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## Lobster Genomics: Fishing for Biomarkers



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4<sup>th</sup> Annual Lobster Science Workshop  
Moncton New Brunswick, November 4-5, 2008

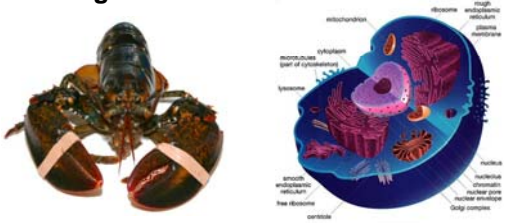


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## Genomics Research

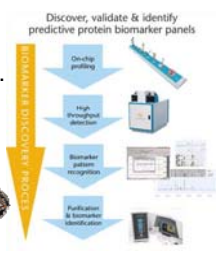
- Genomics** is the study of an organism's entire genome.



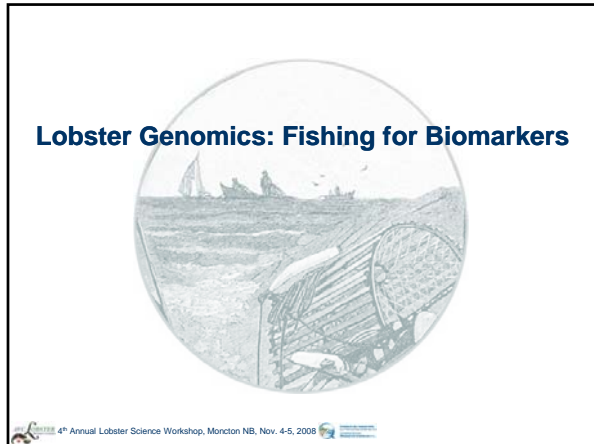
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## Biomarker Discovery

- Biomarker**
  - a substance used as an indicator of a biologic state.
  - objectively measure & evaluate
    - Normal biologic processes
      - Molt cycle
    - Pathogenic processes
      - Gaffkemia
    - Toxin exposure
      - PSP



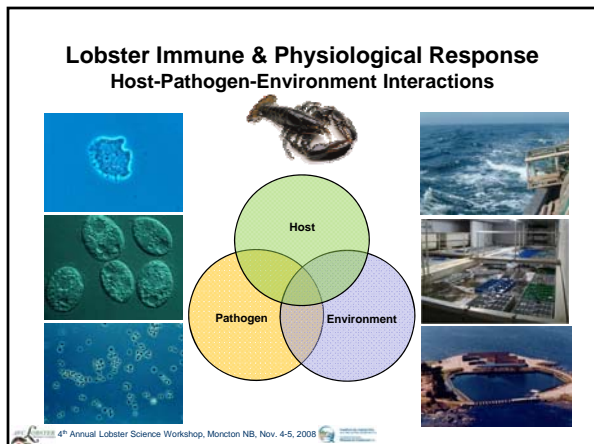
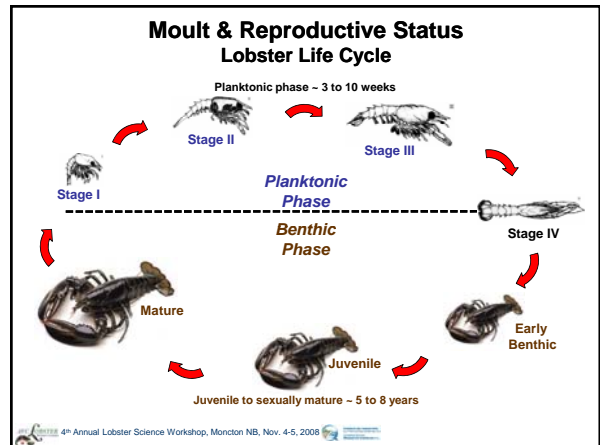
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### Lobster Genomics Atlantic Lobster Molt & Quality Project

- **Develop Biomarkers for:**
  - Molt status = Quality
  - Reproductive status
  - Immune response
  - Physiological response

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### Lobster Immune & Physiological Response Host-Pathogen-Environment Interactions

- **Infection experiments**
  - *Gaffkemia*
    - *Aerococcus viridans*
  - *Bumper Car*
    - *Anophryoides haemophila*

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## Lobster Genomics

- **Construct cDNA library**

- Postmoult & intermoult
  - Adult male & female
- Pool 10 tissues
  - gonad, eye stalk, mandibular organ, supraesophageal ganglion, hepatopancreas, antennal gland, claw muscle, tail muscle, gill, intestine
- Extract total RNA



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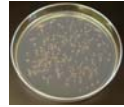
## Lobster Genomics

- **cDNA library**

- Normalize RNA to enrich for rare transcripts
- Synthesize cDNA
- Clone into pCMV Sport 6.1

- **Expressed Sequence Tags (EST)**

- Randomly selected 20,000 clones
- High throughput
  - Single-pass 5' end sequencing

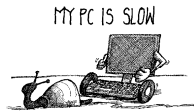


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## Lobster Genomics - Bioinformatics

- **Cluster analysis**

- **CLOBB**
  - Cluster On the Basis of BLAST similarity
  - 16,471 sequences
    - 10,965 EST singletons
    - 5,506 clusters containing 2 or more ESTs.
- **TGICL**
  - TIGR Gene Indices Clustering tools
  - 15,864 "unique" sequences

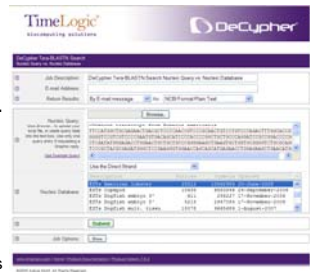


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## Lobster Genomics - Bioinformatics

- **Final BLAST with Decypher**

- Internal *Homarus* database @ MDIBL
- 15,027 sequences
- 12,715 new sequences + 2,312 from previous data
- GenBank total
  - ~ 29,636 sequences



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## Lobster Genomics - Bioinformatics

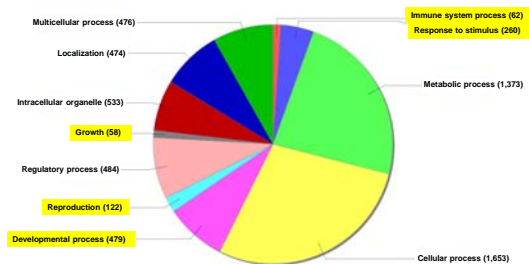
- **Gene Ontology (GO) analysis**

- Blast2GO
  - Automatic annotation of 15,027 sequences
- Data mining
  - Link sequence to predicted biological function



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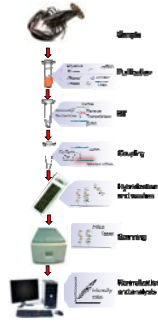
## Biological Process Level 2



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## Lobster Genomics - Microarray

- Develop & Print Microarrays
- Validate & confirm with Q-PCR



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## Lobster Genomics - Microarray

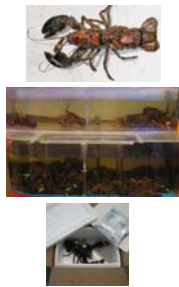
- Gene Expression studies
  - Reproductive cycle
    - Female lobster (Ovary stages 1-5)
  - Moulting cycle
    - Larval lobsters stages 1-4
    - Juvenile lobsters
  - Immune response
    - Gaffkemia infection trial
    - Bumper Car infection trial



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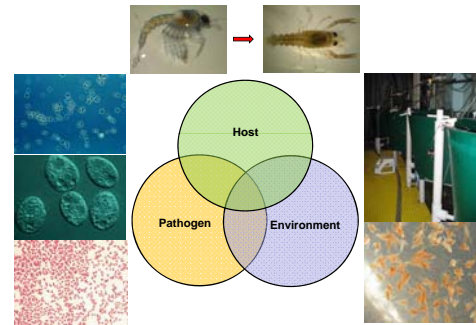
## Lobster Genomics - Microarray

- Future microarray studies
  - Disease
    - Shell disease, Vibriosis...
  - Nutritional studies
    - Ocean, pound, bait?
  - Toxicity
    - Heavy metals, PSP?
  - Environmental stressors
    - Water quality
      - Temperature, salinity, oxygen
    - Live shipping?



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## Microarrays & Lobster hatcheries



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## Collaborators

- Andrea Battison, Fraser Clark, Adam Acorn
- Yingwei Wang
- David Towle, Andrew Christie
- Jeff Shields, Hamish Small
- Michel Comeau, Phil Byrne
- Dounia Daoud
- Martin Mallet



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