Systems Approach to Assessing Cumulative Exposure to Endocrine Disrupting Chemicals

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Cumulative exposure to EDC…

Challenges:

- Conventional analytical methods obsolete
- Reporter genes too specific

Cumulative exposure to EDC…

Systems approach:

- Simultaneously assess multiple endocrine signaling circuits
- Incorporate toxicokinetic and toxicodynamic consideration
- Data output defines modes of activity and interactivity

Objectives

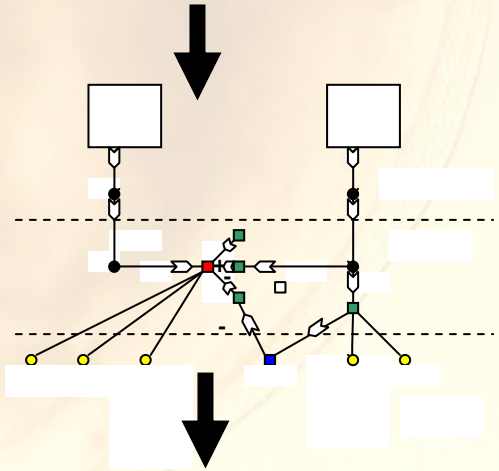
- Identify a suite of gene products (biomarkers) that define an endocrine network
- Assess responses of the biomarkers in to individual EDCs
- Assess performance of the system in response to chemical mixtures

Components

Sensor

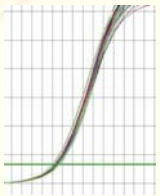
Daphnia magna

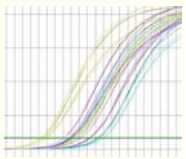
System



Steroid/terpenoid network

Output



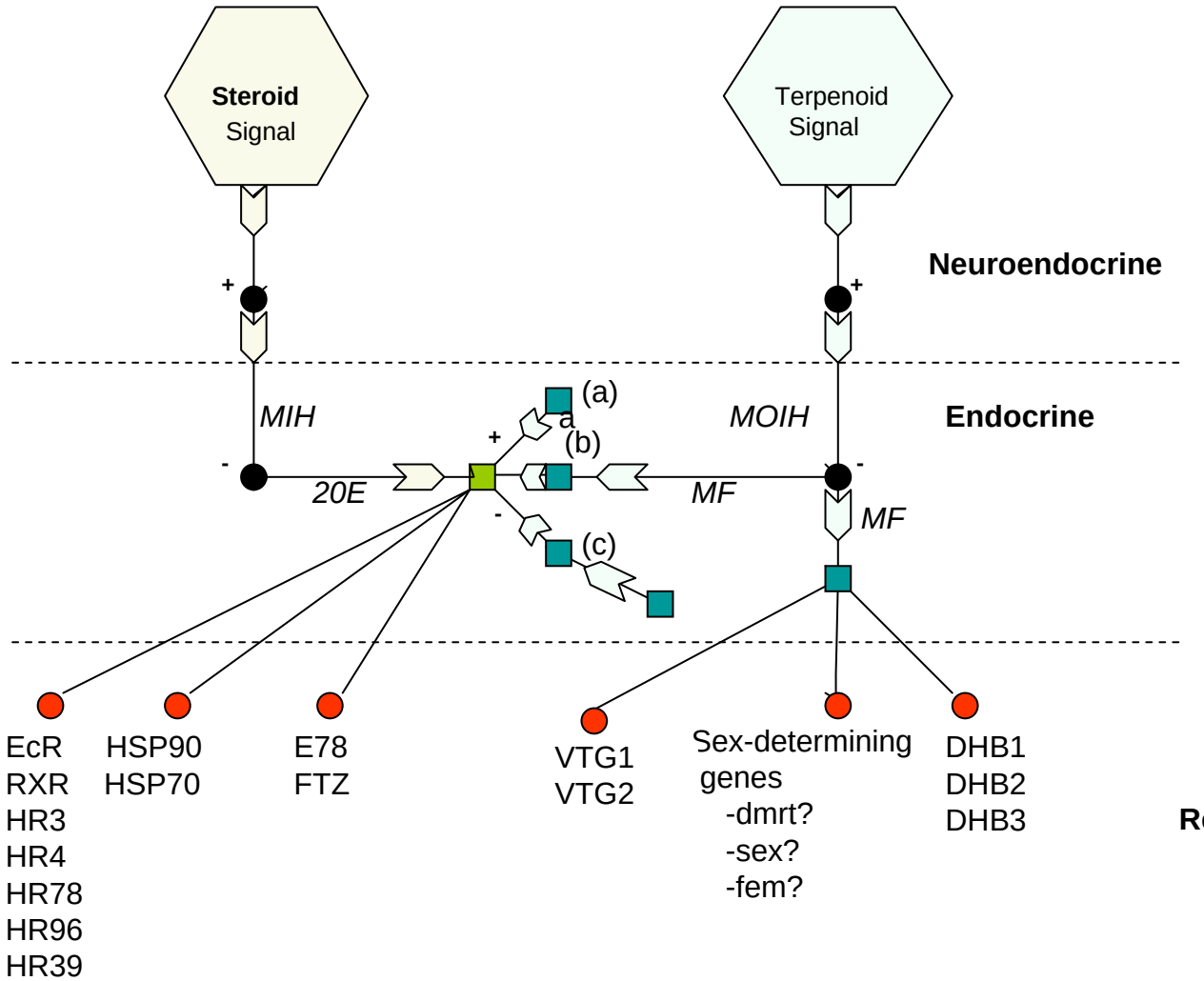


PCaRray

Sensor

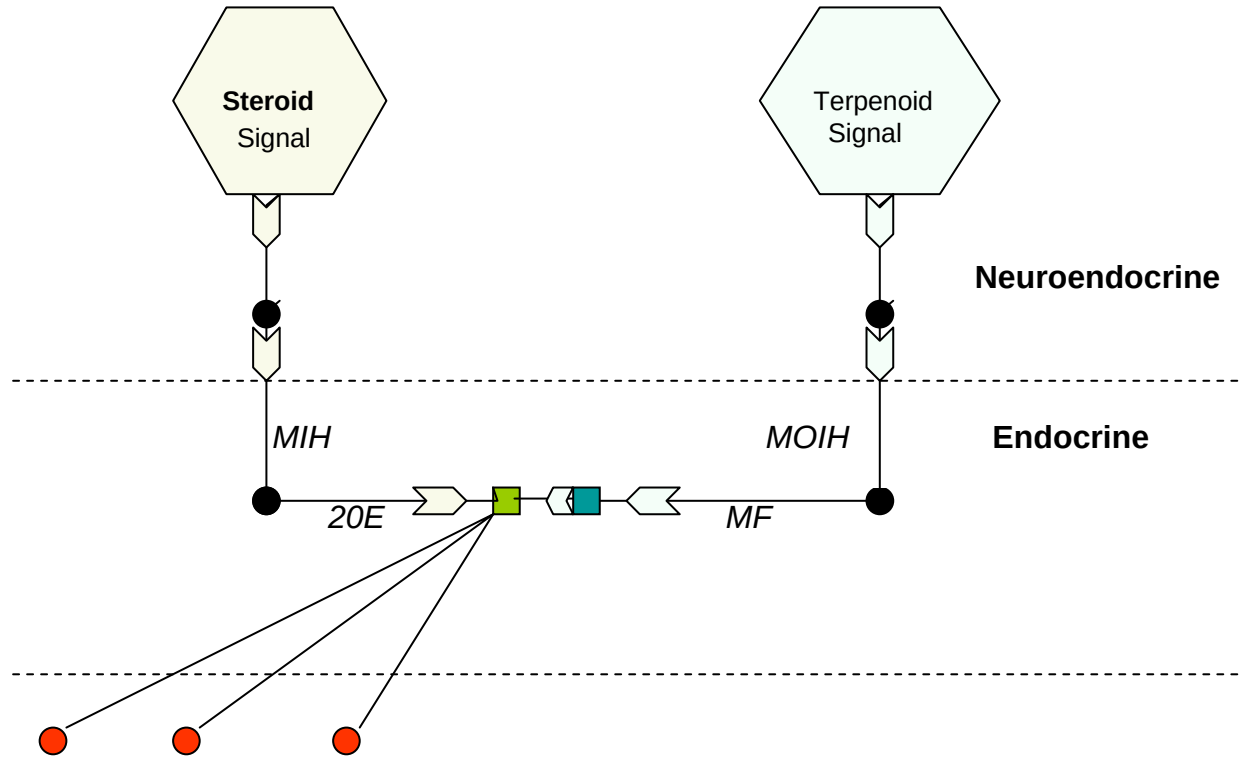
Sensor

System



Regulated genes
(potential biomarkers)

System



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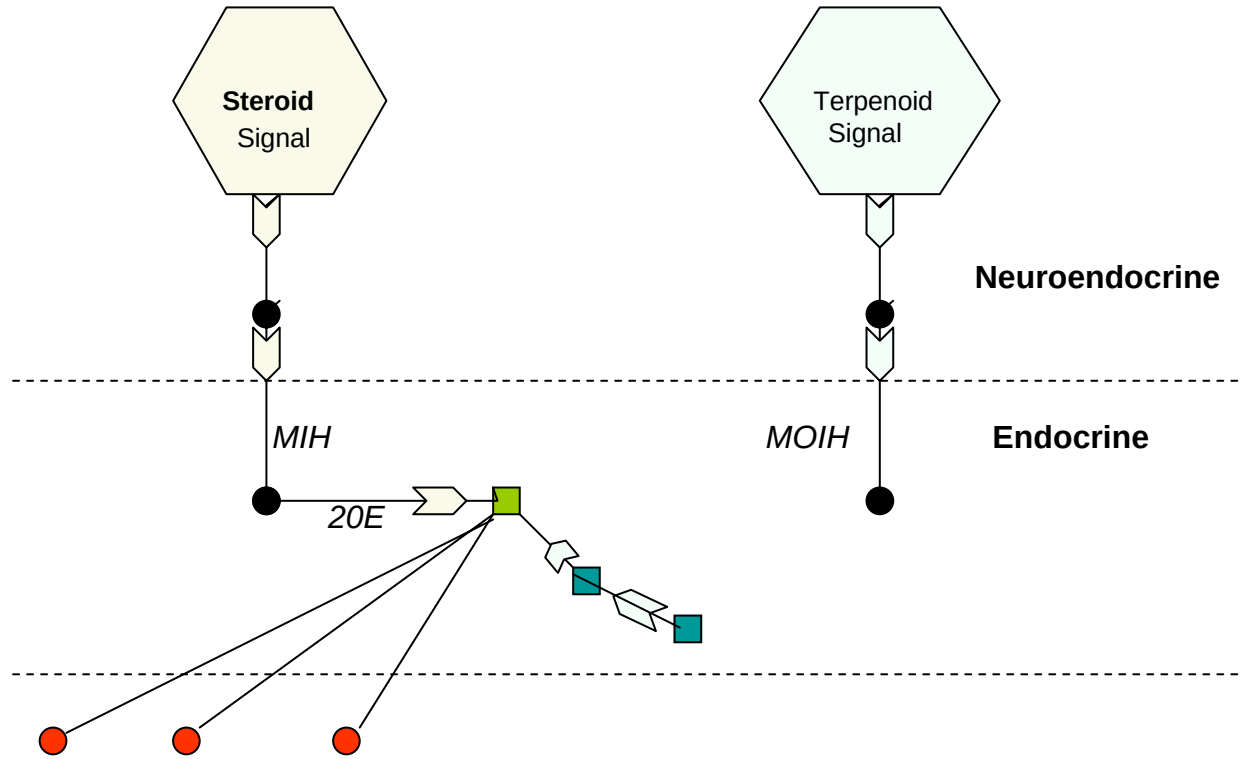

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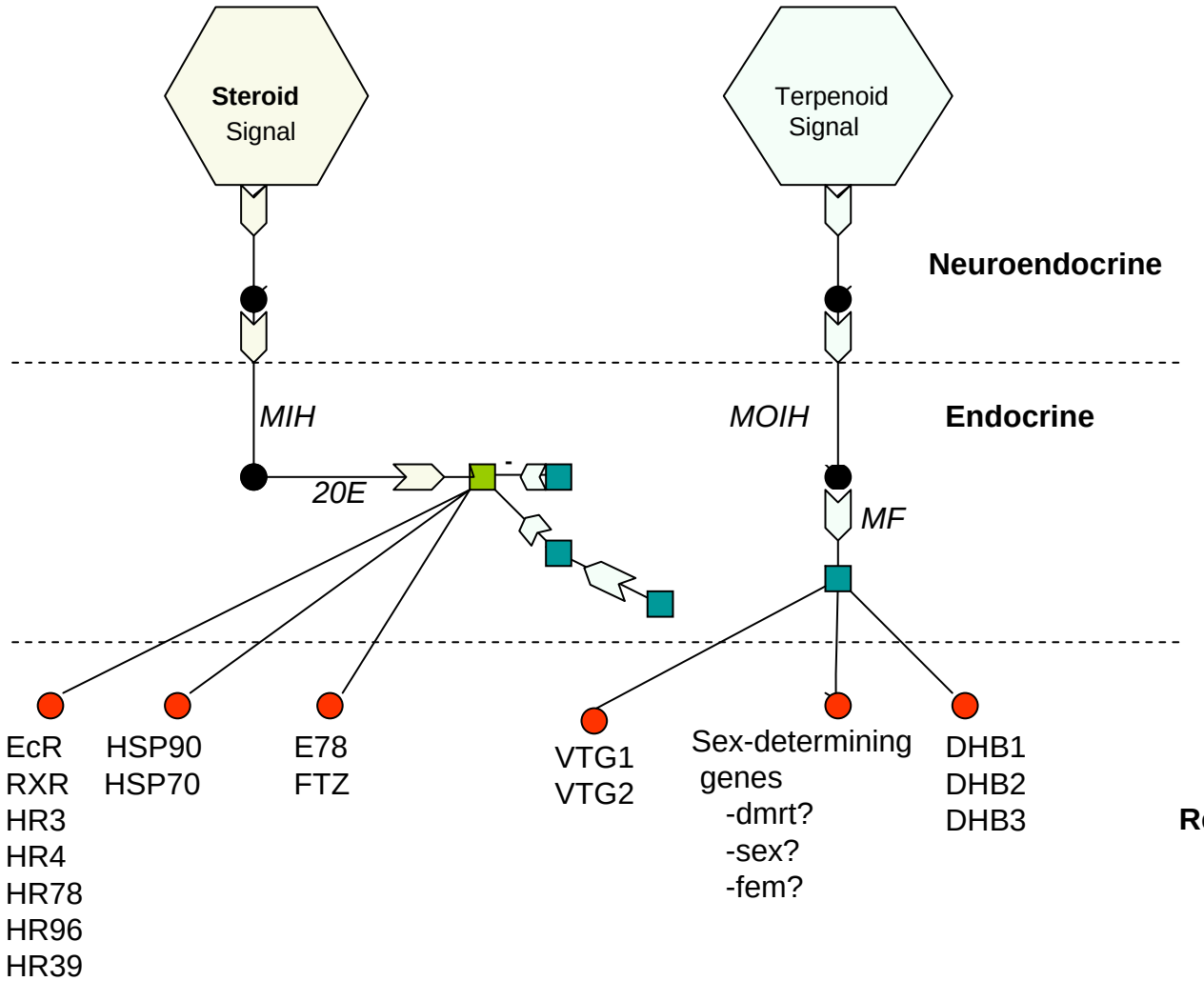
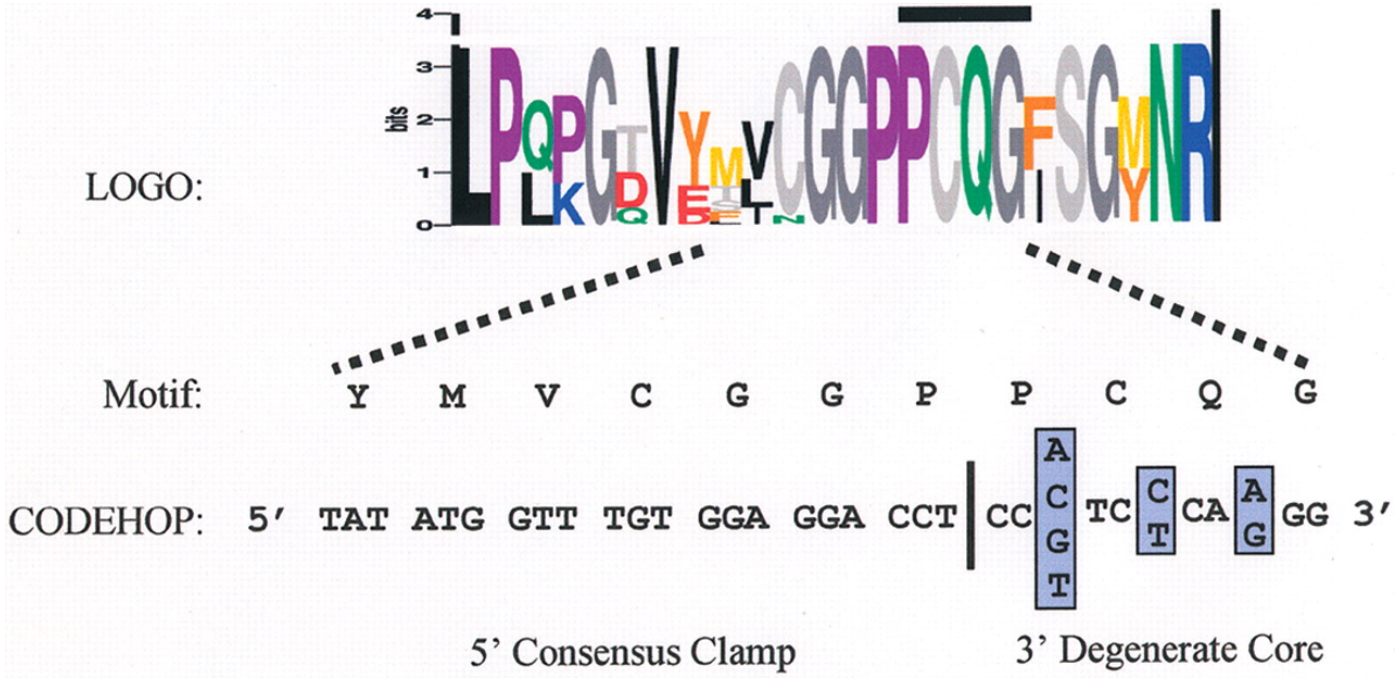


Regulated genes
(potential biomarkers)

Output: PCaRray

- Develop gene product-specific primers
  - CODE-HOP
- Simple gene array
- Real-time PCR analyses of gene expression

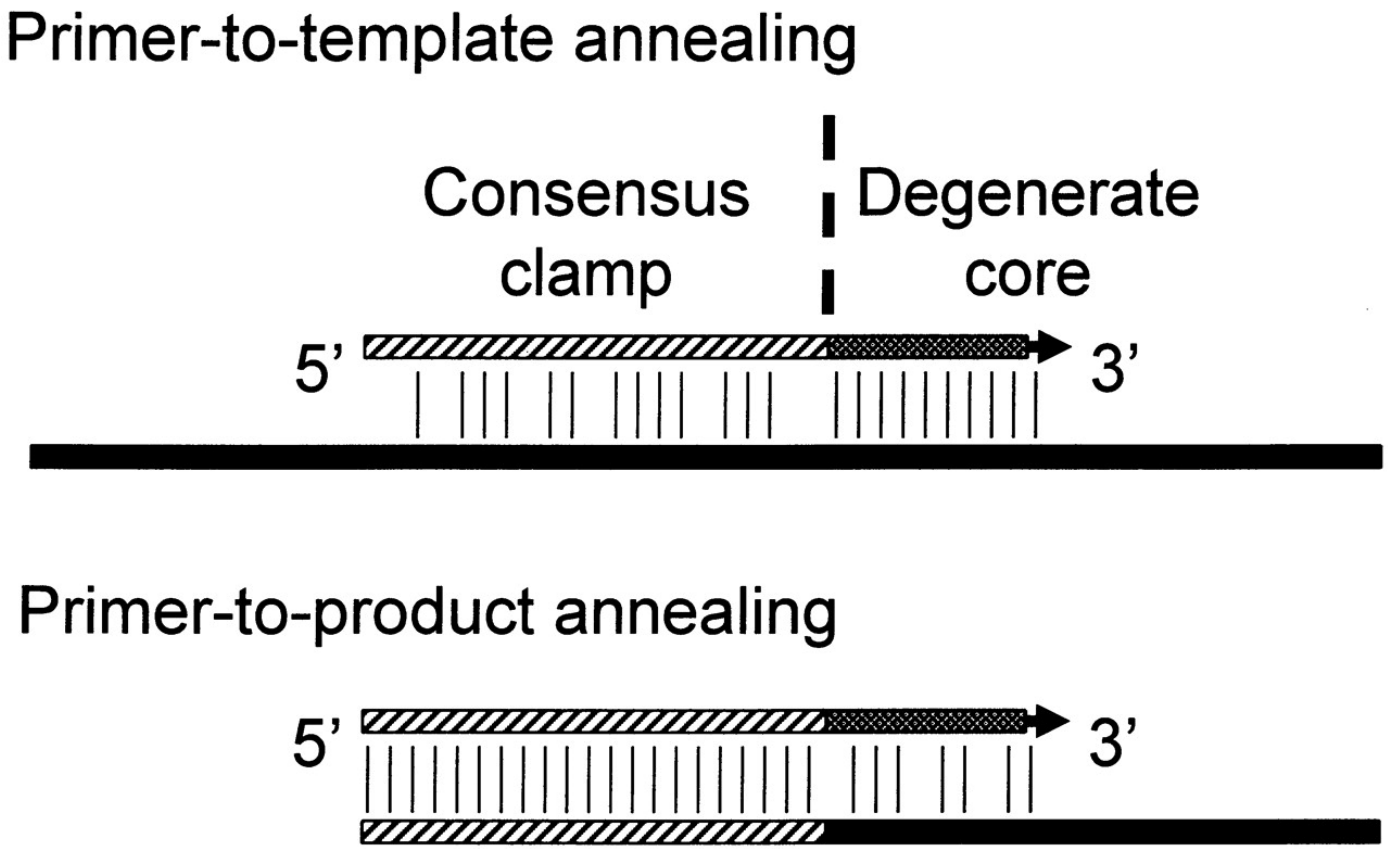
CODE HOP PCR



Rose, T. et al. Nucl. Acids Res. 2003 31:3763-3766; doi:10.1093/nar/gkg524

Nucleic Acids Research

CODE HOP PCR



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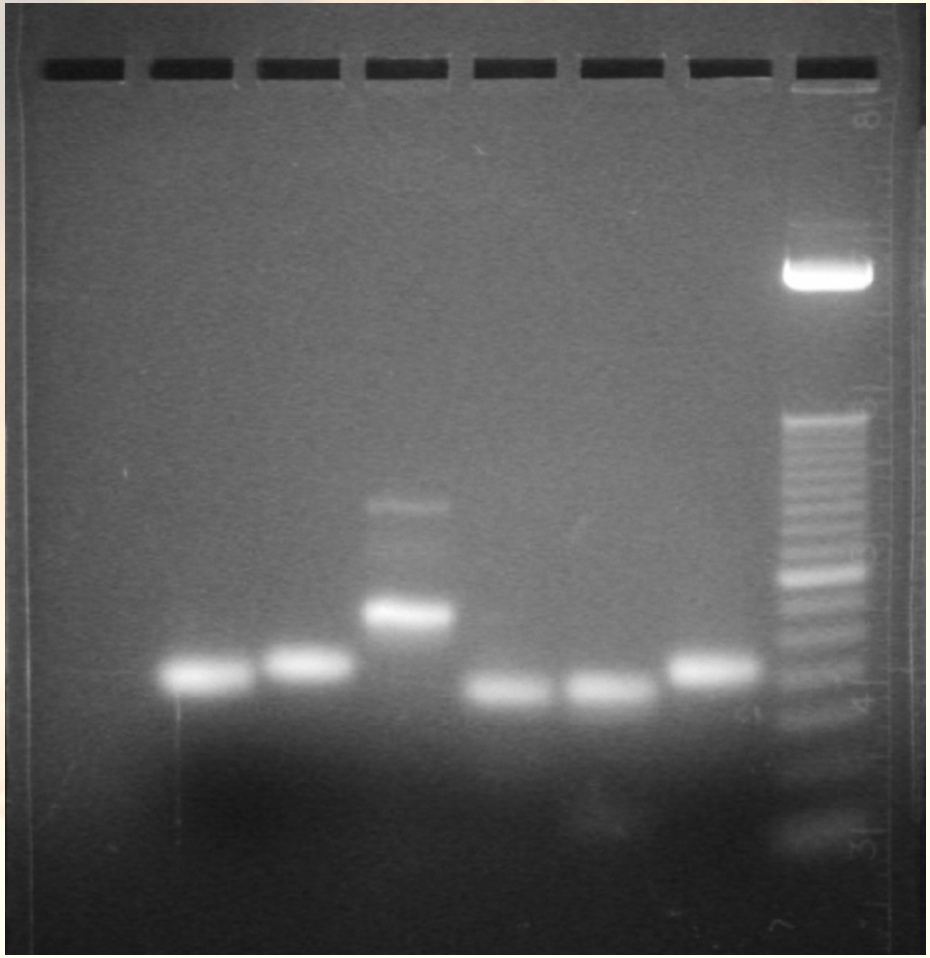
Nucleic Acids Research

PCR amplification of sensor gene products



Identify a suite of gene products (biomarkers) that define endocrine circuits

- NR2B (RXR)
  - 1888 base pairs
  - 400 amino acid protein
  - Five-domain structure of a nuclear receptor

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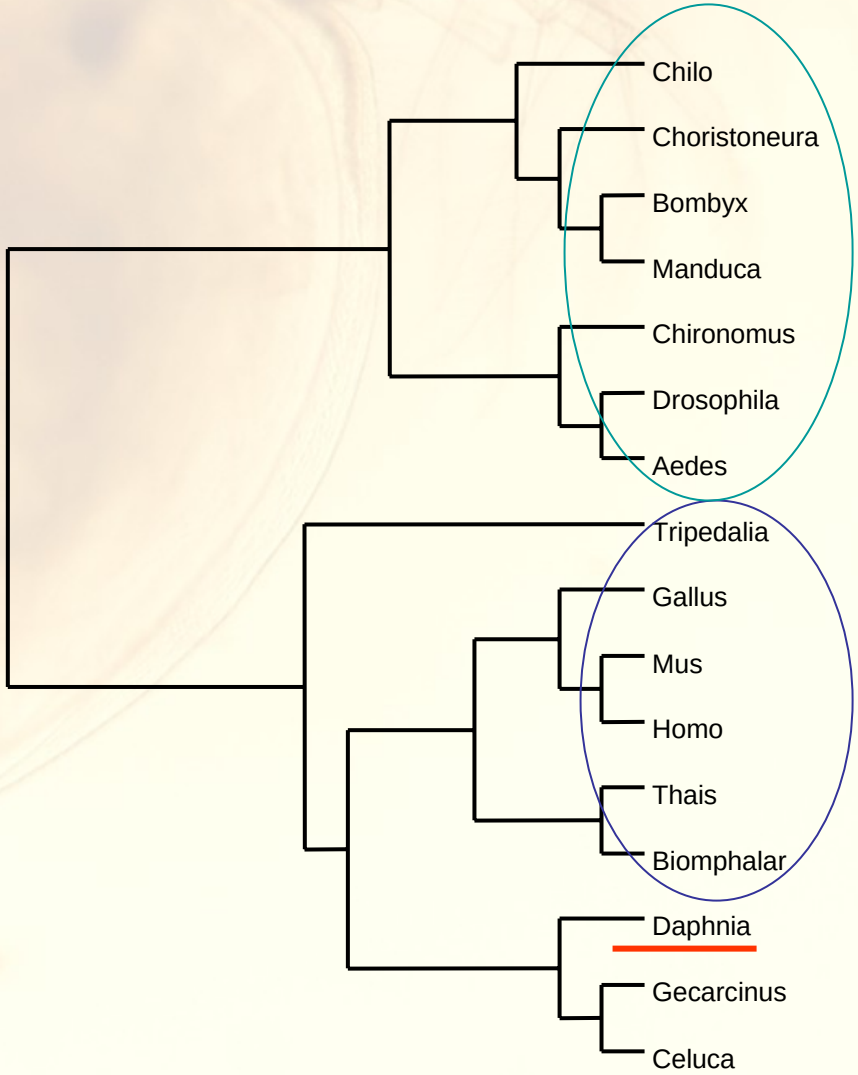
- NR2B (RXR)



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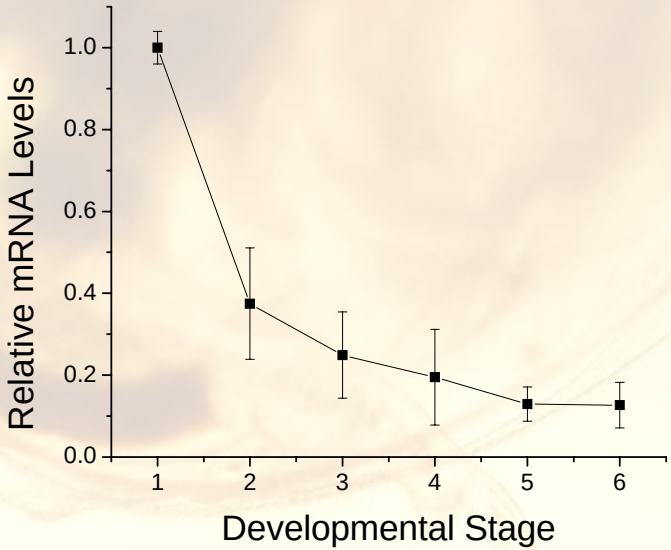
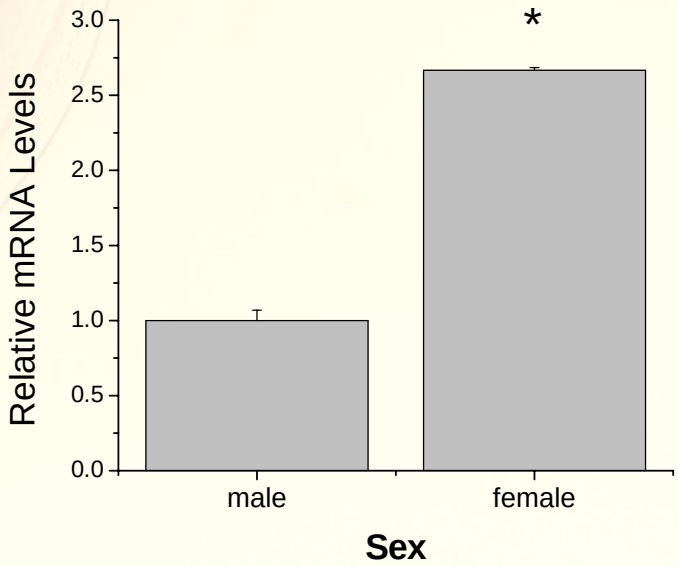




Identify a suite of gene products (biomarkers) that define endocrine circuits

EcR

↑

RXR

↑

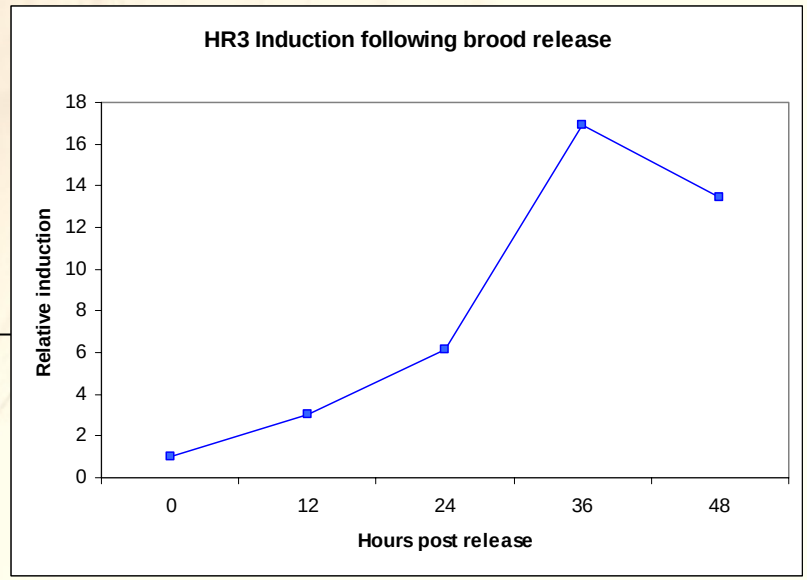
HR3

↑

E75

Heme protein
NO sensor
Regulates HR3

Nitrates/nitrites?



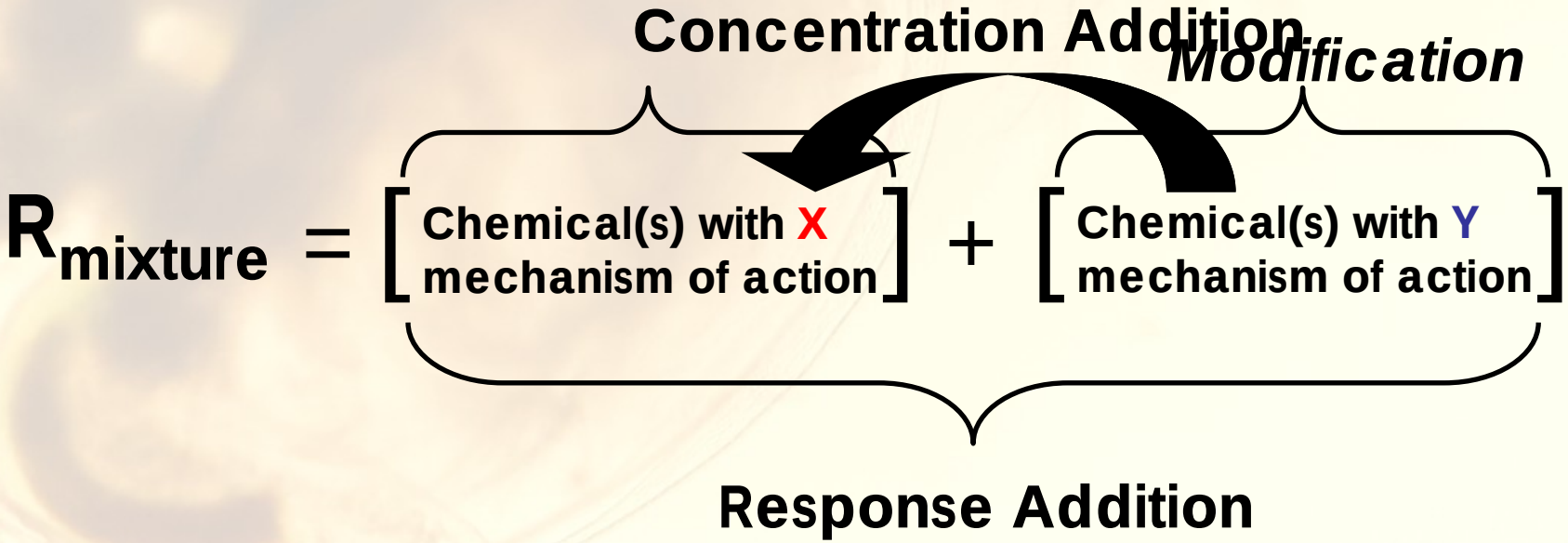
Assess performance of the biomarkers in response to individual EDCs

Lab exposures are complete with:

- EcR agonists
  - 20-hydroxyecdysone
- Ecdysteroid synthesis inhibitors
  - Fenarimol
  - Piperonyl butoxide
- Anti-ecdysteroids
  - 4-nonylphenol
- Terpene mimics
  - Pyriproxyfen
  - Fenoxycarb
  - Methoprene
  - Dieldrin
- Negatives
  - Kinoprene
  - Cis-chlordane

Assess performance of the system in response to chemical mixtures

Integrated Addition and Interaction Model:



http://wang.tox.ncsu.edu/model5/

Conclusions

- A systems approach using a small whole organism sensor will:
  - refine cumulative assessment of exposure to EDC mixtures that target endocrine networks
  - establish a paradigm for application to other sensor species.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