

- Note that the opinions expressed within the abstracts and presentations are those of the authors/presenters alone and do not necessarily reflect the opinions of WADA.

Vectors and Delivery Methods - Vector and transgene vector detection

*The second WADA symposium on Gene Doping
Karolinska Institute, Stockholm
December 4-5, 2005*

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Therapeutic Gene Modulation
University Center for Pharmacy
Rijksuniversiteit Groningen



www.rug.nl/farmacie/tgm

JOS COLLIGNON



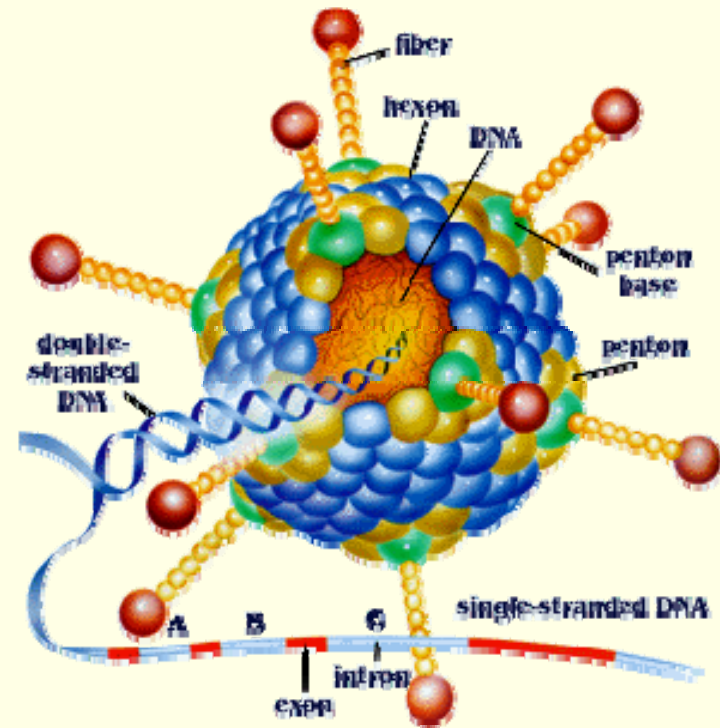
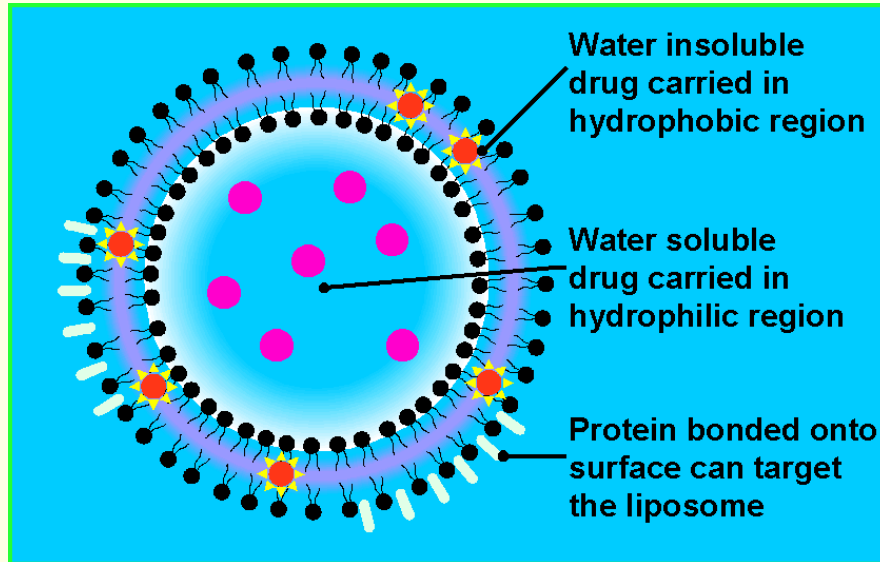
100 M MENS FINAL

1	GENE THERAPIST H. HAISMA	9.85
2	GENE THERAPIST T. FRIEDMANN	9.87
3	GENE THERAPIST O. RABIN	9.90



GENE DOPING

Vectors



Shedding Data

Gene Therapy Studies

Shedding

Excreta	Semen
	Stool
	Saliva
	Urine
	Blood

Germ line	Sperm
	Ovum

-Environment
-Next of kin

GENE DOPING

Detection?

<i>Vector</i>	<i>Tissue</i>	<i>Shedding</i>	<i>Persistence</i>
DNA	Muscle	-	months
Adenovirus	Tumor	Serum, saliva, urine	days
AAV	Muscle	Serum, saliva, urine	weeks
Retrovirus	I.v. Blood	Semen	weeks

GENE DOPING Detection?

Vector:

Protein

No, requires biopsy

DNA, RNA

Yes, blood, urine

Chemicals

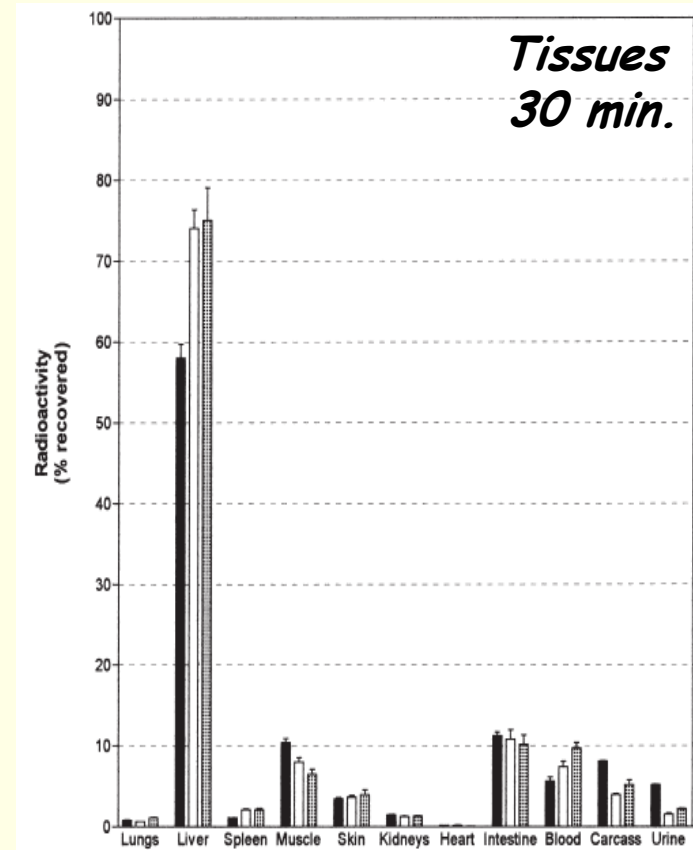
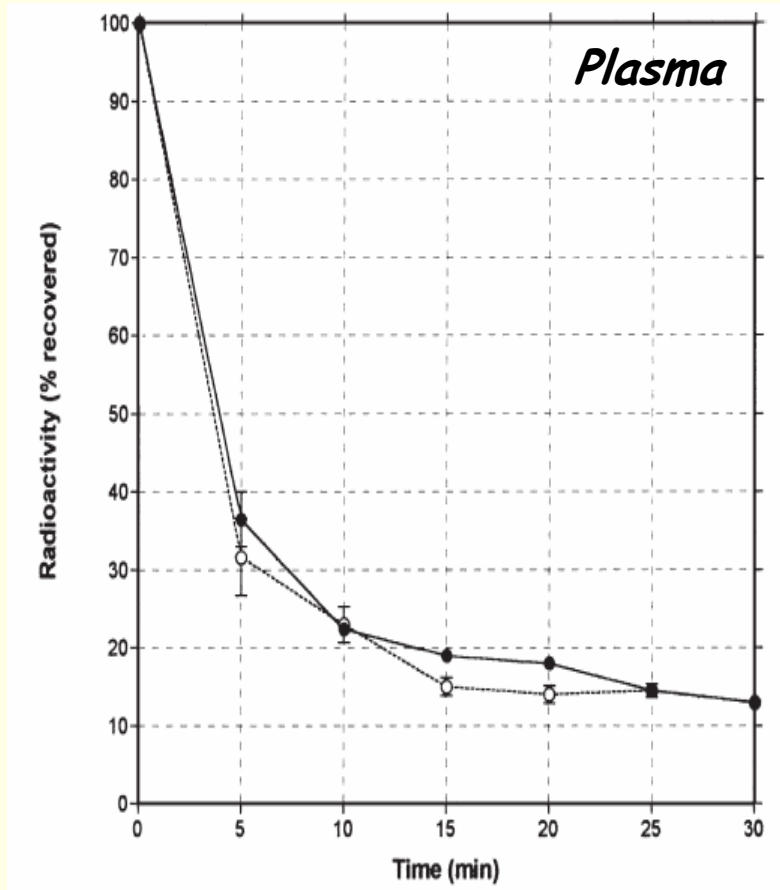
No, requires biopsy

Antibody response

Yes, blood

GENE DOPING

Clearance of free DNA



GENE DOPING

DNA detection?

Bacterial DNA is immunogenic

CpG DNA:

unmethylated CpG motifs are abundant in bacterial DNA;

the frequency of the CpG motif is suppressed and highly methylated in mammalian DNA

GENE DOPING Detection?

Transgene:

protein

- Human original Yes, elevated blood, urine
- 'New' modified Yes, if in blood, urine
- Human modified Yes, if in blood, urine

effect

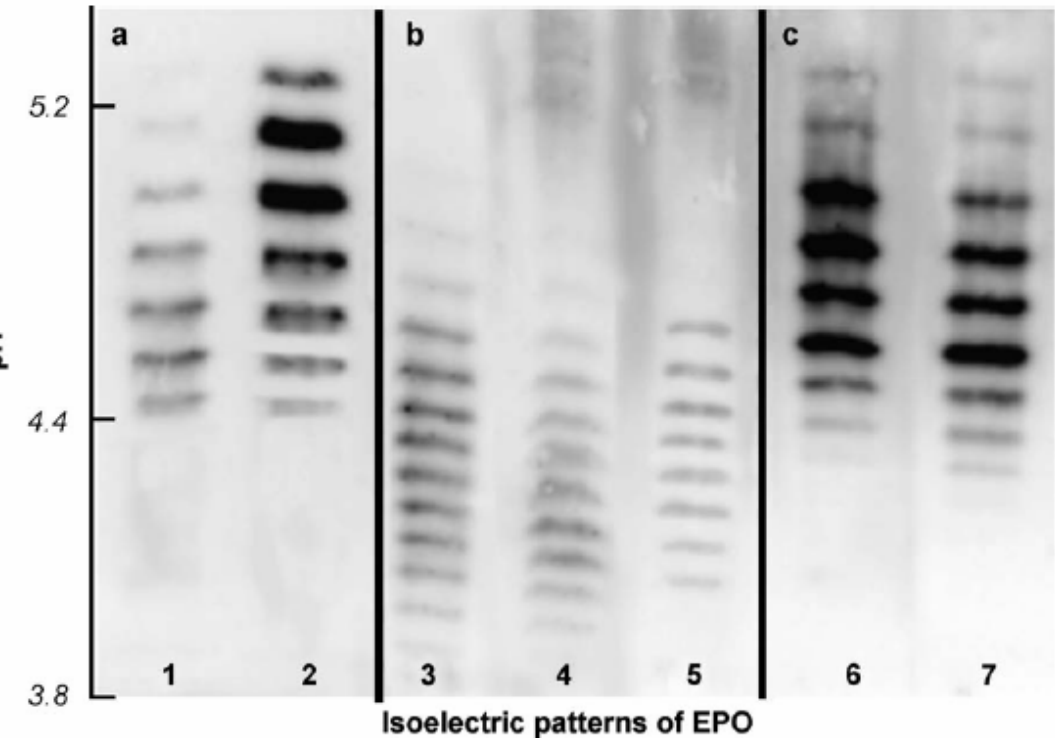
Yes, if in blood, urine

GENE DOPING

Specific Detection?

Isoelectric patterns of erythropoietin.

CHO BHK human macaque
urine serum macaque serum
after gene transfer
in skeletal muscle



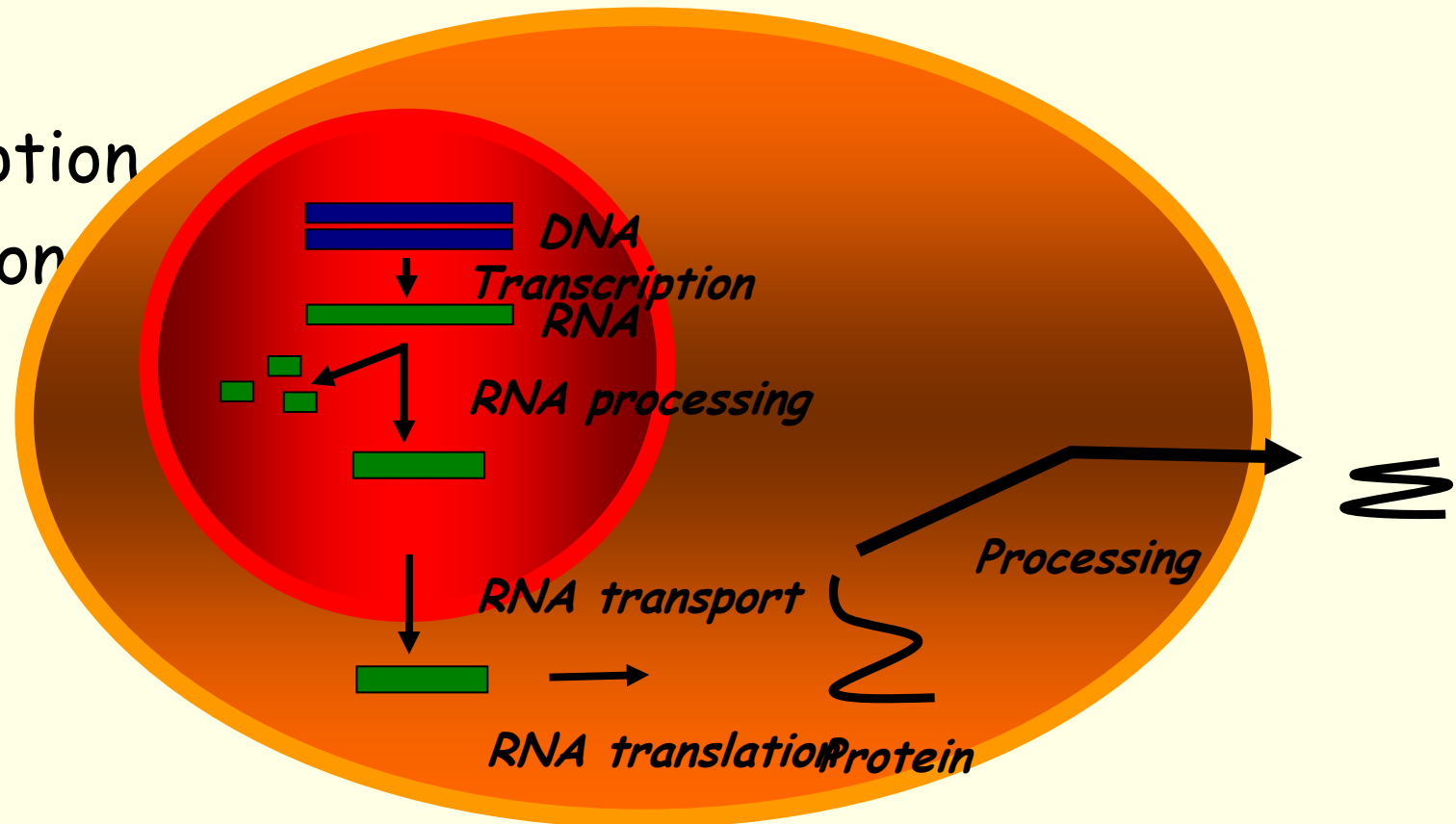
Lasne F. et al. Mol Ther. 2004; 10:409-10.

Gene Doping detection

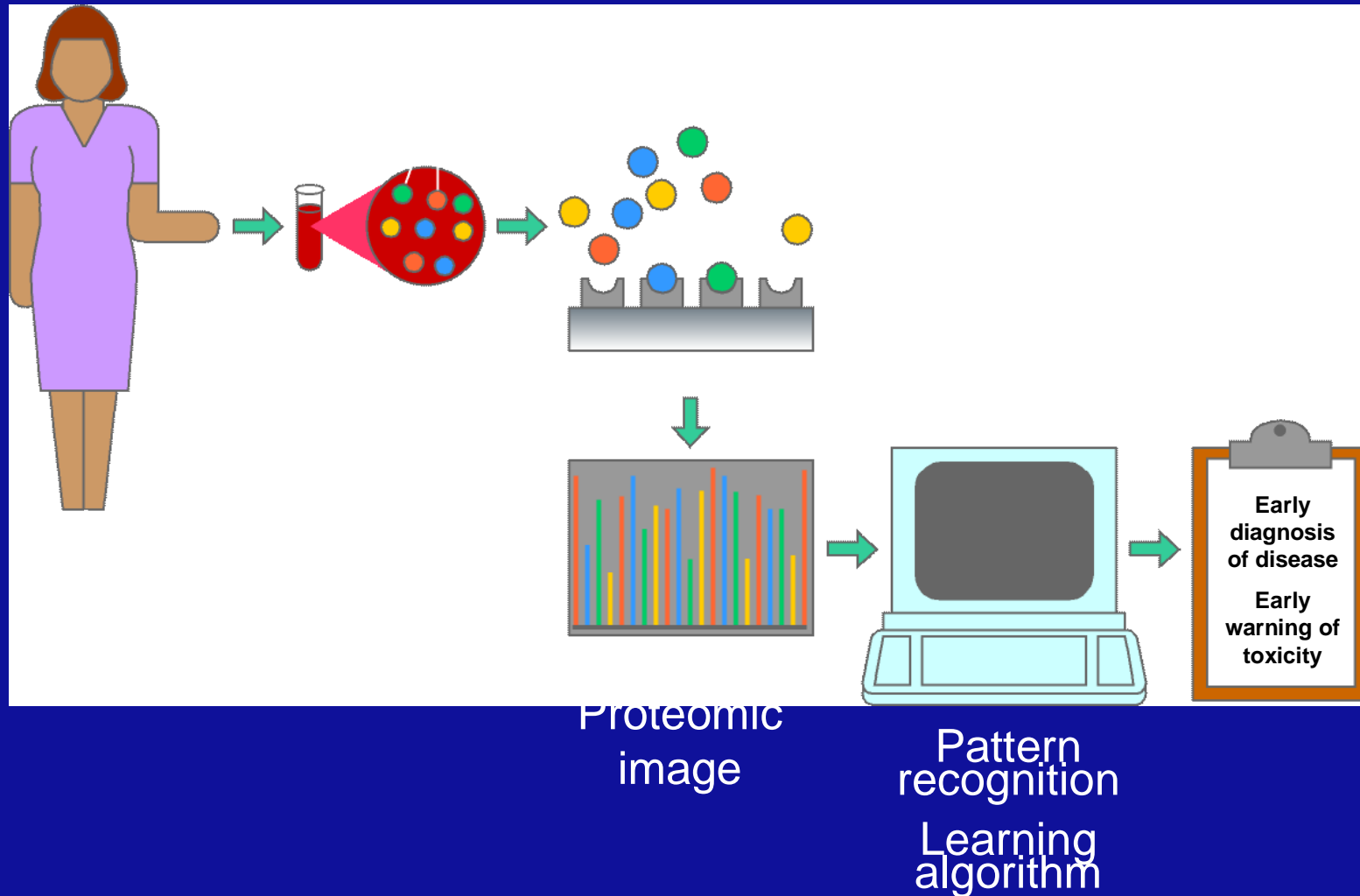
- **Specific**
- Every potential drug needs a specific sampling and analysis method
- **General**
- Profiling allows the determination of (major) changes in gene expression patterns by: gene array or proteomics

GENETIC INTERVENTIONS

- Gene
- Transcription
- Translation
- Protein



Serum Protein Pattern Diagnostics



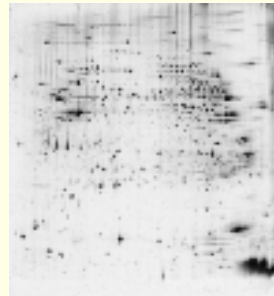
Proteomics

Sample Preparation



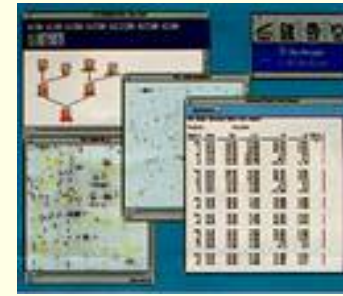
(I)

2-D Electrophoresis



(II)

Spot Detection & Image Analysis



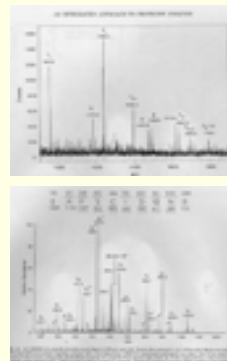
(III)

Enzymatic Digestion



(IV)

Peptide-Mass Fingerprinting



(V)

Protein Identification



(VI)

Peptide Sequencing via MS

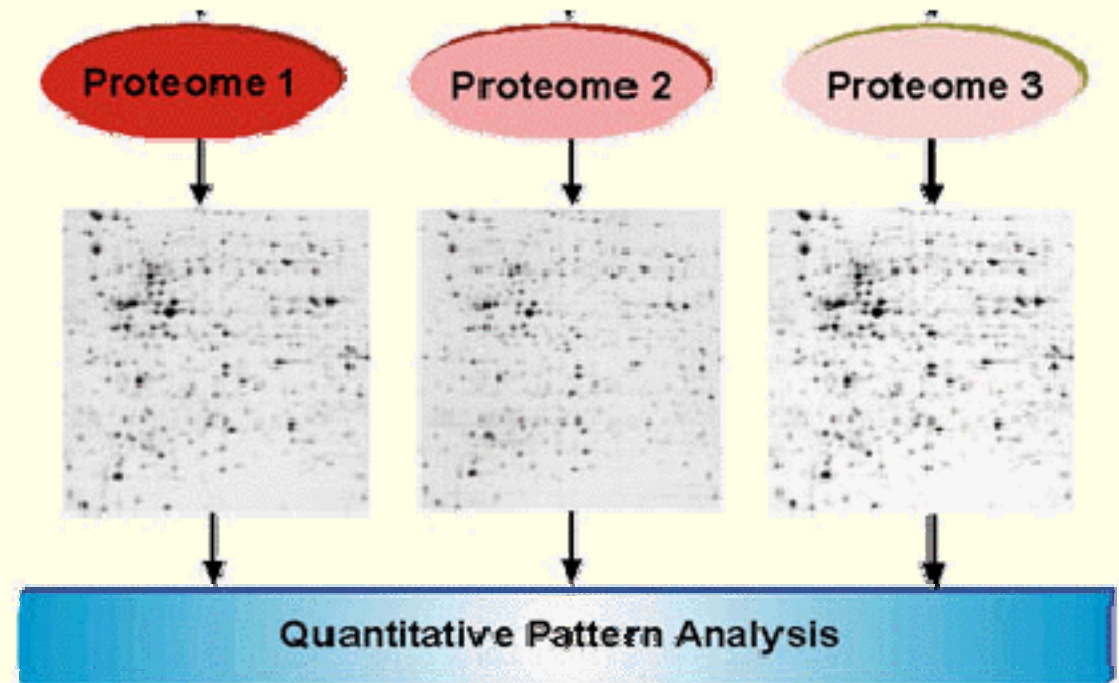
Database Search

GENE THERAPY AND DOPING

Detection by proteomics

Physiological profiling

- Serial Blood sampling
- Assesment of protein markers



Post Translational Modifications

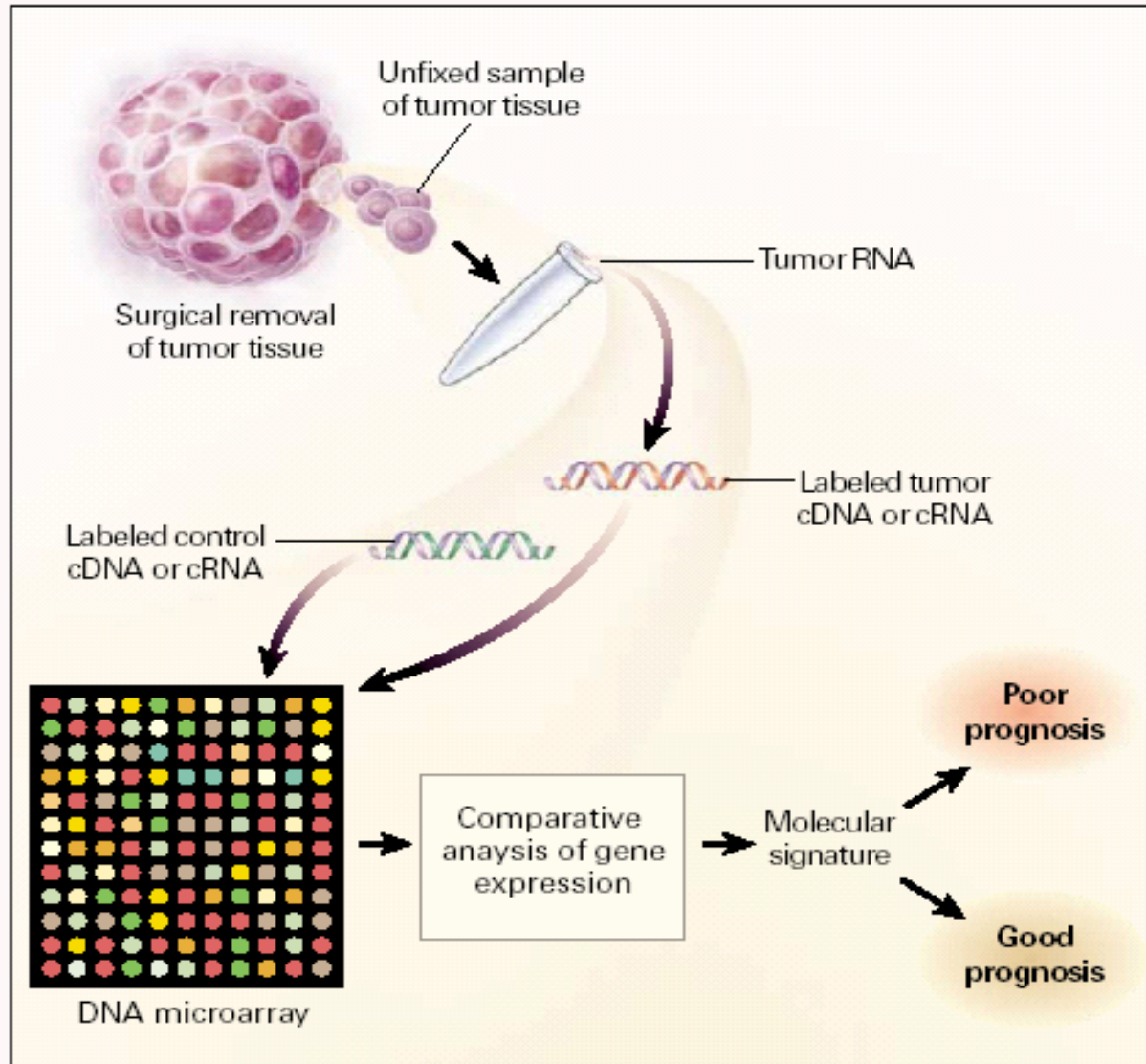
Table 1. Some common and important post-translational modifications

PTM type	Δ Mass ^a (Da)	Stability ^b	Function and notes
Phosphorylation pTyr pSer, pThr	+80 +80	+++ +/++	Reversible, activation/inactivation of enzyme activity, modulation of molecular interactions, signaling
Acetylation	+42	+++	Protein stability, protection of N terminus. Regulation of protein–DNA interactions (histones)
Methylation	+14	+++	Regulation of gene expression
Acylation, fatty acid modification Farnesyl Myristoyl Palmitoyl etc.	+204 +210 +238	+++ +++ +/++	Cellular localization and targeting signals, membrane tethering, mediator of protein–protein interactions
Glycosylation N-linked O-linked	>800 203, >800	+/++ +/++	Excreted proteins, cell–cell recognition/signaling O-GlcNAc, reversible, regulatory functions
GPI anchor	>1,000	++	Glycosylphosphatidylinositol (GPI) anchor. Membrane tethering of enzymes and receptors, mainly to outer leaflet of plasma membrane
Hydroxyproline	+16	+++	Protein stability and protein–ligand interactions
Sulfation (sTyr)	+80	+	Modulator of protein–protein and receptor–ligand interactions
Disulfide bond formation	–2	++	Intra- and intermolecular crosslink, protein stability
Deamidation	+1	+++	Possible regulator of protein–ligand and protein–protein interactions, also a common chemical artifact
Pyroglutamic acid	–17	+++	Protein stability, blocked N terminus
Ubiquitination	>1,000	+/++	Destruction signal. After tryptic digestion, ubiquitination site is modified with the Gly-Gly dipeptide
Nitration of tyrosine	+45	+/++	Oxidative damage during inflammation

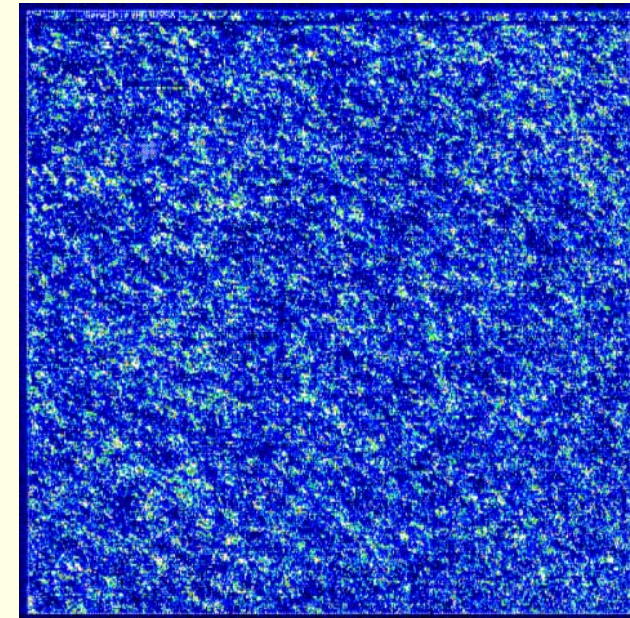
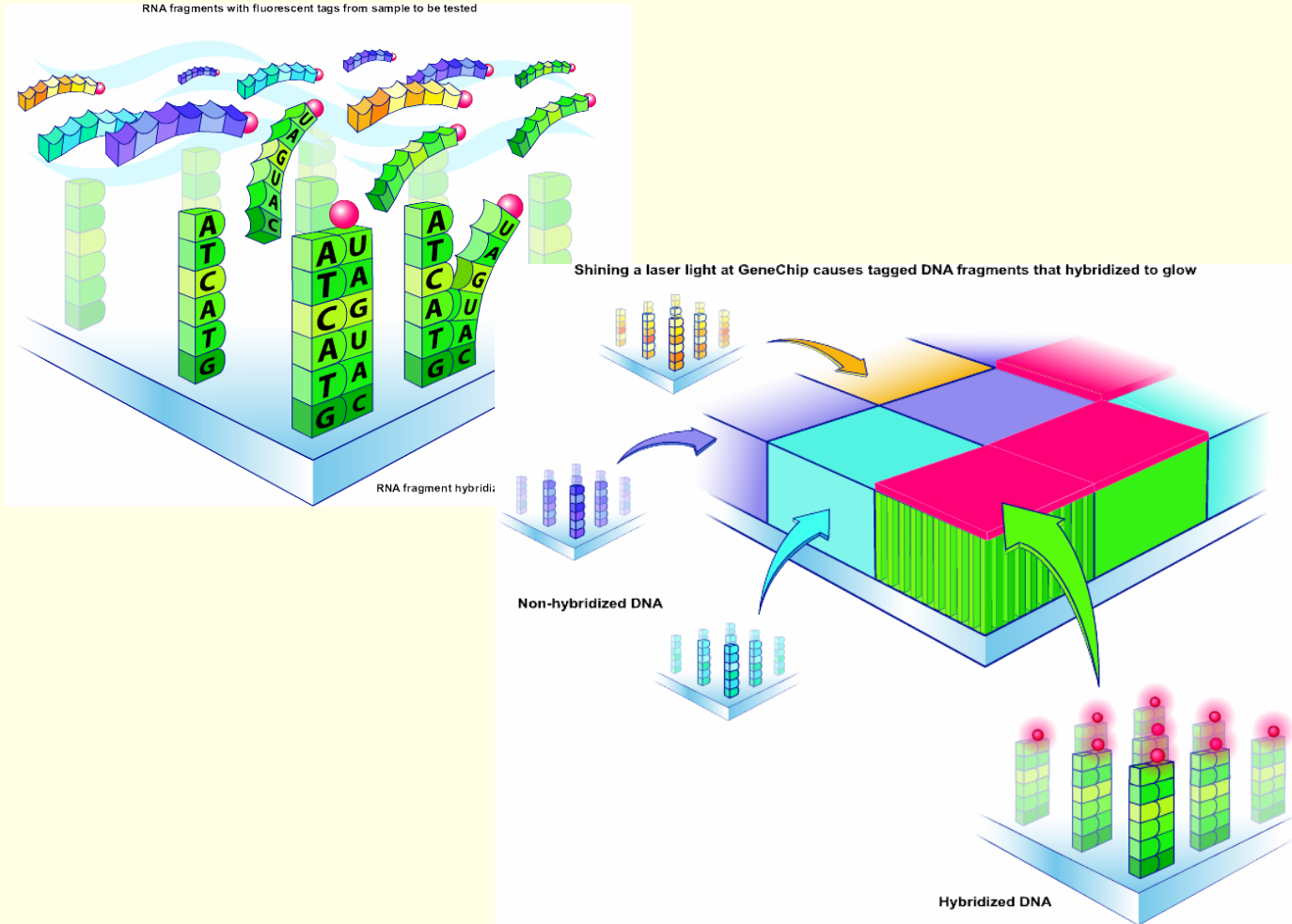
^aA more comprehensive list of PTM Δ mass values can be found at: <http://www.abrf.org/index.cfm/dm.home>

^bStability: + labile in tandem mass spectrometry, ++ moderately stable; +++ stable.

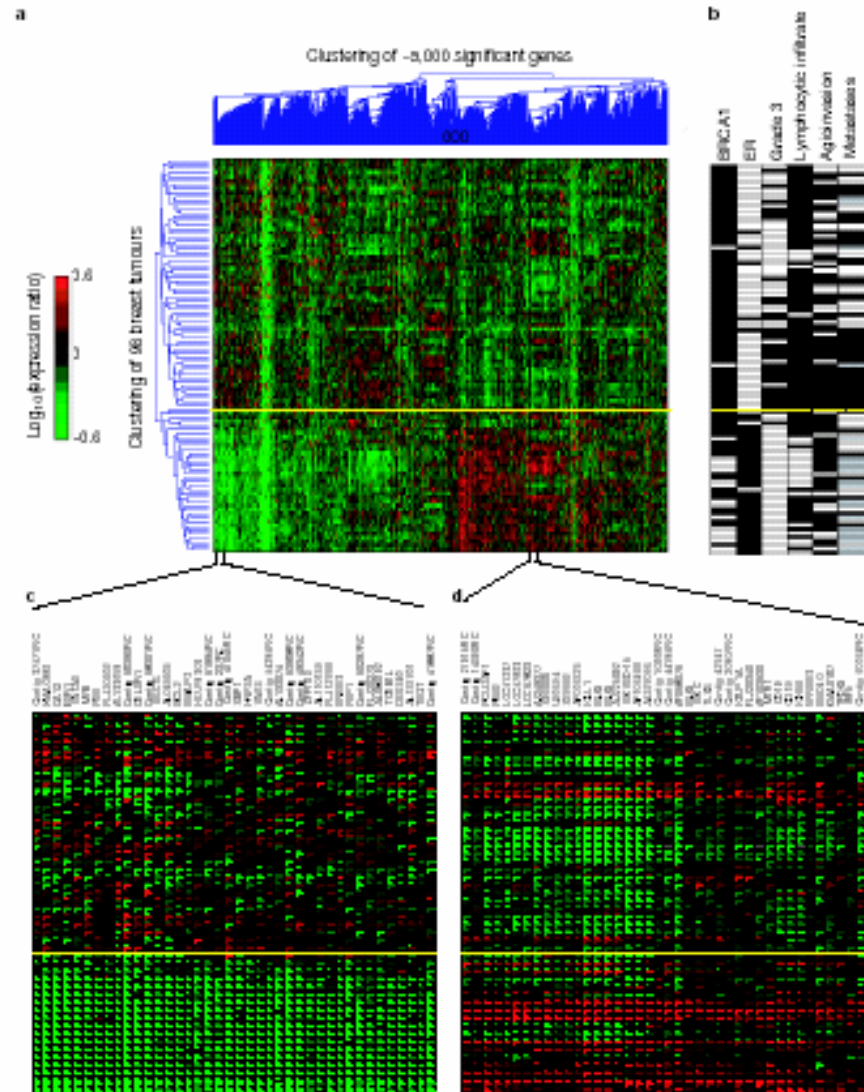
Gene expression profiles



Gene array



Gene Array



Discussion

- Gene doping vectors will be undetectable
- Proteomics and Gene expression profiling are powerful generally applicable methods and will be part of diagnosis and therapy in the future
- Requires fresh tissue, urine or blood sample of good (RNA or protein) quality
- Logistics (handling, storage)
- A global change in sample handling is needed