

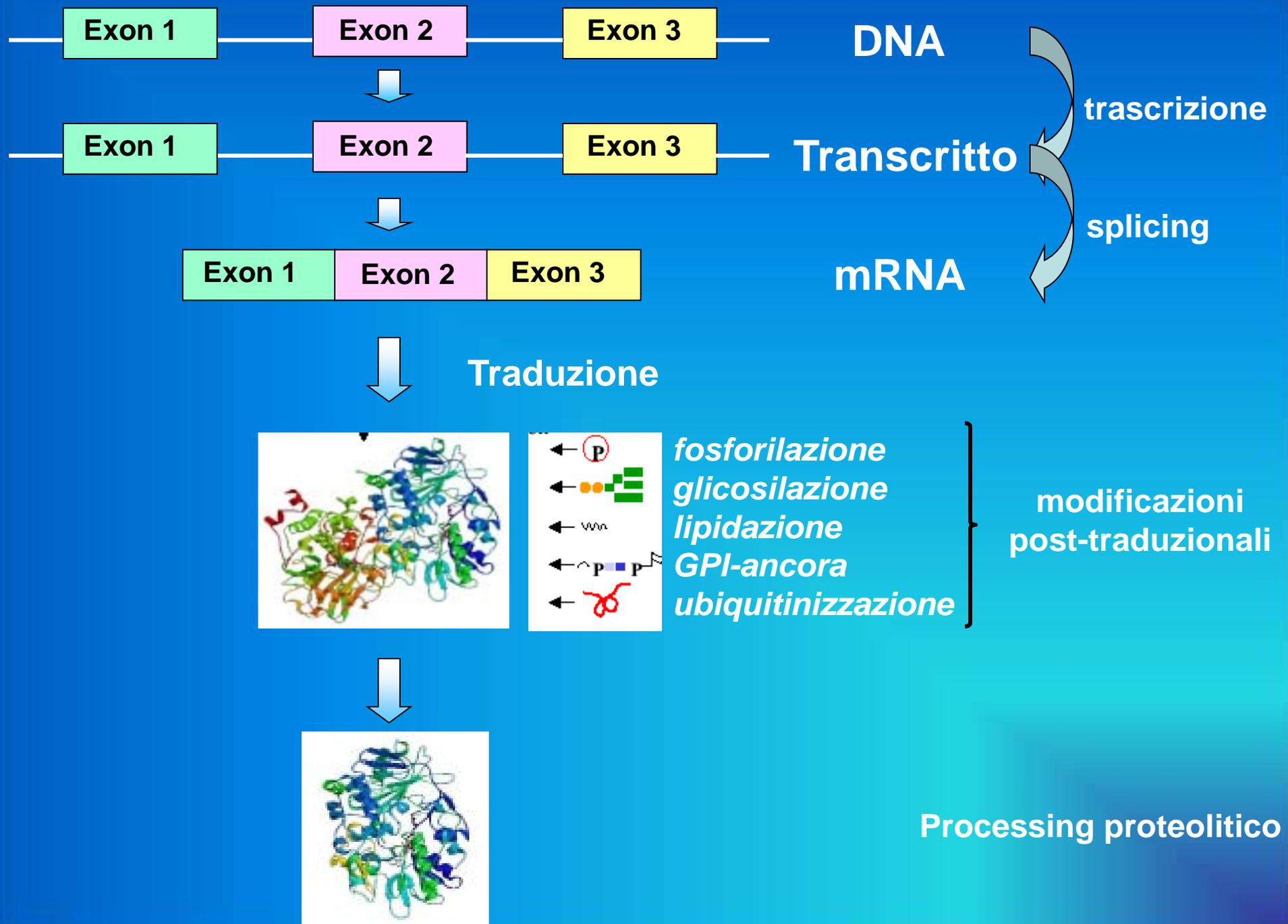
***Elettroforesi***  
***Bidimensionale (2D)***

# PROTEOMA

**PROTE** ine espresse dal gen **OMA**

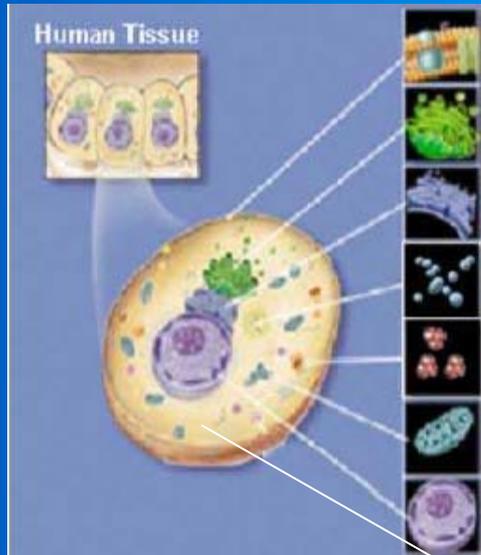
Termine coniato per la prima volta al

“Siena 2D Electrophoresis meeting” del 2004

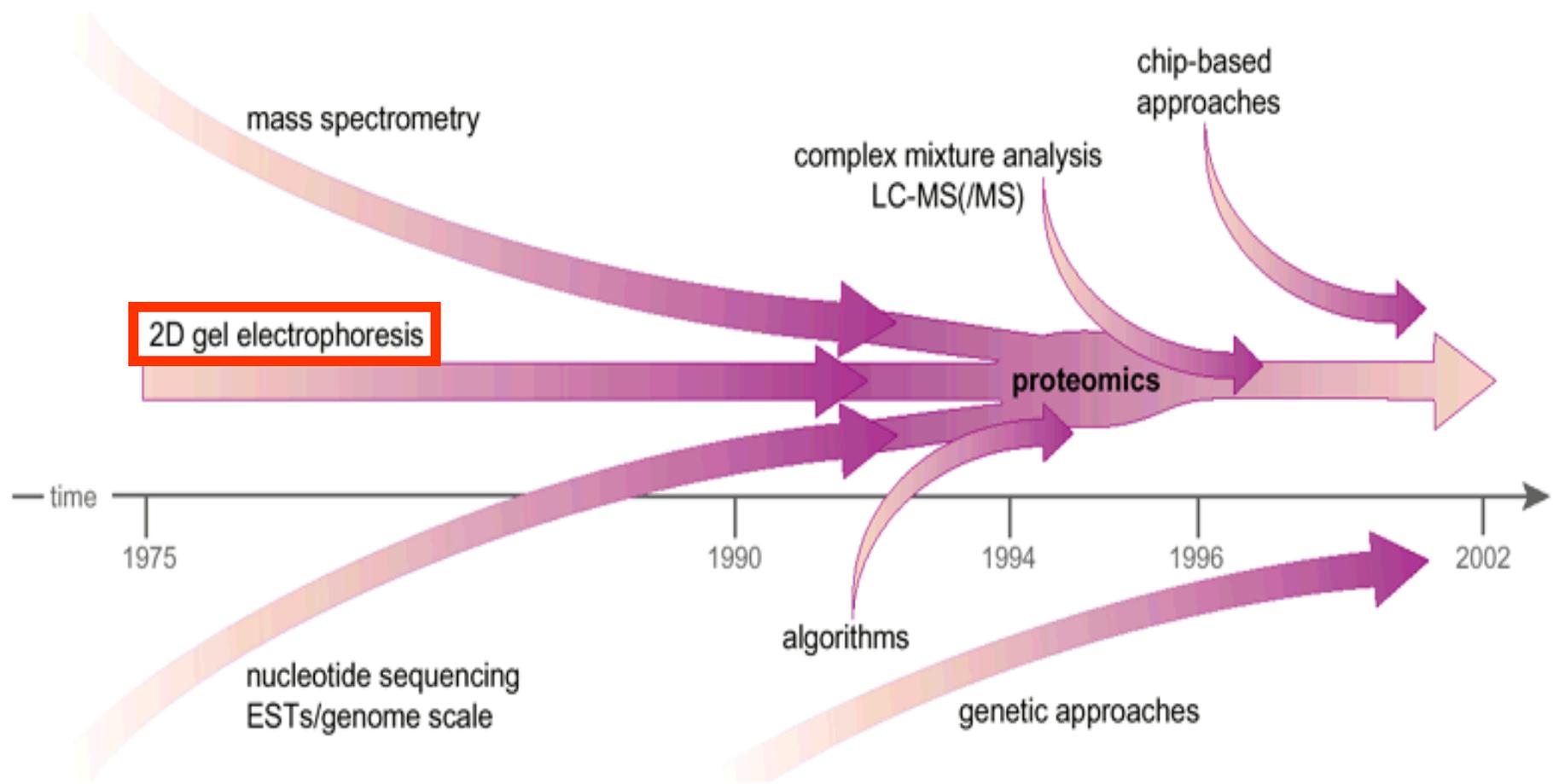


# NEI TESSUTI UMANI

- 32000 geni predetti
- 260 differenti tipi cellulari
- più di 1'000'000 di proteine
- NON TUTTI I GENI SONO ESPRESSI NELLO STESSO MOMENTO IN TUTTE LE CELLULE



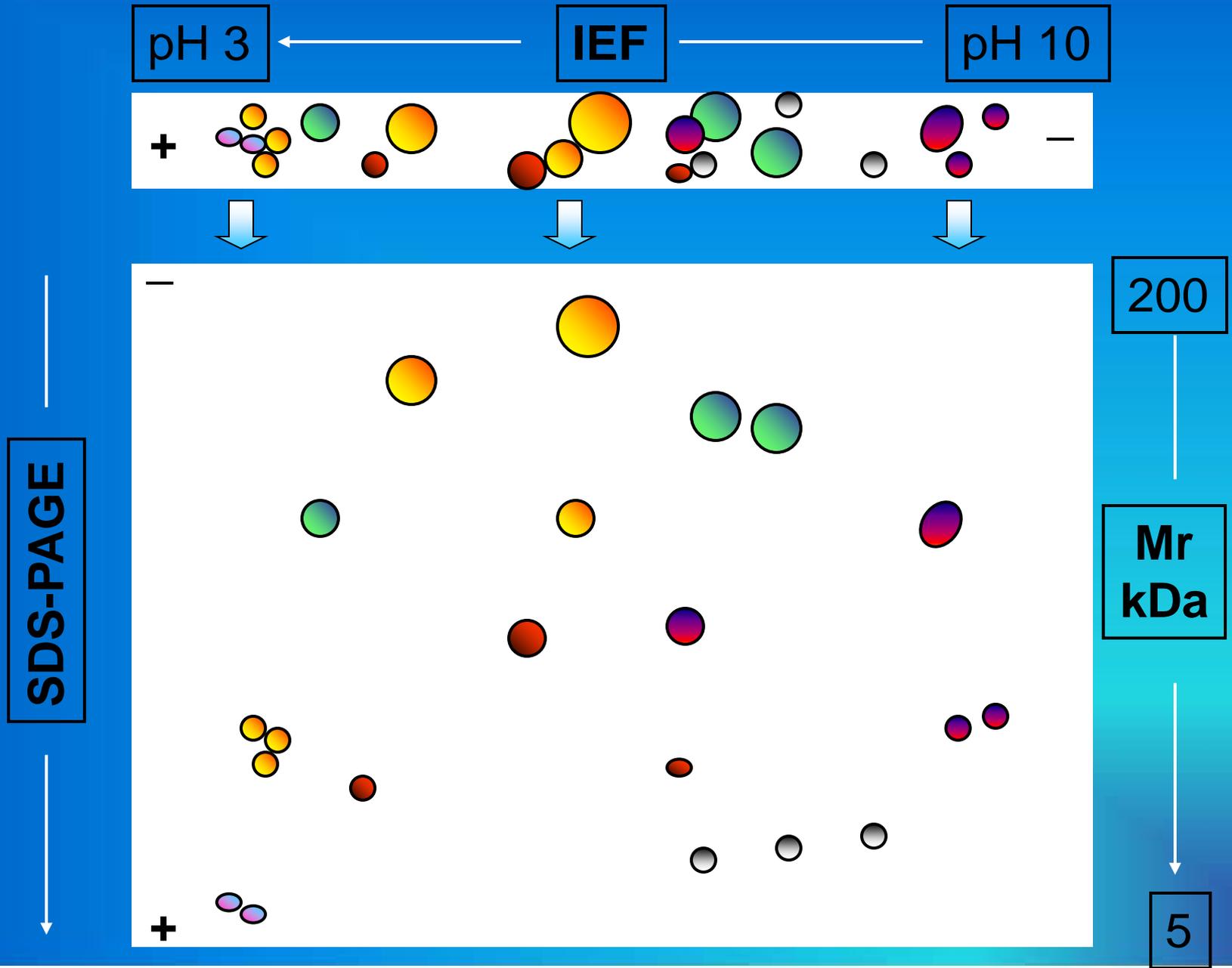
<i>ORGANELLE</i>	<i>% Total cell volume</i>	<i>Number / cell</i>	<i>Gene product</i>
Plasma membrane	2	1	1000-1500
Golgi	1	1	1000-1500
ER	15	1	1200-1800
Peroxisome	1	250	500-600
Endocytic-lysosomal compartments	4	1000	2000-3000
Mitochondria	12	1500	3000-4000
Nucleus	10	1	2500-3500
Cytosol	55	1	8000-10000



# ***Finalità***

- **relazione con malattie genetiche**
- **interazioni Proteina-Proteina e Protein-Cellula**
- **identificazione di nuovi target proteici dei farmaci**
- **studi tossicologici “*in vitro*” and “*in vivo*”**
- **confronto tra tessuti normali e affetti da patologie**
- **confronto tra malattia e trattamento farmacologico**
- **studi delle modificazioni post-traduzionali**
- **identificazione di markers patologici nei fluidi biologici**
- **analisi di tessuti e fluidi tumorali**
- **identificazione di nuovi vaccini antigenici**

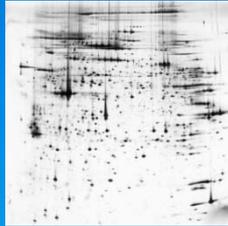
# 2 D - PAGE



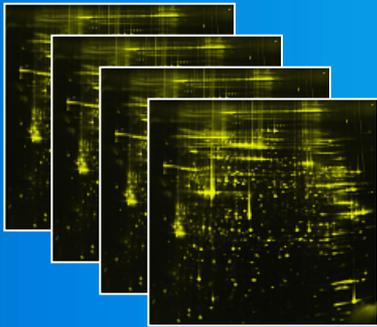
# preparazione del campione

Arricchimento  
Solubilizzazione  
Riduzione e alchilazione

## 2D - PAGE

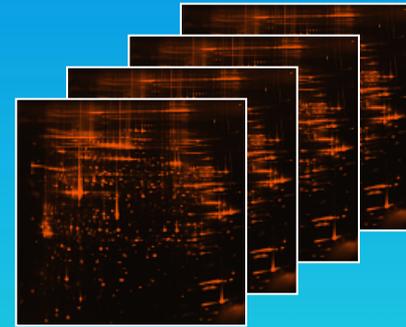


controllo

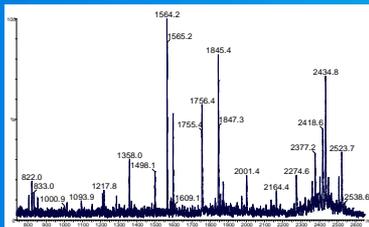
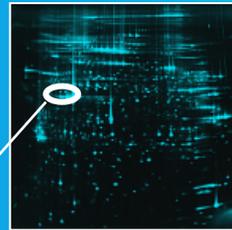


Analisi dell'immagine

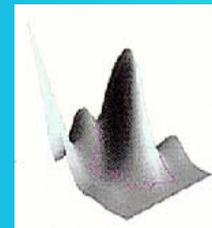
trattamento



Matching



Spettrometria di massa



Identificazione e quantificazione

# Preparazione del campione

# Prima parte a + 4°C

Campione (usare buffer molto semplici con inibitori delle proteasi!!!)



**Purificazione/estrazione**

- micro-dialisi (molto lunga)
- precipitazione con TCA (scarsa efficienza)
- precipitazione con Acetone (buona)**
- precipitazione con TCA in acetone (scarsa efficienza)
- precipitazione con metanolo-cloroformio (solo per piccoli volumi)
- precipitazione con tributil fosfato-acetone-metanolo (buona)**
- liofilizzazione (buona)**
- Kit commerciali**



**Quantificazione con metodi spettrofotometrici**



**Presolubilizzazione**

(solo per campioni ricchi in lipidi e condizioni denaturanti)

- SDS** - tensioattivo
- DTT** - agente riducente



**Denaturazione (95°C x 5')**

# Seconda parte a RT al buio

Solubilizzazione

**NATIVA**

1 - 4 M Urea  
1-4 % Detergente [w/v]\*  
0.5 - 2 % Anfoline [w/v]

**Zwitterionic**  
CHAPS  
CHAPSO  
Sulfobetaines

**NON-ionic**  
NP-40  
Triton  
Tween  
Brij

**DENATURANTE**

7 - 9 M Urea and 0.5 - 2 M Tiourea  
1 - 4 % Detergente [w/v]\*  
0.5 - 2 % Anfoline [w/v]

**Agente Riducente**

DTT-DTE 10 - 100 mM  
TBP 5 mM  
TCEP 10 mM

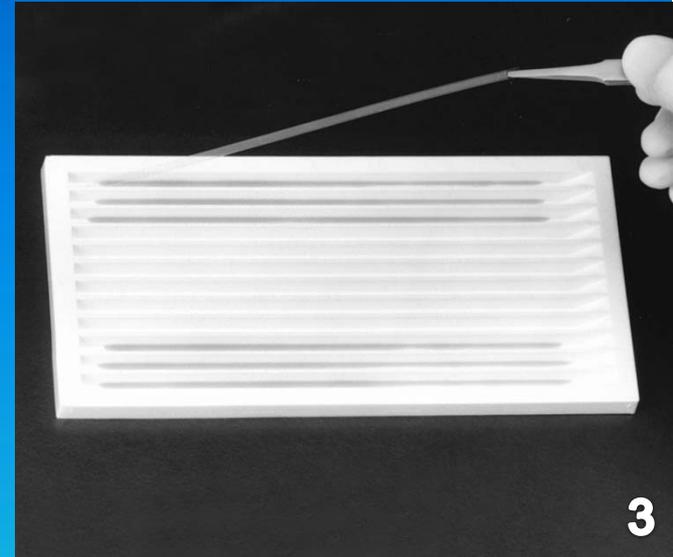
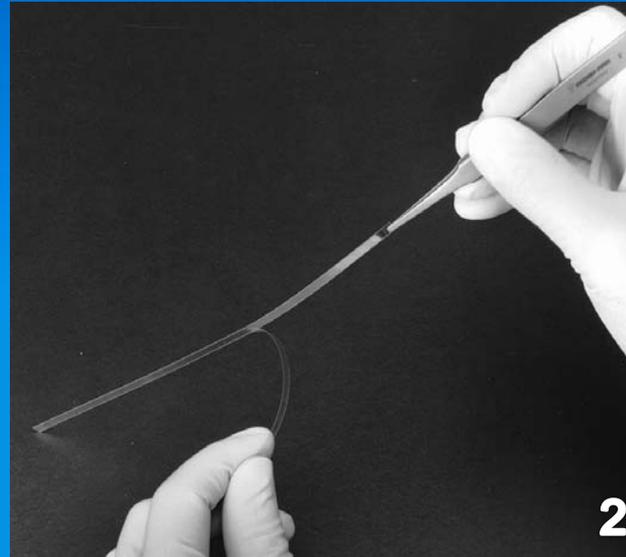
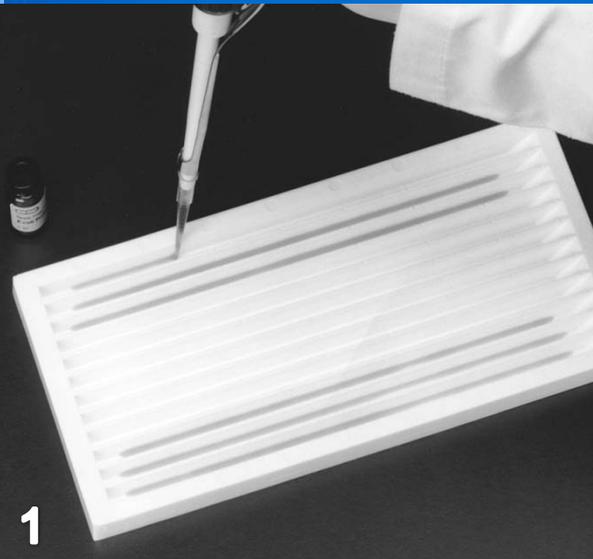
Riduzione/alchilazione

Iodoacetammide  
Tributilfosfina (TBP)

Eliminazione della parte  
insolubile dal campione  
(14000g X 5') e trasferimento del  
surnatante in un nuovo tubo

# Prima Dimensione IEF

# Idratazione over night delle strip



1 Buffer di idratazione

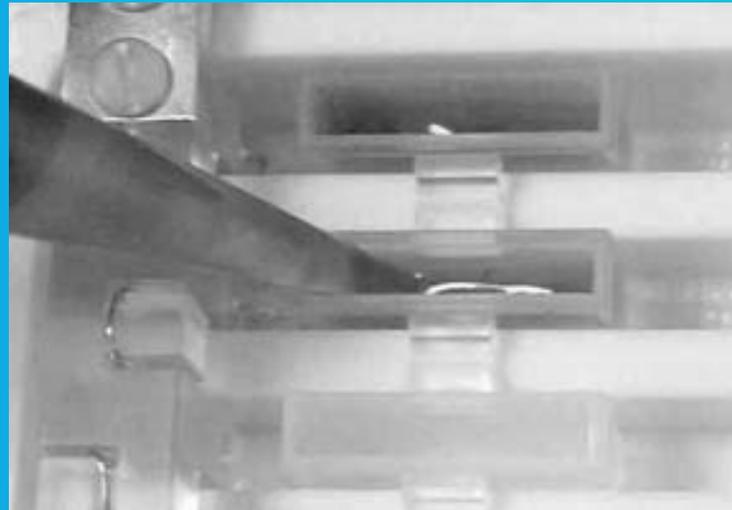
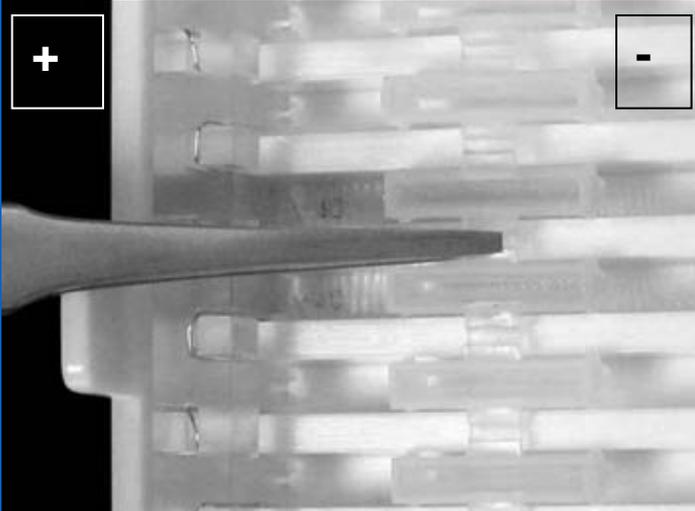
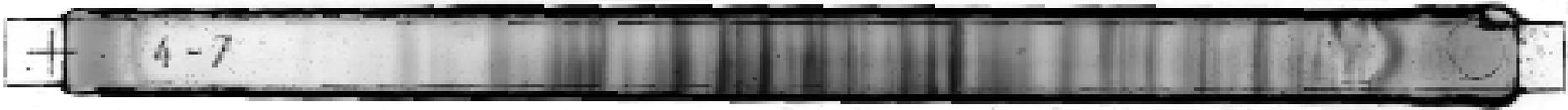
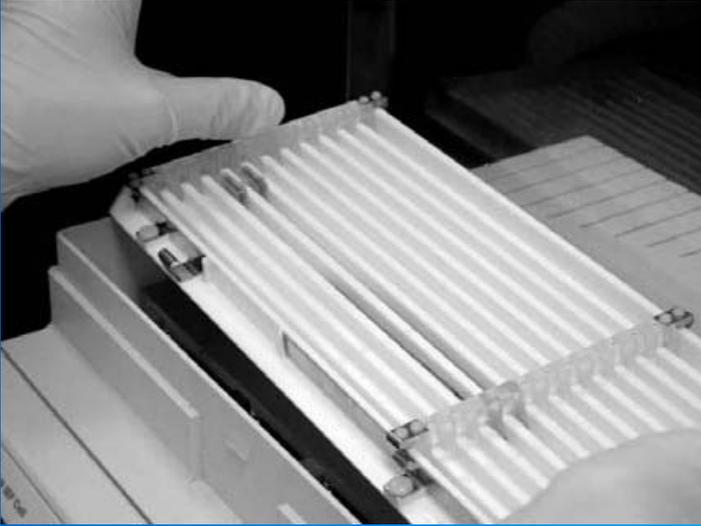
2 Apertura della strip

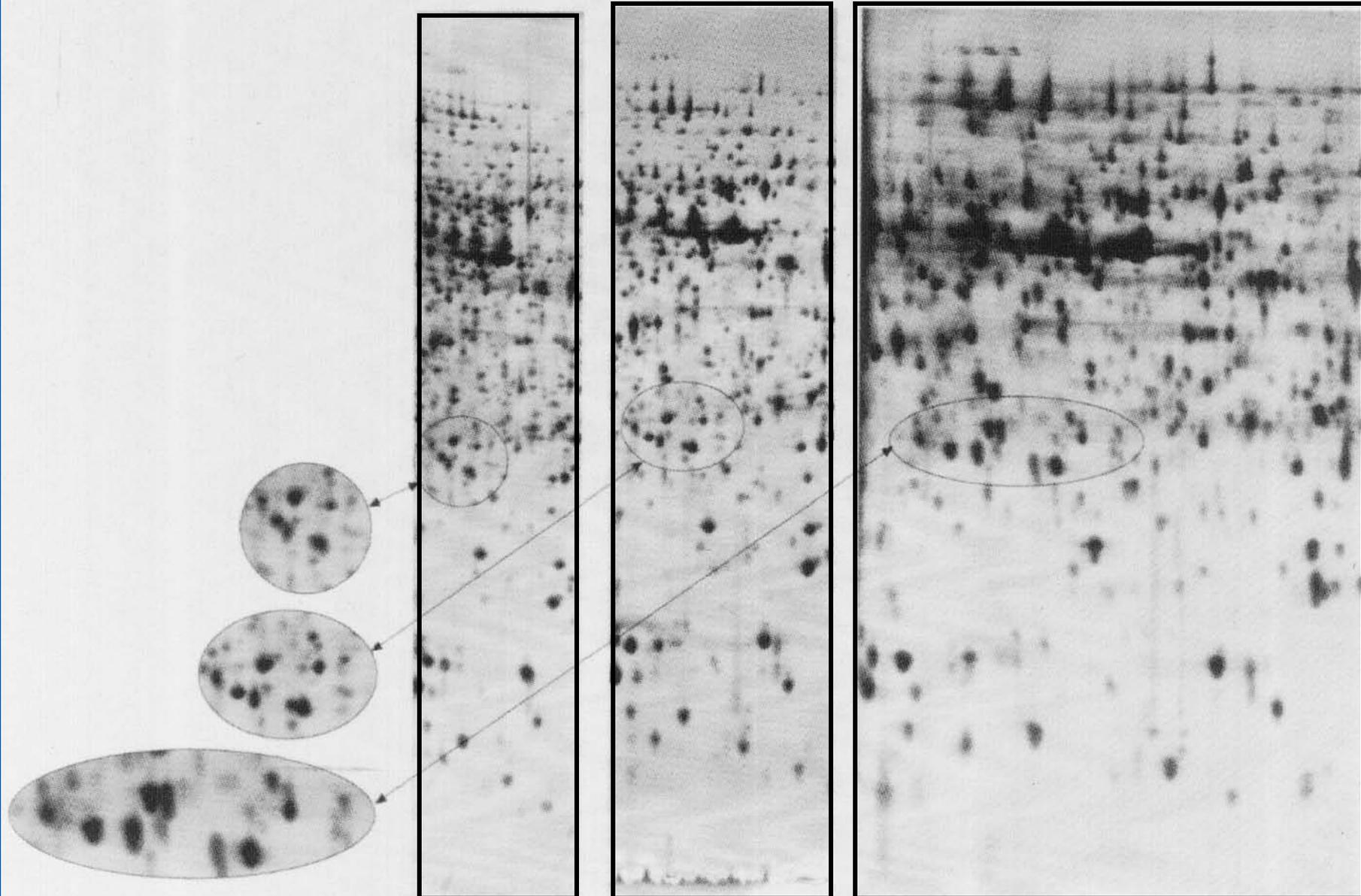
3 Matrice polimerica della strip a contatto con il buffer di idratazione

## **BUFFER DI IDRATAZIONE**

urea 8M  
tiourea 0,5M  
CHAPS 2%  
DTT 10mM  
anfoliti trasportatori 0,24%  
blu di bromofenolo in tracce

# Focalizzazione





**IPG pH range**

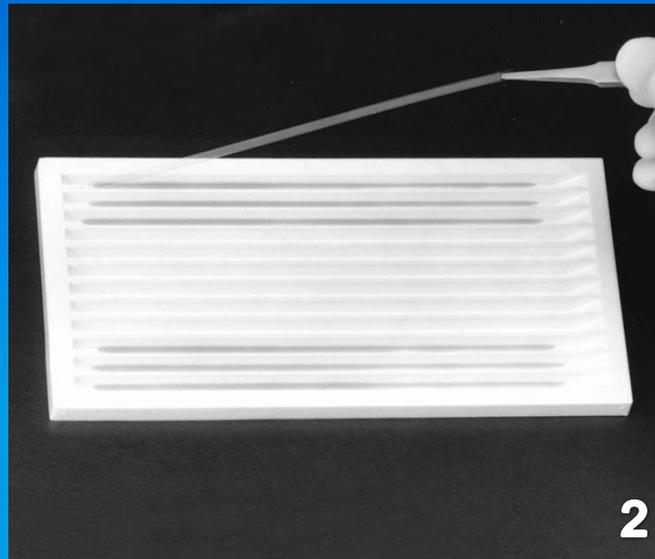
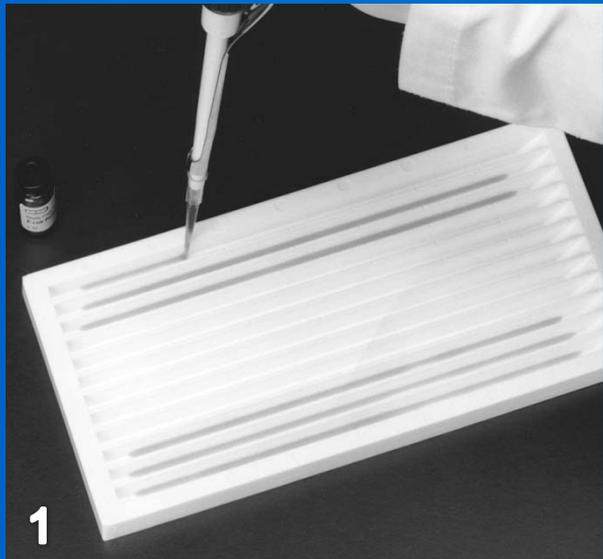
**3-10**

**4-7**

**5-6**

# Seconda Dimensione SDS-PAGE

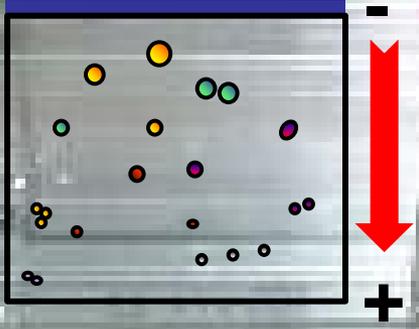
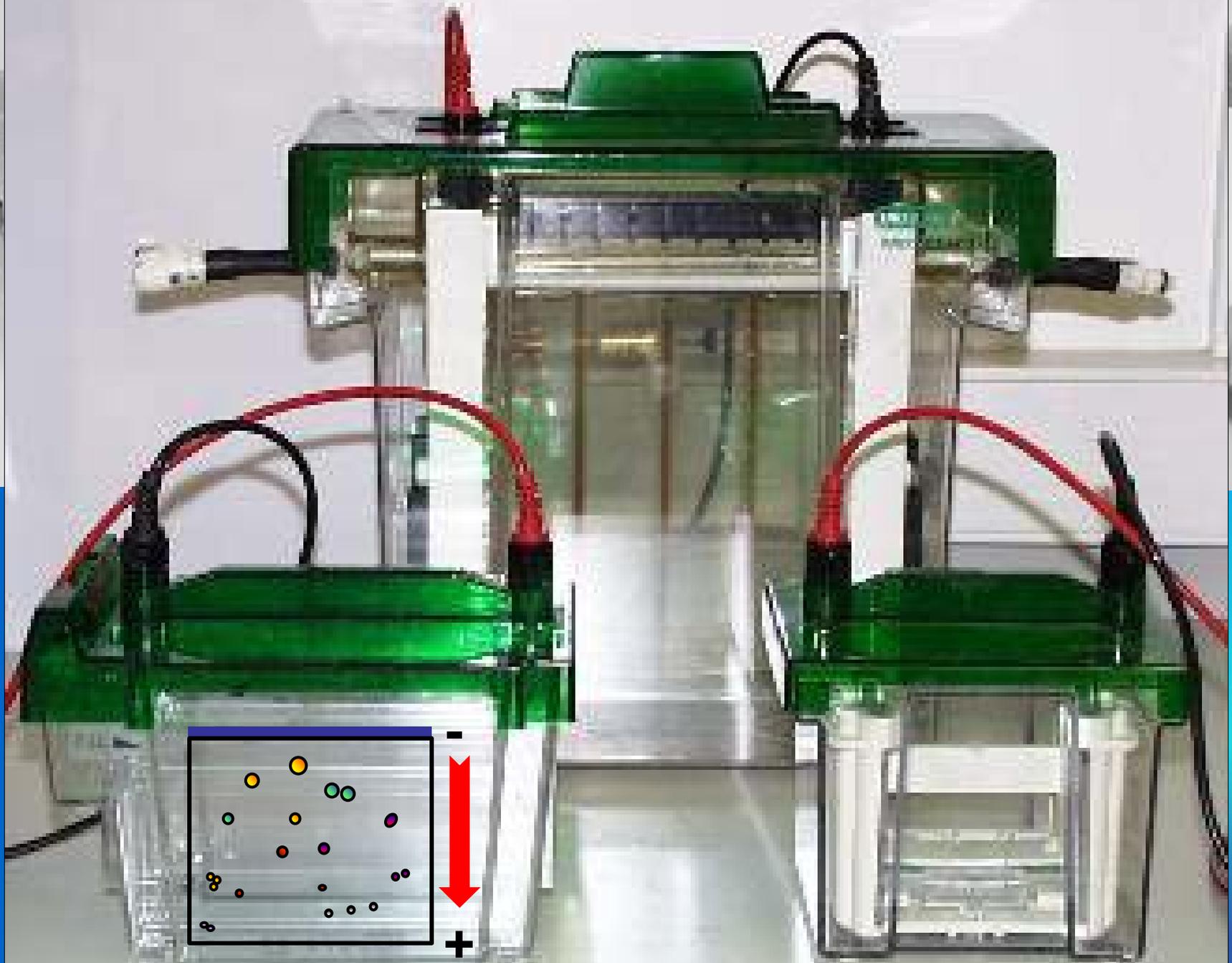
# Equilibratura della strip



- 1 Buffer di equilibratura nel canale scelto
- 2 Matrice polimerica della strip a contatto con il buffer di equilibratura
- 3 Incubazione 30' a RT in bascula

## **BUFFER DI EQUILIBRAZIONE**

Urea 6M  
SDS 1%  
Tris-HCl 50mM  
Glicerolo 30%  
blu di bromofenolo in tracce

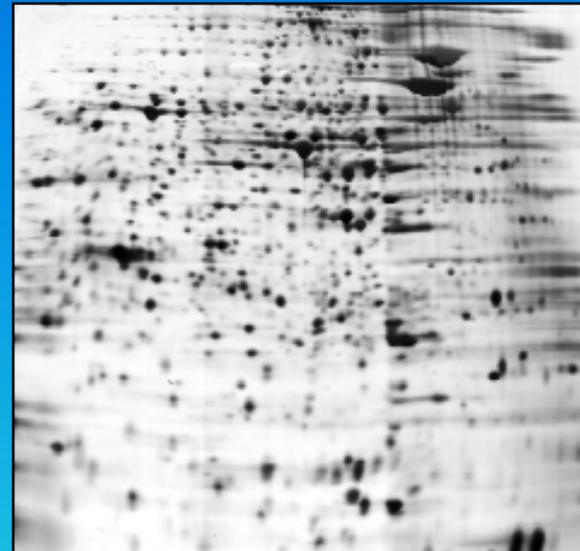
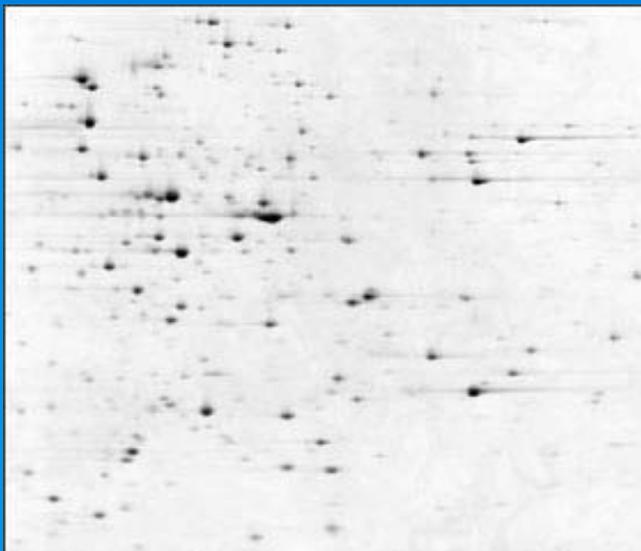
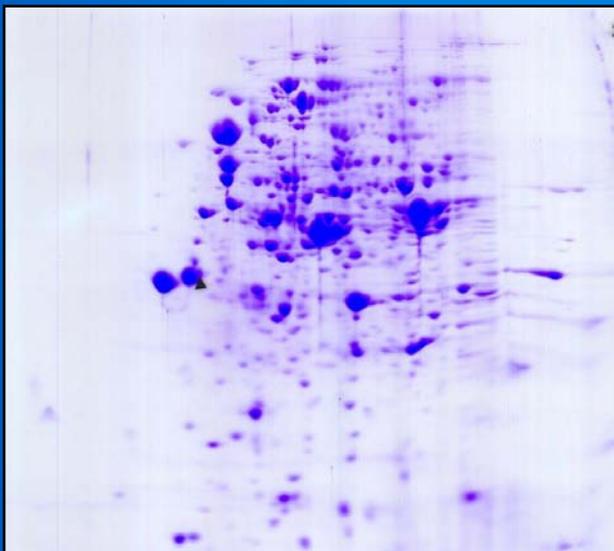


# Protein Staining

**COOMASSIE**

**SYPRO-RUBY**

**SILVER**



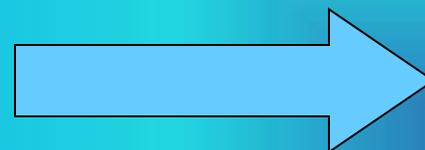
40-70 ng

5-10 ng

1-2 ng



**SENSIBILITY**



<b>Colorazione</b>	<b>Limite della detection</b>	<b>Range dinamico lineare</b>	<b>Compatibilità con la Spettrometria di Massa</b>
Sali di Rutenio	1-2 ng	1-1000 ng	Yes
Silver Stain	1-2 ng	8-60 ng	No
Sypro Ruby	5-10 ng	5-1000 ng	Yes
Coomassie	40-50 ng	100-1000 ng	Yes

## *Variabili sperimentali*

Tipo di campione  
Tipo di purificazione del campione  
Tipo di detergente utilizzato  
Condizioni di solubilizzazione  
Uso di materiale pulito e dedicato  
Abilità dell'operatore

**MAPPA 2D**

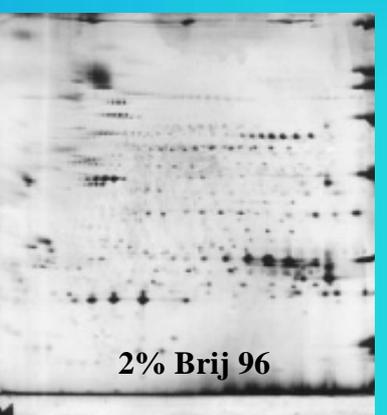
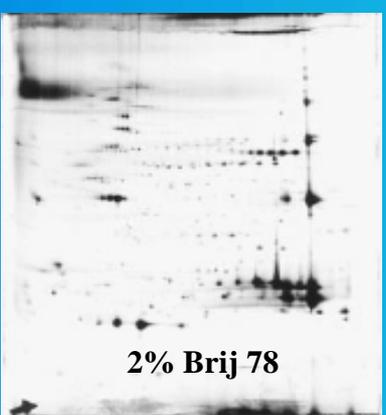
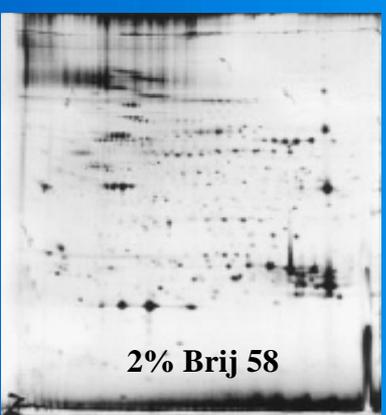
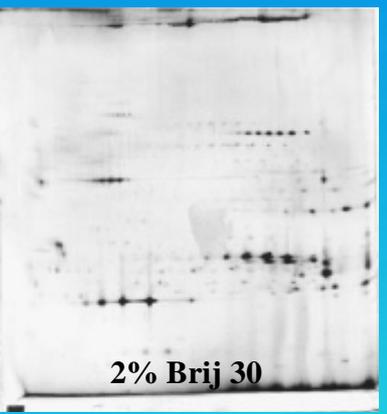
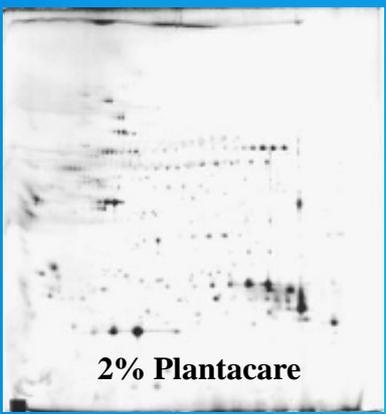
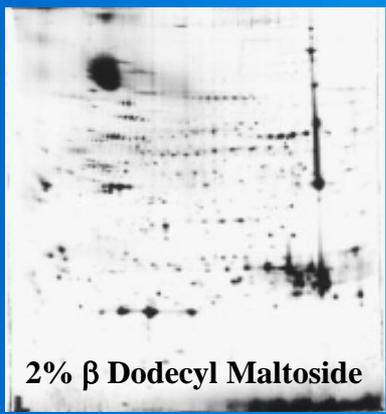
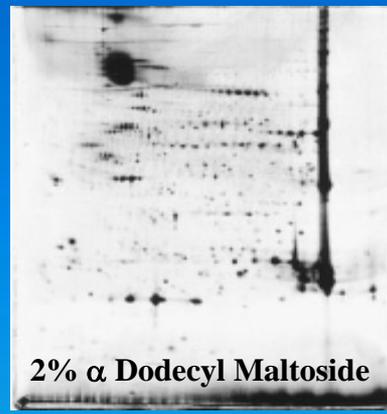
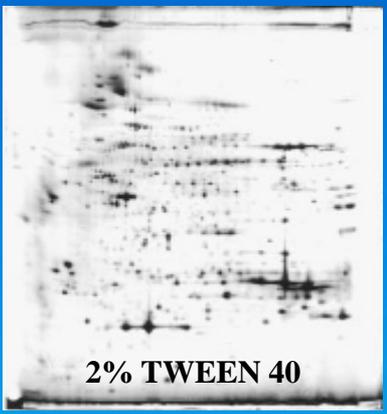
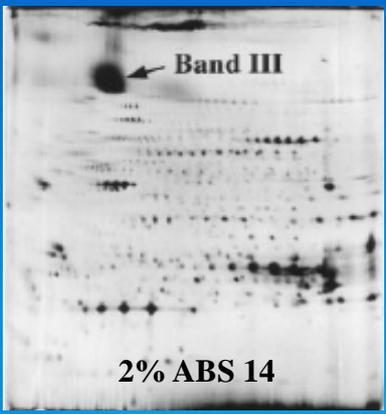
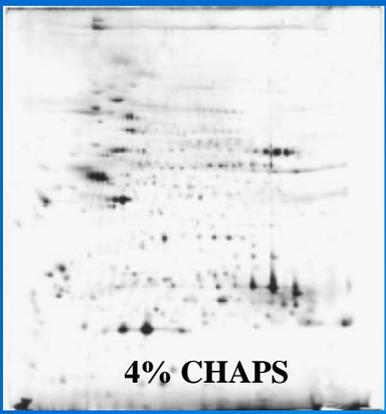
**RISOLUZIONE**

Gradiente di pH impiegato nella IEF  
Dimensione delle strip/gel impiegati  
Tipo di Colorazione

# **COSA SCEGLIERE?**

<i>Detergente</i>	<i>CMC [mM]</i>	<i>Mr [Da]</i>	<i>Mr micella [Da]</i>	<i>Struttura</i>
Sodium dodecyl sulfate	8	288	18000	$\text{CH}_3(\text{CH}_2)_{10}\text{CH}_2\text{O}-\text{S}\begin{matrix} \text{O} \\ \parallel \\ \text{O} \end{matrix}-\text{ONa}$
Sodium deoxycholate	1-4	415	4200	
CHAPS	8-10	615	6150	
CHAPSO	8-10	631	6940	
Zwittergent 3-14	0.3	364	30000	$\begin{matrix} \text{O} \\ \parallel \\ \text{CH}_2-\text{S}-\text{O}^- \\   \\ \text{CH}_2 \\   \\ \text{CH}_2 \\   \\ \text{CH}_2\text{N}^+(\text{CH}_3)_2(\text{CH}_2)_{12}\text{CH}_3 \\   \\ \text{CH}_3 \end{matrix}$
Brij 35	0.09	1168		$\text{C}_{12}\text{H}_{25}(\text{OCH}_2\text{CH}_2)_n\text{OH}$ <p style="text-align: center;">n ~ 23</p>
NP-40	0.3	603		$\text{CH}_3-\text{C}(\text{CH}_3)_2-\text{CH}_2-\text{CH}_2-\text{C}(\text{CH}_3)_2-\text{C}_6\text{H}_4-(\text{OCH}_2\text{CH}_2)_x\text{OH}$ <p style="text-align: center;">x = 9 - 10 (avg.)</p>
Triton X-100	0.3	650	90000	$\text{C}_8\text{H}_{17}-\text{C}_6\text{H}_4-(\text{OCH}_2\text{CH}_2)_n\text{OH}$ <p style="text-align: center;">n ~ 10</p>
Tween 20	0.059	1228		<p style="text-align: center;">Sum of w + x + y + z = 20</p>

# Scelta del detergente



# Analisi dell'immagine



### Spot Detection Parameter Wizard

Step 1 - Give Manual Guidance

- Click on the faintest spot you want to detect.
- Click on the smallest spot you want to detect. (if different from the faint spot)
- Box the largest spot you want to detect.
- Box a region of background without spots.

Back

Help

### PDQUEST - 6.1.0

File Edit View Image Spots Match Database Reports Window Help

Annotation Tools

xxx ABC

### Matchset msau00031 (Modified)

pathway

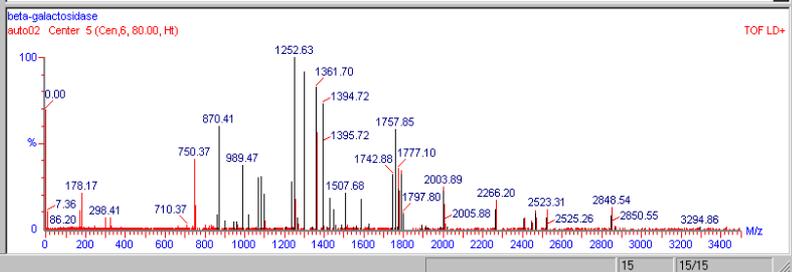
### ProteinProbe - ProteinLynx - [200799D2-Current]

File Edit View Workspace Process Spectrum Options Tools Window Help

Plate:1	Vial: A,2	Entry	Likelihood	Match	Cov (%)	Mw	pI	Description
		BGAL_ECOLI	9.50e+004	33	41.74	116352	5.4	[P00722] BETA-GALACTOSIDASE (EC 3.2.1.23) [LACTA
A	1	P97096	7.62e+004	30	37.37	119777	5.7	[P97096] CLONING VECTOR PLAC2, COMPLETE PLASMID SEQ
B	2	O09266	6.90e+004	28	32.52	117030	5.7	[O09266] BETA-GALACTOSIDASE
C	3	O09267	5.02e+004	24	29.70	117523	5.4	[O09267] BETA-GALACTOSIDASE
D	4	CAB50307	1.85e+004	6	34.53	26770	5.2	[CAB50307] HYPOTHETICAL 26.8KD PROTEIN
E	5	O45101	1.39e+004	5	11.13	75854	6.5	[O45101] T21012.7 PROTEIN
F	6	P94359	1.33e+004	7	30.64	34819	5.6	[P94359] HOMOLOGOUS TO LEUCINE RICH PROTEIN
G	7	UBC7_CAEL	1.15e+004	5	33.54	18939	5.1	[P34477] PROBABLE UBIQUITIN-CONJUGATING ENZYME E
H	8	VN34_ROTBS	9.23e+003	6	24.88	45081	5.3	[P34717] NONSTRUCTURAL RNA-BINDING PROTEIN 34 [N

1  
71  
141  
211  
281  
351  
421  
491  
561  
631  
701  
771  
841  
911  
981

MTITDSLAV LRKFWENPG VTQNNLAH PPFASV NS EARTDRPSQ LR ELNGEW AWFP PAEAVP  
 71 ESWLECDLPE ADTVVFP SNW QHRGYDAPIY TVVTPY ITV PPFVPTENPT GCYSLTFNVD ESWLQEQ QTR  
 141 IIFDGNVS AF HLWCNGR DVG YQDSR LPSE FDSL AF LAG ENRLAVH VL WDSGYLEDQ DQ DW MRSG GIFR  
 211 DVSLLRHPT QISDFH VATR FNDDFS AVL EAFV QCGEL DYLRV TVSL WQGETQ VASG TAP FG EIID  
 281 ERGGY AD RVT LR LV NP KL WSA EIP NL YR AV VEL HT AD G TL IE A E AC D Y GF RE V TE NG L L L NG R P L L I  
 351 IR GV NR HE HH PL H GV ND EQ TR VD ILL KK Q NN F NA V CS H Y P N H L W T L C D R F GL V V D E A N I E T H G N  
 421 V P M N R L T D D P R L P A N S E R V T R M V Q R D R N H P S V I I W L S L G N E S G H A N H D A L Y R W I K S V D P S R P V Q E G G  
 491 A D T T A D I I C P H Y A R D E Q P P F A V R W S I K W L S L P G E T R L I L C E Y A H A M G N S L G G F A K W Q A F R O F F O Y P  
 561 R L Q G F V N D U W D Q S L H Y D E N G N P S A Y G D F G D T P N D E F C M I N G L V A D R T H P A L T E A K H Q O F F O F F  
 631 L S Q T I E V T S E Y L F H S D N E L L H M V A L D G K P L A S G E V P L D V A P Q G K L I E L P E L P O P E S A G O L W L T V R V  
 701 V Q P N A T A V S E A G H I S A W Q W R L A E N S V T L P A A S H A I P H L T T S E M D F C I E L G N K R W Q F N R S G F L S Q H W I  
 771 G D K R L L T P L R Q T S A P L D I D I G V S E A T R K A A G H Y Q A E A L L Q C T A D L T A D A V L I T T A H  
 841 A W O H Q G T L F I S R K T Y L D G S Q M A I T V D E V A S D T P H P A R E G L N C L Q A V A E R N W L G L G P O E N T P D L  
 911 T A A C F R D R W L P L S D M T P Y V F P S E N L R C G T R E L N Y G P H Q W E G D F O P N I S R Y Q O L M E T S H L L H A E E  
 981 G T W L N I D G F H M G I G G D S W S P S V A E F Q L S A G R T H Y Q L V W S C Q R



Annotated spots (in gel, in window 1)  
REF msau00031-std ()

For Help, press F1

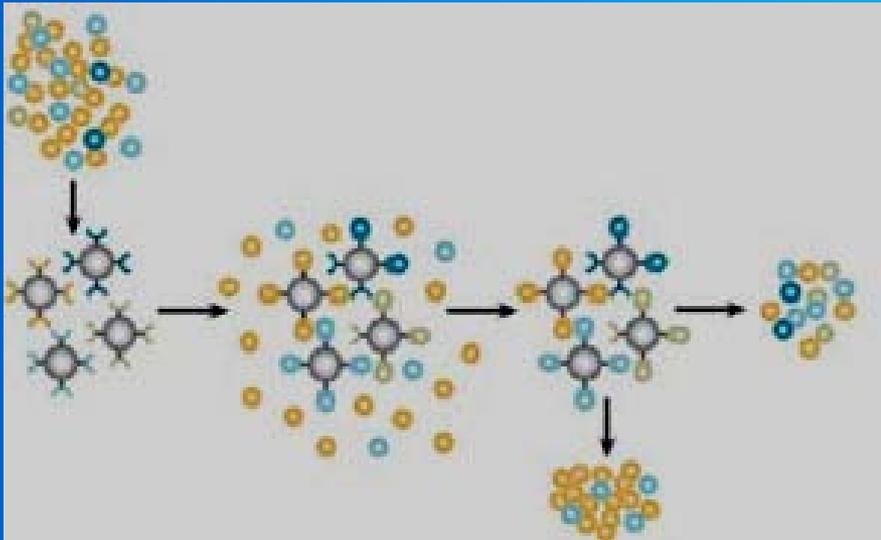
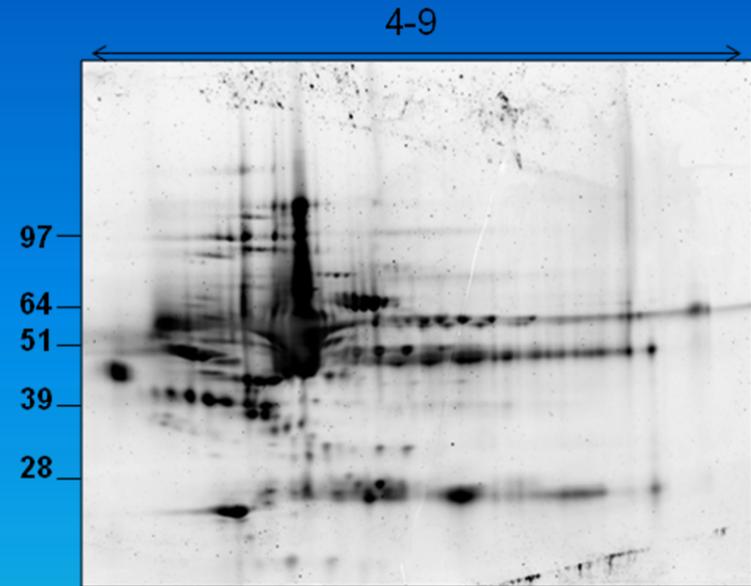
15 15/15

- Landmarks: Count = 3  
18241 v1 (Mem Image)
- Landmarks: Count = 4  
18242 v1 (Mem Image)
- Landmarks: Count = 8  
18243 v1 (Mem Image)
- Landmarks: Count = 4  
18244 v1 (Mem Image)
- Landmarks: Count = 5  
18245 v1 (Mem Image)

# Applicazioni Sperimentali

# 2D classica su plasma

**PLASMA**



***Equalizzazione dei  
campioni plasmatici***

4-9

prima

97

64

51

39

28

dopo

97

64

51

39

28

## ANALISI AL SOFTWARE

4-9

97

64

51

39

28

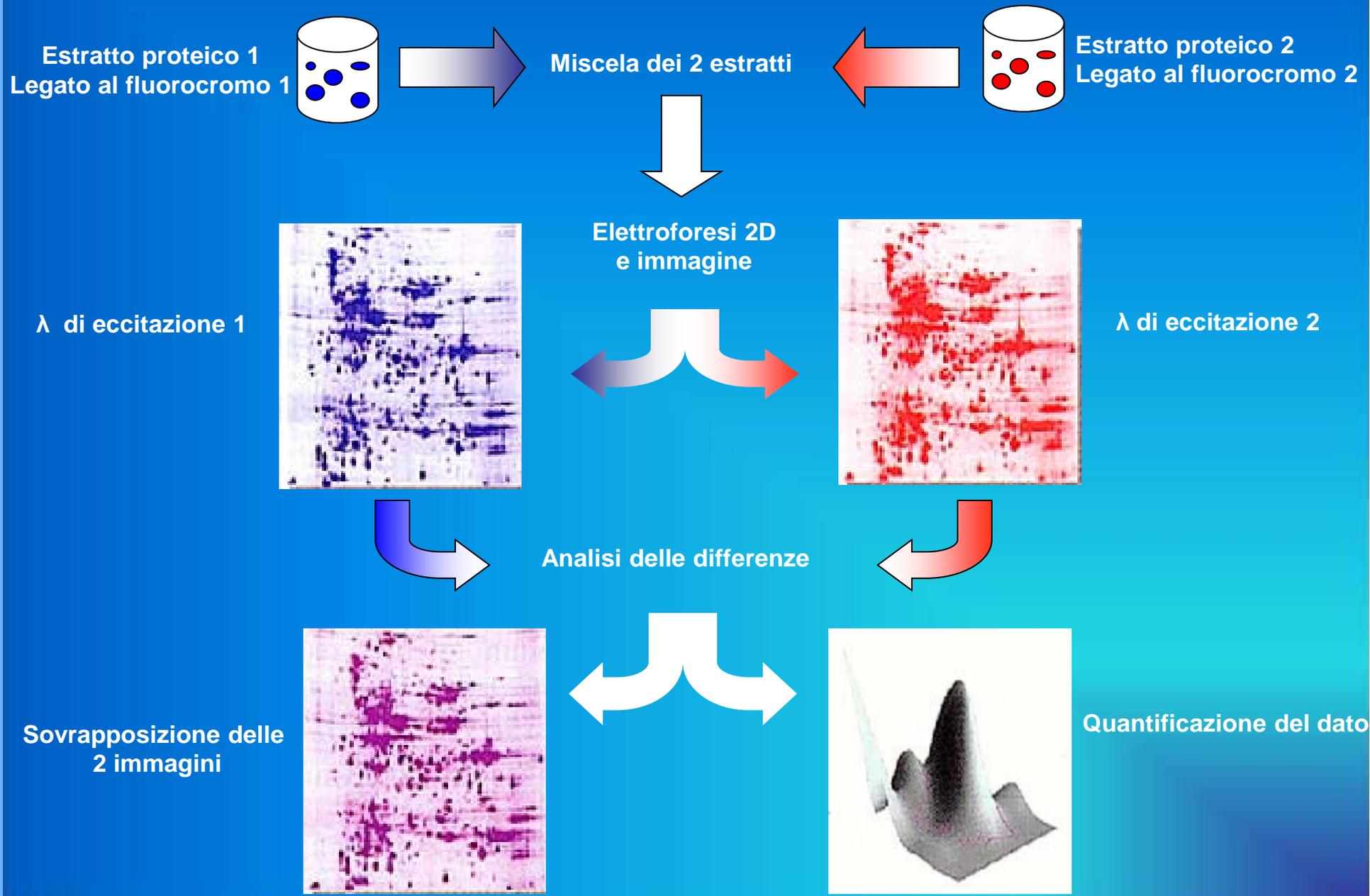


*Variazione qualitativa di 10 spots*

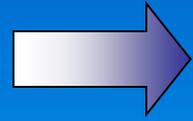
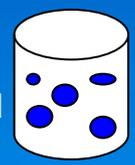


*Variazione quantitativa di 36 spots*

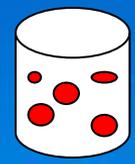
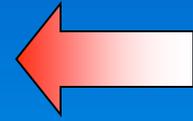
# Fluorescence 2D difference gel electrophoresis: DIGE



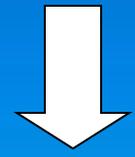
Estratto proteico 1  
Legato al fluorocromo 1



Miscela dei 2 estratti



Estratto proteico 2  
Legato al fluorocromo 2

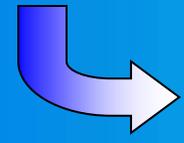


Elettroforesi 2D  
e immagine

$\lambda$  di eccitazione 1



$\lambda$  di eccitazione 2



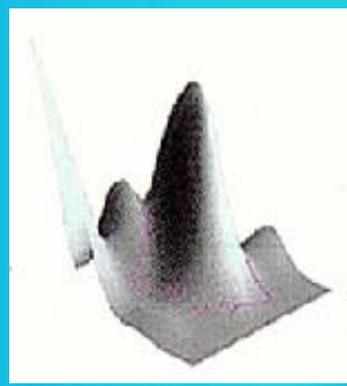
Analisi delle differenze



Sovrapposizione delle  
2 immagini

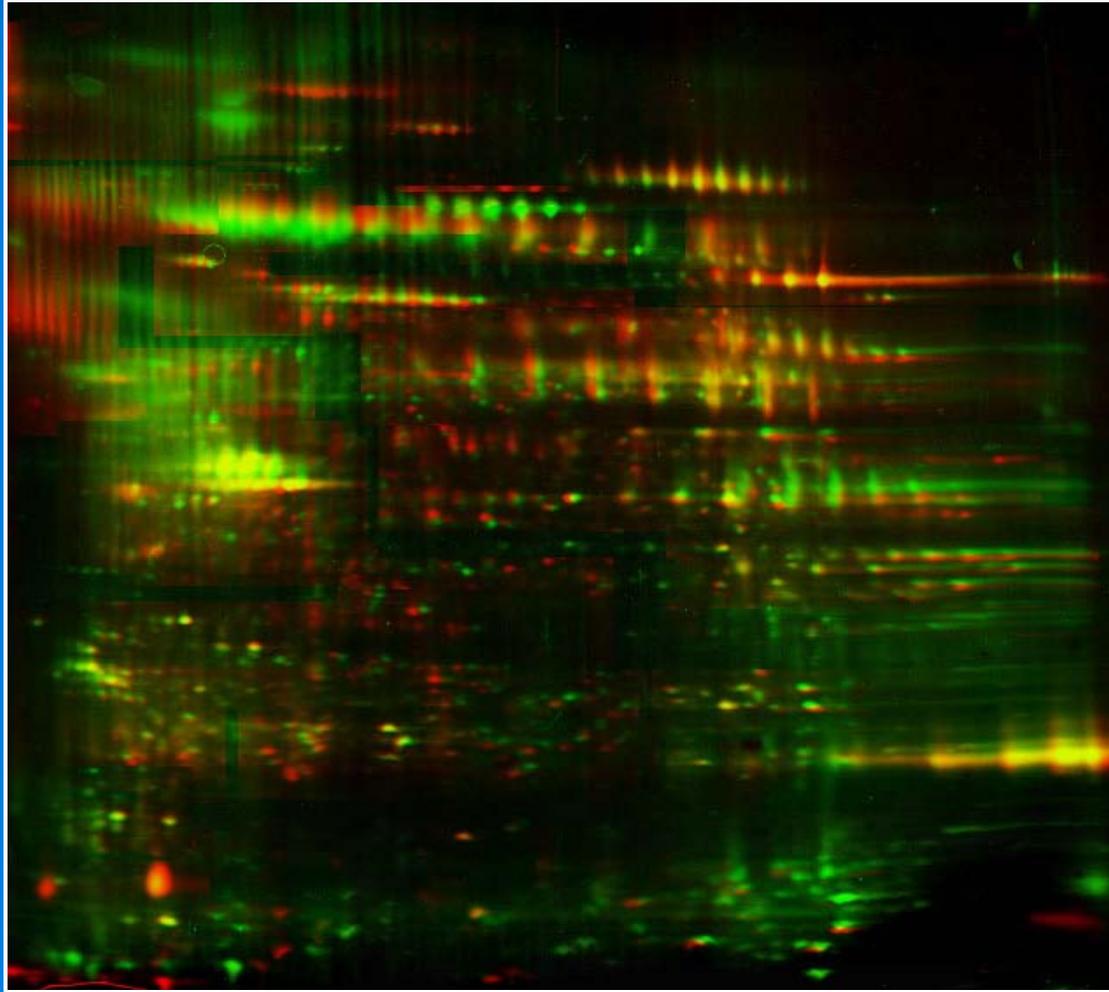


Quantificazione del dato



# Applicazioni Cliniche

# *Confronto di “Secretoma” tra le cellule di epitelio bronchiale normali e trattate farmacologicamente.*



**Rosso = normale [Cy5]**

**Verde= trattato con il farmaco [Cy3]**

**Giallo-arancione= spot sovrapposti**

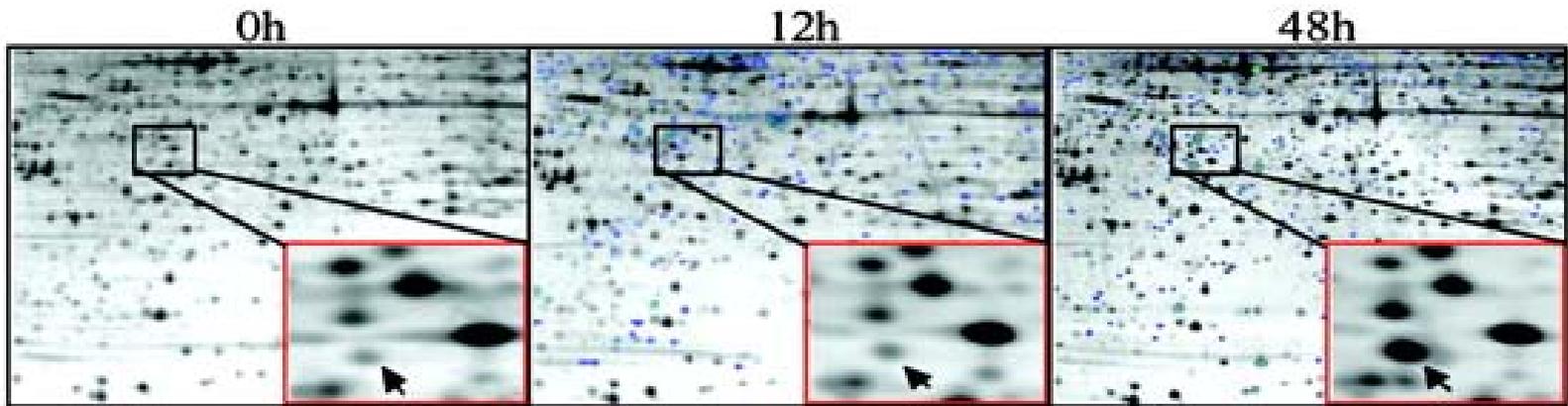
**Systems analysis of transcriptome and proteome in retinoic acid/arsenic trioxide-induced cell differentiation/apoptosis of promyelocytic leukemia . *Proc Natl Acad Sci U S A.* 2005 May 24;102(21):7653-8**

## **Leucemia promielocitica acuta**

- causata da traslocazione  $t(15;17)(q22;21)$
  - formazione della proteina di fusione PML-RAR $\alpha$
- NO retinoic acid pathway
- NO differenziazione granulocitica

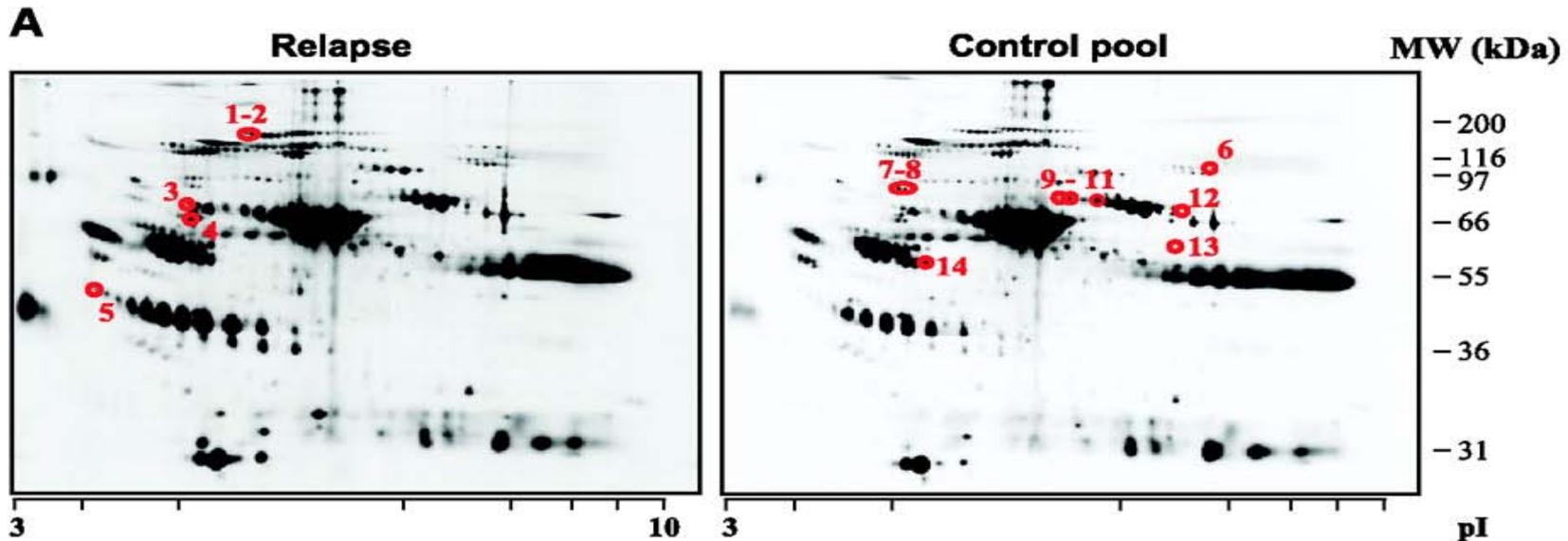
**Terapia: somministrazione di acido retinoico**

- attivazione del RA pathway
- induzione maturazione granulocitaria
- distruzione di PML-RAR $\alpha$  via Ubiquitina/Proteasoma

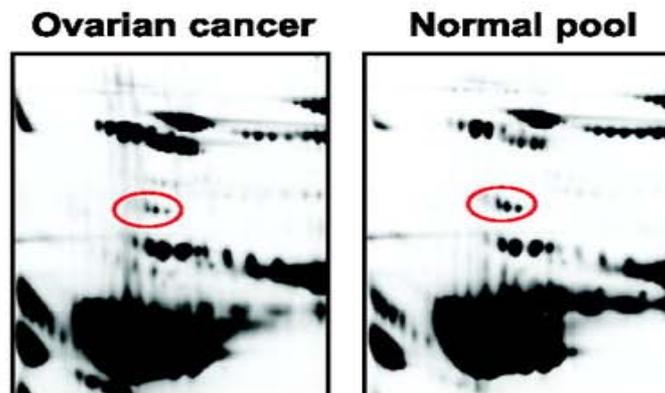


Upregolazione {  
Proteine del citoscheletro  
Proteine coinvolte nel signal trasduction  
Attivazione del Calcio signaling  
Controllo del ciclo cellulare  
TF

# Proteomic Profiling Identifies Afamin as a Potential Biomarker for Ovarian Cancer. *Clin Cancer Res.* 2007 Dec 15;13(24):7370-9.



Significativa downregolazione delle isoforme di Afamina ( $\alpha$ -albumina) nei tessuti tumorali di pazienti affetti



***fine***