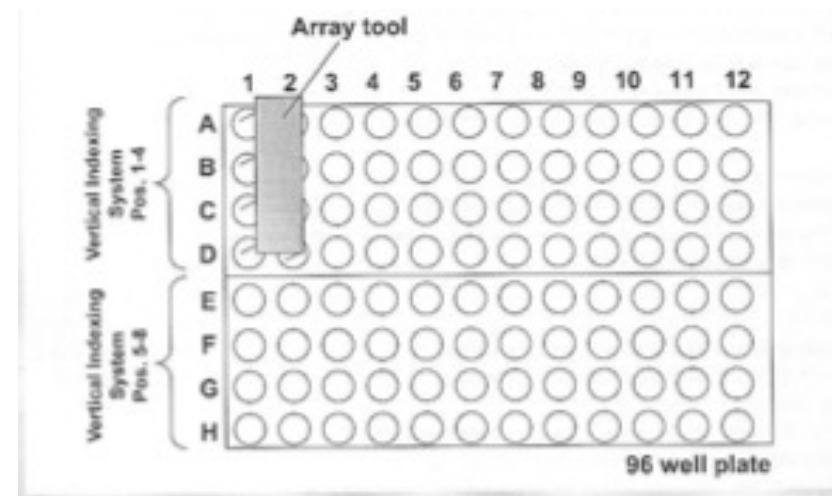
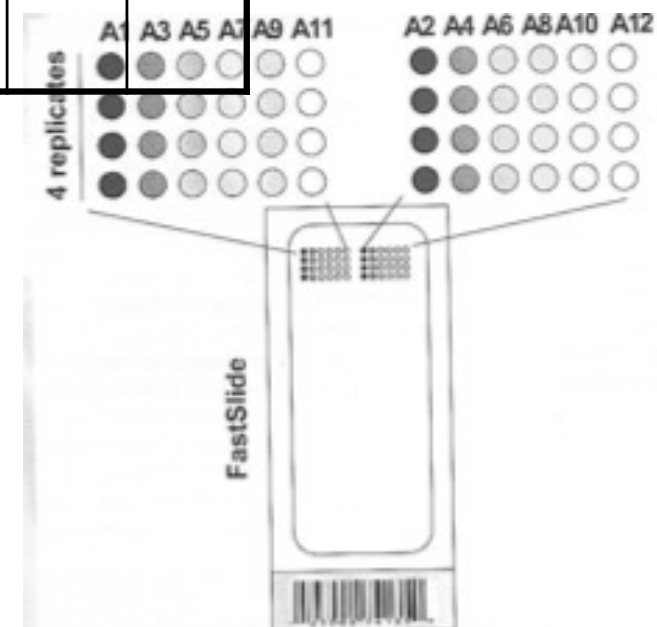


Plate with samples to spot

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1 undil	S2 undil	S1 1:2	S2 1:2	S1 1:4	S2 1:4	S1 1:8	S2 1:8	S1 1:16	S2 1:16	Buff.C trl.	Buff.C trl.
B	S5 undil	S6 undil	Ect.									
C												
D												
E	S3 undil	S4 undil	S3 1:2	S4 1:2	S3 1:4	S4 1:4	S3 1:8	S4 1:8	S3 1:16	S4 1:16	Buff.C trl.	Buff.C trl.
F	S7 undil	S8 undil	Ect.									
G												
H												

From sample to array



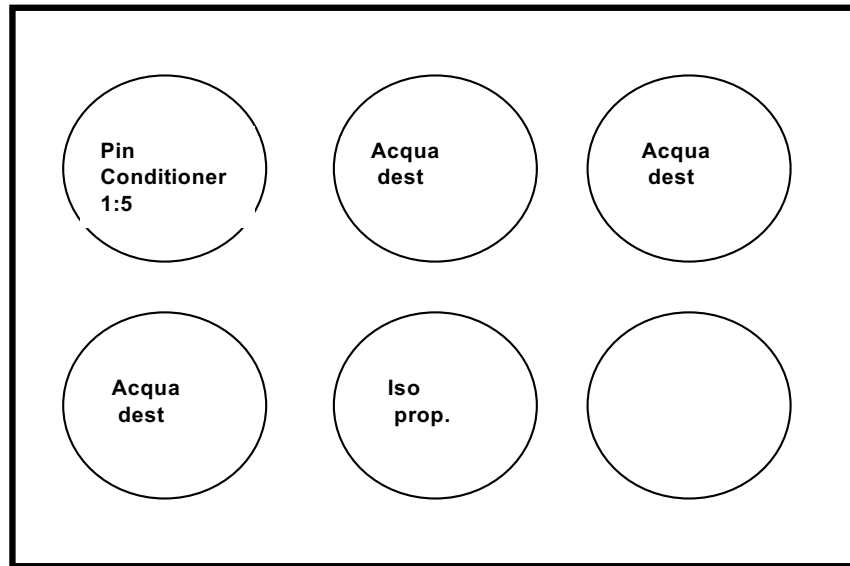


Plate A: for coating

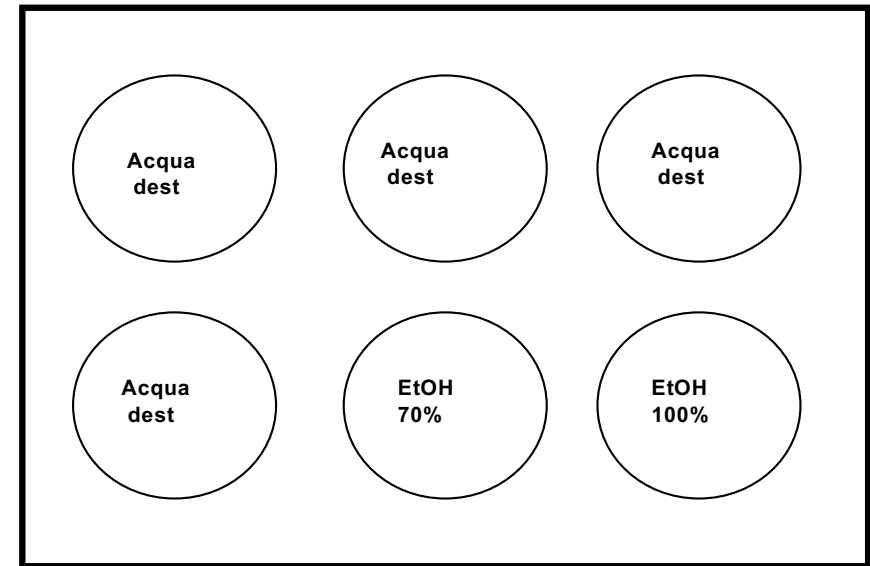


Plate B: for washing

Steps:

- 1. Prepare the plate with the dilutions, seal it, centrifuge it and keep it on ice.**
- 2. Wash and balance pins**
- 3. Array set up**
- 4. Wash between one spot and another**

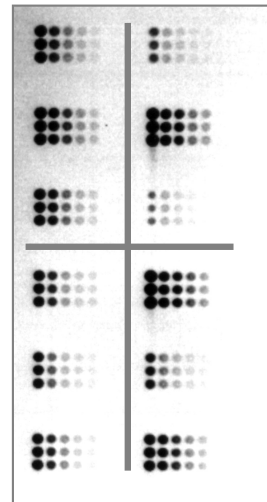
Protein microarrays

FFPE

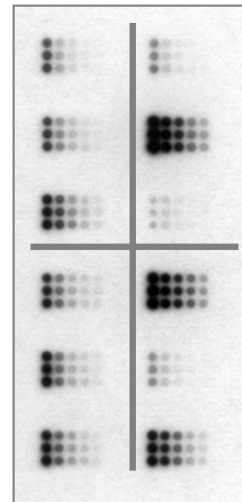
Pools	Lung		Breast
	1	7	
	2	8	
	3	9	
	4	10	
	5	11	
Colon	6	12	

Outline

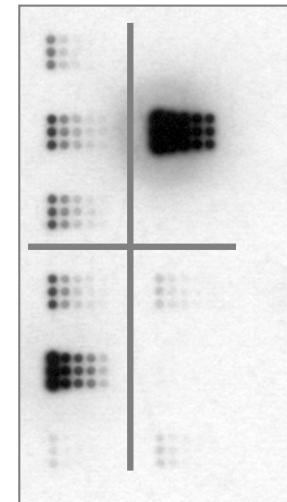
FineFix



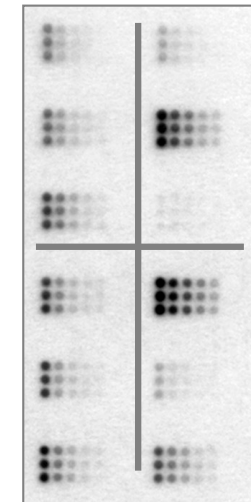
Sypro



E-cad



EGFR



HER2

