



Stress Response & Adaptation: A New Molecular Toolkit for the 21st Century

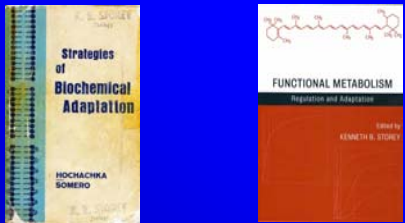
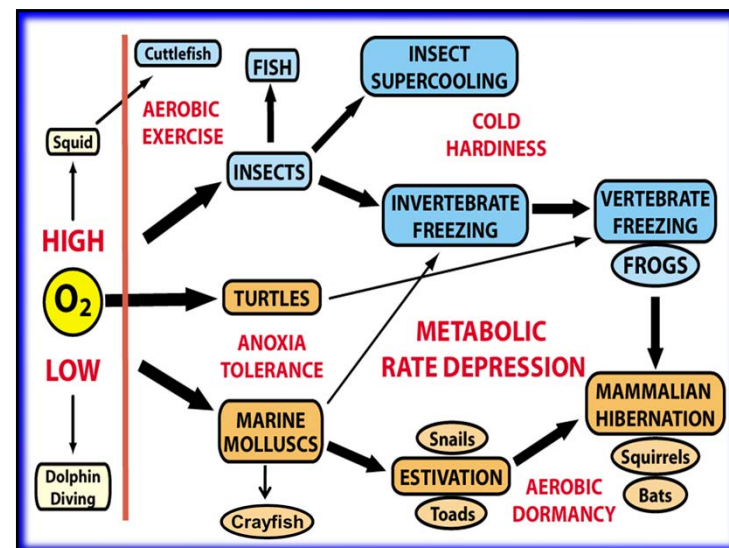
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Multiplex: Next Wave of Experimental Methodologies

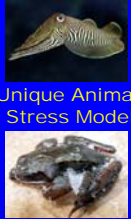


What are we interested in? Biochemical Adaptations

- Search to identify principles of biochemical regulation across the animal kingdom
- Biochemical Unity: Principles of Biochemical Adaptation

Experimental Approach



Unique Animal Stress Model

Comparative Experiments

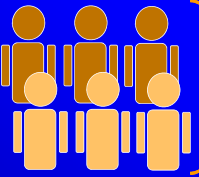
Diverse Techniques

Underlying Molecular Mechanisms

- **Experiments:** Adaptations to environmental stress
- **Over 100 Technical Bulletins**
- **Techniques:** Enzyme assay, protein purification, protein ID + quantification, Western blotting, transcription factor profiles, DSF, PCR, cDNA array, microRNA analysis, etc.

Current Core focus: Gene Expression

GENES $\xrightarrow{\text{Transcription}}$ RNAs $\xrightarrow{\text{Translation}}$ PROTEINS (ENZYMES)



'Single-plex' Experiments

Western blots, PCR, ELISA, etc.

Results

- Multiple trained students
- Long periods of experiments, data mining
- Animal tissues needed in large amounts

In 2006...

- Ready availability of **single-plex** technology: cDNA array, Western blotting, qPCR

Genomic and Proteomic Approaches in Comparative Biochemistry and Physiology*

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ABSTRACT


Introduction
Multiple new technologies in biochemistry and molecular biology have arisen in the last decade that are now available and adaptable for use by the nonspecialist to investigate problems in organismal adaptation to environmental stress. A revolution in approach is coming to the fields of physiology and eco-

In 2012...

- Ready availability of **multi-plex** technology: Luminex, multiplex-PCR, multiplex-Western blot

Current Core focus: Gene Expression

GENES $\xrightarrow{\text{Transcription}}$ RNAs $\xrightarrow{\text{Translation}}$ PROTEINS (ENZYMES)



'Multi-plex' Experiments



Luminex, multi-PCR, etc.

Results

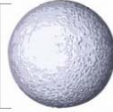
- Single trained student
- SHORT periods of experiments / data mining
- Minimum amounts of tissues required
- MONEY needed. BIG MONEY !!

Multiplex technology

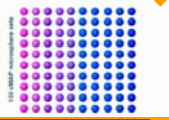
- Quantitatively measure multiple analytes in a single assay
 - i.e. 3-50 protein targets in 1 well
- Primarily nucleic acid and protein –based techniques
- Luminex**: Best type of MULTIPLEX technology

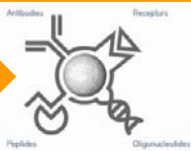
Luminex




BASICS
The xMAP technology uses 5.6 micron polystyrene microspheres which are internally dyed with red and infrared fluorophores.



BASICS
Different microsphere sets may be combined within an assay. Because each microsphere carries a unique signature, the xMAP-processor system can identify to which set it belongs. Therefore, multiplexing up to 100 tests in a single reaction volume is possible.

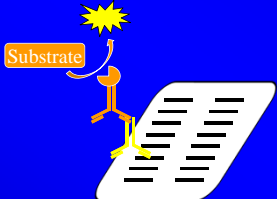


Antibodies
Receptors
Proteins
Oligonucleotides



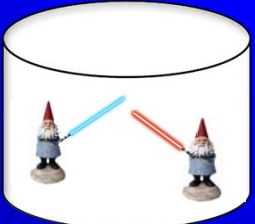
Luminex

How does it REALLY work



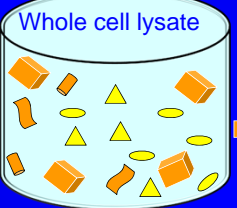
Western Blot

- Antigens immobilized on membrane
- Antibody detects Antigens of interest
- Visualization of DATA!



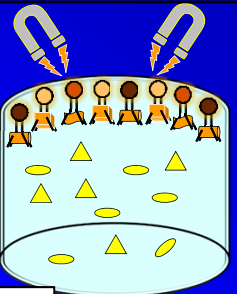
Luminex

- Magnets
- Lasers
- Shiny machines
- Magical appearance of DATA!



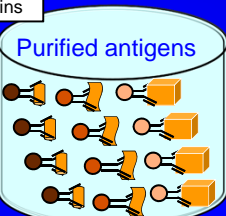
Whole cell lysate

Incubate with magnetic bead conjugated antibodies



1. Whole cell lysate in microplate
2. Bead conjugated antibodies detect antigens of interest
3. Magnetic retention of antibodies & bound antigens
4. Elution of non-specific proteins
5. Immunoprecipitation of target antigens

Elution of non-specific proteins



Purified antigens

Non-target proteins
Target antigens
Bead conjugated antibody

Luminex

- Antigens attached to bead-conjugated antibodies are probed with detection antibody
- Microplate content passed through a flow cytometer detection chamber
- Two separate lasers excite fluorescence
 - A:** Identifies bead type based on microsphere fluorescence
 - B:** Measures fluorescence associated with detection antibody

- Target antigens
- Bead conjugate antibody
- Detection antibody

Luminex data output

- Relative fluorescence allows comparison of antigen levels between samples
- Also allows comparison of different antigen levels within single samples (Relative abundance of protein A vs. B)

Applications

- Covalent attachment of: Antibodies, Oligonucleotides
- Capture of proteins, peptides, coding and non-coding RNAs, miRNA targets and more !
- Create a snapshot of **Global Cellular Functions**
 - Use this ‘snapshot’ to identify mechanisms of metabolic regulation

Application: Adaptation to environmental stress

Metabolic Regulation

Cellular pathways that are responsible for major biological processes required to sustain basic functions.
E.g. glycolysis, transcription, translation, lipid metabolism, etc.

Cellular Defense

Cellular pathways that are responsible for responding to &/or repairing cellular damage.
E.g. antioxidant enzymes, heat shock proteins, anti-apoptosis, etc.

Protein Applications

- Commercial kits: Key targets of metabolic pathways
 - Detects **total** and **phosphorylated** targets
- Thousands of biomarkers available

Browse Research Focus Area
Select one of the Research Focus Areas below to learn more about our Immunoassay and Multiplex Solutions for your area of interest.

- Neuroscience
- Metabolism
- Immunology
- Cancer
- Toxicity
- Cell Signaling
- Cellular Metabolism
- Cardiovascular

Wide range of research interests
e.g. Immunology, MAPK, PDH etc.

Human T Cell Receptor - 7 Plex
Catalogue Number: 48-690 (nonmagnetic)

- CD3ε (pan Tyr)
- CREB (Ser132)
- ERK1/2 (Thr185/Tyr187)
- LAT (pan Tyr)
- Lck (pan Tyr)
- Syk (pan Tyr)
- ZAP-70 (pan Tyr)

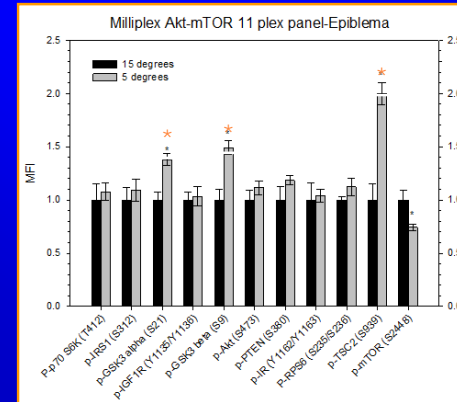
Human MAPK/SAPK 10 Plex
Catalogue Number: 48-680 (nonmagnetic)

- ATF2 (Tyr87/1)
- c-Jun (Ser73)
- ERK1/2 (Thr185/Tyr187)
- HSP27 (Ser78)
- JNK/SAPK1 (Thr183/Tyr185)
- MEK1 (Ser222)
- MSK1 (Ser222)
- p38/SAPK2AB (Thr180/Tyr182)
- p53 (Ser15)
- STAT1 (Tyr701)

Multiplex for Both Species Pyruvate Dehydrogenase (PDH) Complex
Catalogue Number: PDH94G-1300 (magnetic)

- PDH (total)
- PDH (pS232)
- PDH (pS330)
- PDH (pS380)

Luminex: Akt-mTOR network in Insect Cold Hardiness



Epiblema scudderiana, cold-hardy insect - Freeze-avoiding

(Storey, unpublished)

Luminex: Heat Shock Proteins in Anoxia & Freezing

Heat shock proteins and hypometabolism: adaptive strategy for proteome preservation

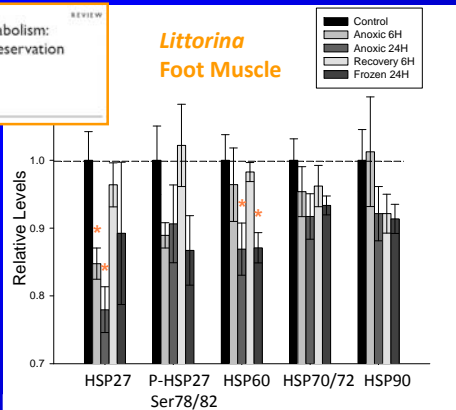
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Littorina littorea, intertidal periwinkle

(Storey, unpublished)

Littorina Foot Muscle



Advantages of Multiplex

Human Akt/mTOR 11 Plex
Catalogue Number: 48-611 (nonmagnetic)

- Akt (Ser473)
- GSK3α (Ser21)
- GSK3β (Ser9)
- IGF-1R
- IR (Tyr1150/Tyr1153)
- IRS1 (Ser312)
- mTOR (Ser2448)
- p70S6K (Thr412)
- PTEN (Ser380)
- RPS6 (Ser235/Ser236)
- TSC2 (Ser909)

- Akt / mTOR study**
- Insulin signaling, protein synthesis regulations
 - 11 phospho-protein targets

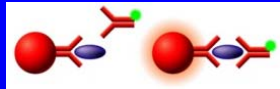
Single-plex

- 6 tissues to analyze
- 11 protein targets
- 66 Western blots
- 6 control + 6 expt lanes per gel
- 300 µg protein per gel
- 792 data points
- **Time: 12-14 weeks**

Multiplex

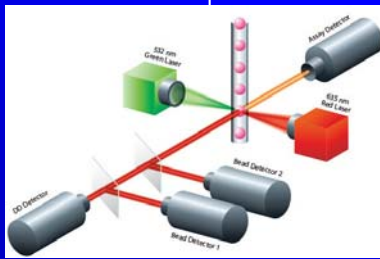
- 1 well = 11 protein targets
- 1 kit = 96 individual sample wells
- < 30 µg protein per well
- 1 kit = >1000 data points
- **Time: < 24 hours**

Oxidative Stress Markers using Luminex Technology



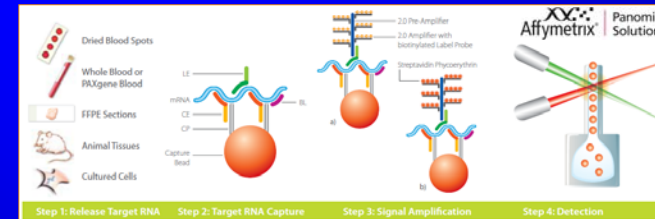
MILLIPLEX® Oxidative Stress Panel

- Catalase
- PRX2 (PRDX2)
- SOD1
- SOD2
- TRX1



All analytes measured in a single well!

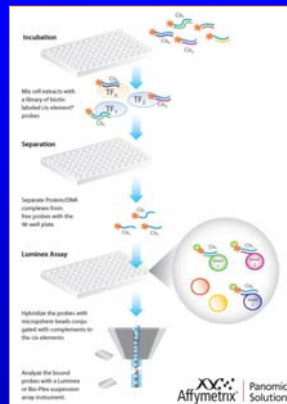
mRNA applications



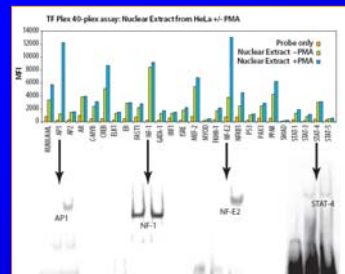
- QuantiGene Plex
- Same technology: Immobilized oligonucleotides
- Direct measure of mRNA levels
- Custom-plex can measure 3-80 genes in 1 sample

Transcription Factor ELISA

- Procarta® TF Plex Assays: Luminex based

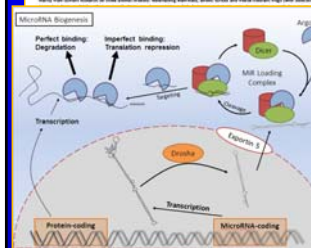


Profile the DNA binding activity of up to 44 different transcription factors (TFs) in a single well



miRNA: Multiplex them all

Review
The emerging roles of microRNAs in the molecular responses of metabolic rate depression



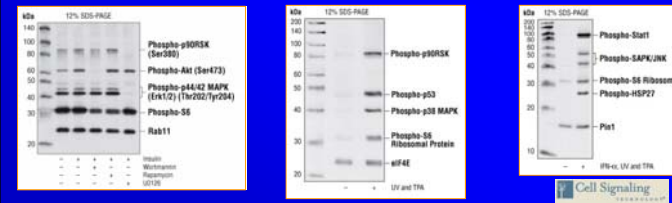
- Size ~22 nucleotides
- Highly conserved across species
- Bind to 3' UTR of mRNAs
- Exact repression mechanism(s) yet to be defined, but seem to include
 - Block translation of mRNA
 - Help bind mRNA into stress granules
 - Target mRNA for degradation

Summary: Multiplex in Functional Genomics

- Single assay, generate large amount of gene expression data
 - Maximize use of biological samples
 - Minimize experimental time
- Specialized equipment
 - Expensive start up cost \$\$
 - Cost of commercial kits (“home-made” kits can be created)

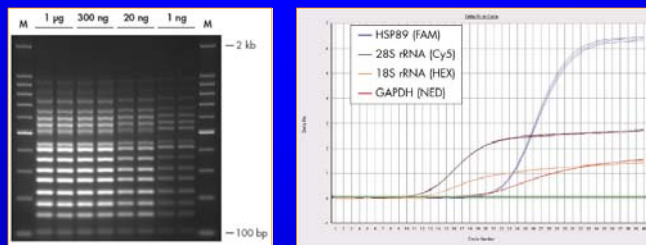
Multiplex Options: Classic Methods

- Multiplex (not Luminex)
 - Efficient, but costly \$\$\$
- Multiplex Western Cocktail
 - Multiple antibody
 - Simultaneous detection of targets



Multiplex Options: Classic Methods

- Multiplex PCR / qPCR
 - Multiple primer pairs
 - Simultaneous quantification of mRNA transcripts



19-plex end point PCR

4-plex real time PCR

Oxidative Stress

Oxidative stress: animal adaptations in nature

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Abstract
As a consequence of aerobic life, an organism must deal with the continuous generation of reactive oxygen species (ROS), O₂, O₂⁻, H₂O₂, and HO₂, in the presence of mitochondria and aerobic metabolism. Organisms protect themselves from such damage with both enzymatic and nonenzymatic antioxidant defenses. However, the specific responses varied after induction of an oxidative stress and resulted in a loss of reactive oxygen species production in some organisms. Specific antioxidant responses are discussed.

Keywords
• Reactive oxygen species
• Free radical damage
• ROS generation
• Phospholipase
• Lipid peroxidation
• Oxidative stress
• Antioxidant enzymes
• Antioxidant defenses

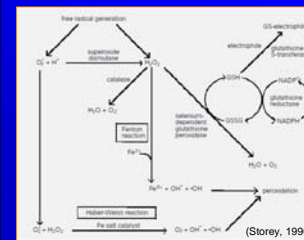
CBP

Review
Antioxidant defenses and metabolic depression. The hypothesis of preparation for oxidative stress in land snails

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Received 4 November 1997; accepted in revised form 18 May 1998; accepted 1 June 1998



- Central theme in oxygen metabolism
- Linked with cancer, aging, diabetes, hypoxia, etc.
- Antioxidant enzymes as indicators of oxidative stress

Hunting for Oxidative Stress

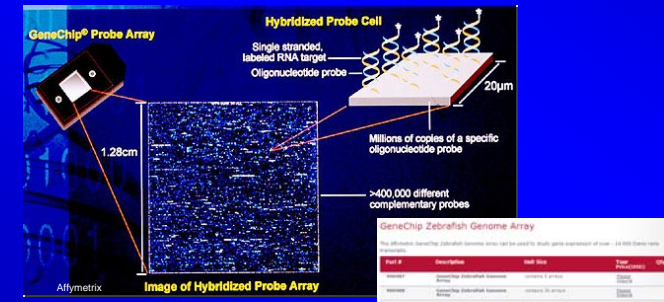
- Where to begin?
 - Identification of regulatory markers
- High-throughput vs. Multiplex

- GeneChip Genome Arrays
- Ability to screen thousands of genes
- Limited biological replicates (cost \$)
- Use as lead generators

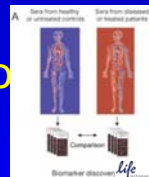
- Pathway specific study
- Quantify complete metabolic pathways
- Limited genes compared to GeneChips
- Large number biological replicates/run
- Used for gene expression profiling

High-throughput technologies

- “Intellectual Fishing”
- Results are hugely informative, potentially generate hundreds of new directions



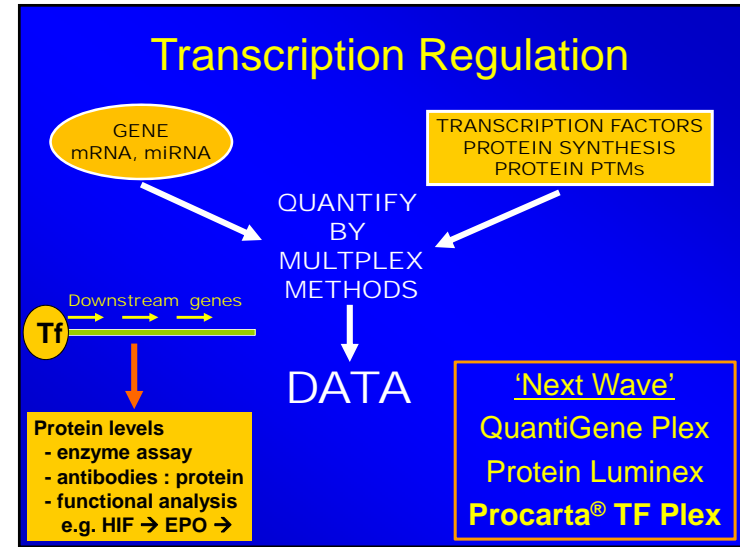
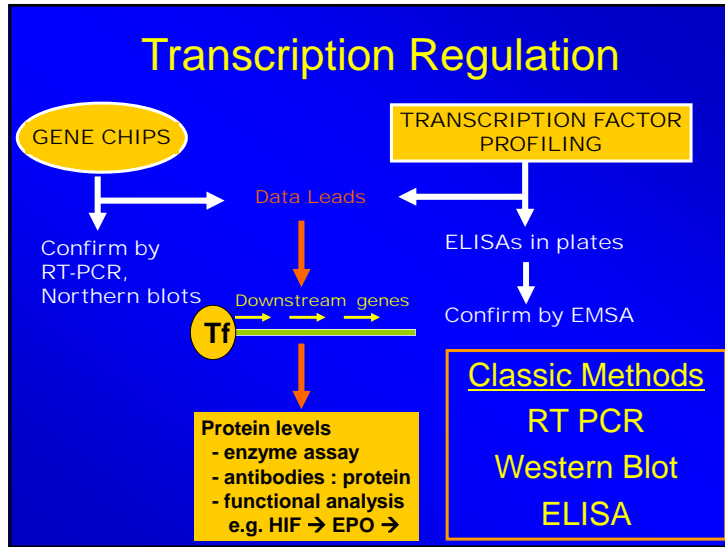
High-throughput technology



- Arrays are available for mRNA, proteins, and microRNA
 - mRNA GeneChip: 10,000-20,000 gene expression
 - Protein Microarray: ~10,000 protein expression
 - microRNA GeneChip: 1,000 – 2,000 mature miRNAs

High-throughput screenings

- ‘Old technology’, still the principal approach
- Snapshot status of thousands of genes
- Identify unknown gene regulations
- Develop testable molecular hypotheses to support physiological observations



Going forward



- Old vs. new technologies
 - Classic methodologies are still perfectly functional
 - New technologies provide same results at faster rate, higher efficiency
- Tailor your technologies to your lab!
 - **Must be sustainable!** \$\$\$
- In 2013, there **WILL** be a new machine / technology / assay.....

Stay Tuned!

Thanks to:

C-W. Wu
S.N. Tessier
J.M. Storey

Funded by NSERC Canada



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