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A Systematic approach to the Large-Scale Analysis of Genotype-Phenotype correlations

Paul Fisher

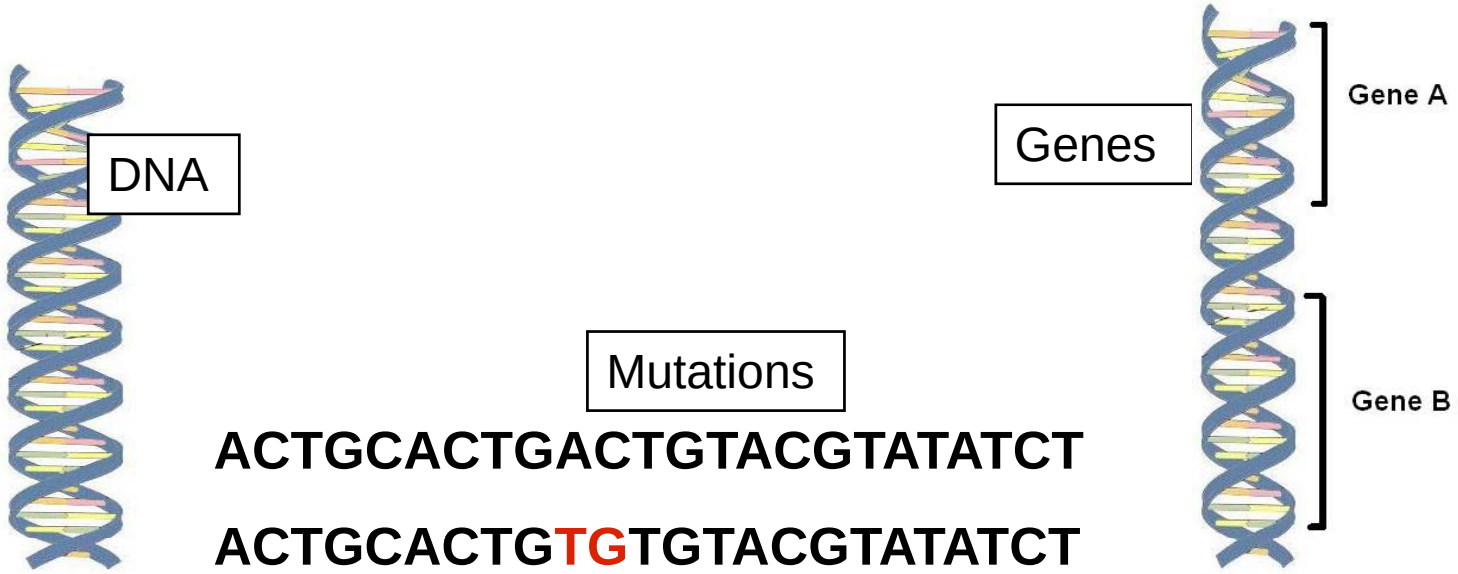
Dr. Robert Stevens

Prof. Andrew Brass

BHIG

Genotype

The entire genetic identity of an individual that **does not show** any outward characteristics, *e.g.* Genes, mutations



Phenotype

(harder to characterise)

The observable expression of gene's producing **notable characteristics** in an individual, *e.g.* Hair or eye colour, body mass, resistance to disease

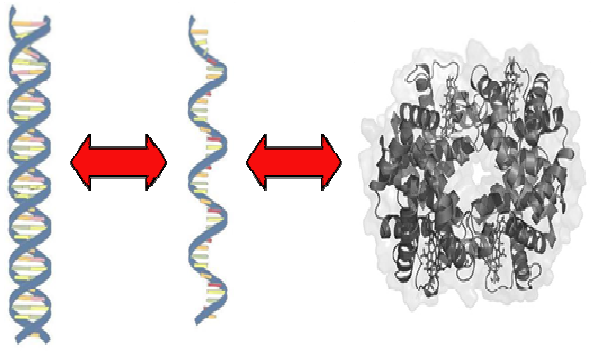
Brown

vs.

White and Brown

Genotype to Phenotype

Genotype



Phenotype

DNA RNA Protein Protein-Protein interaction Pathway Trait

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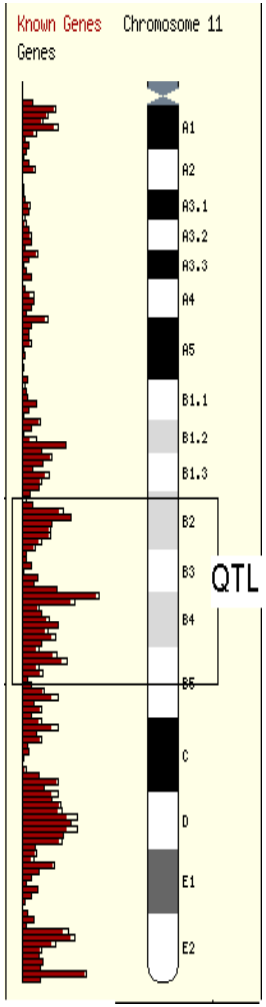
Current Methods

Genotype



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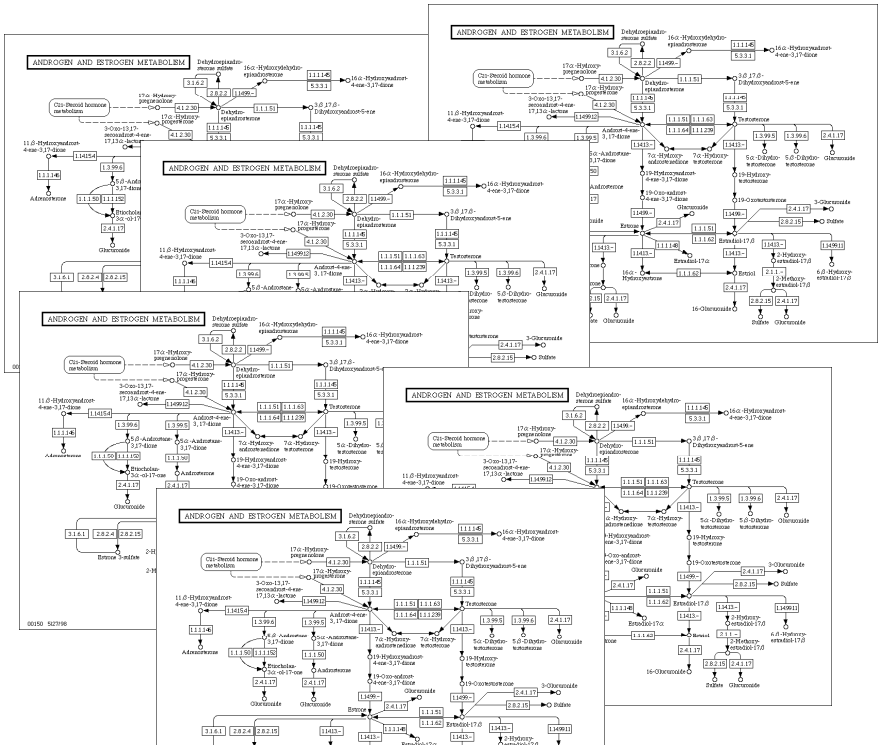

Phenotype

?

What processes to investigate?

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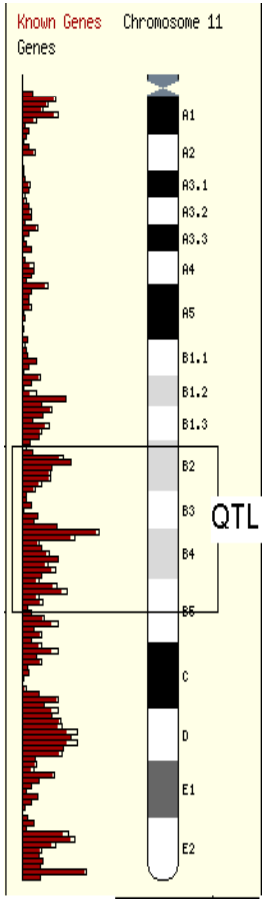
Genotype

Phenotype



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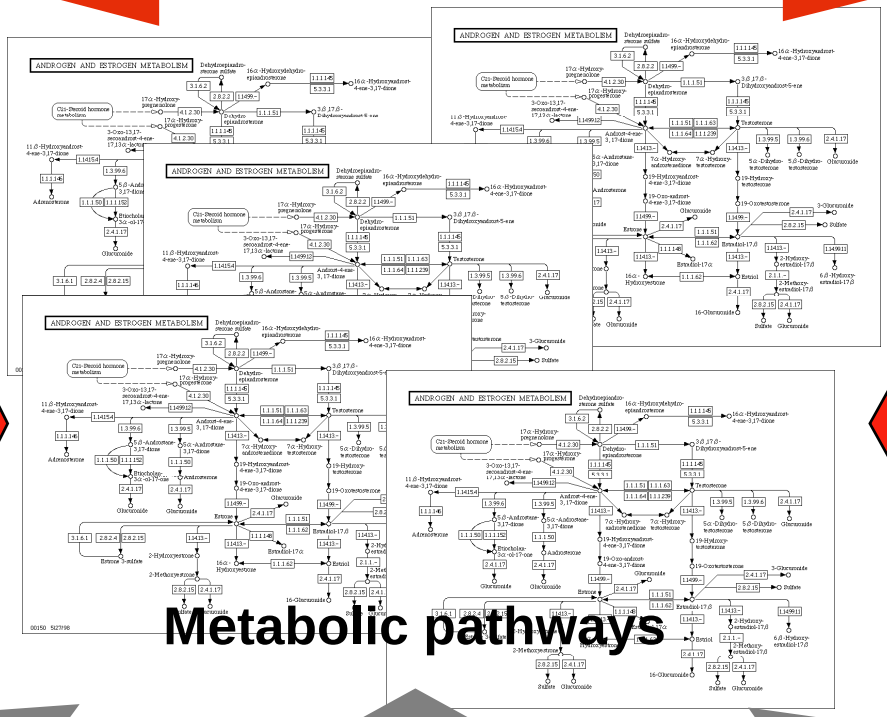
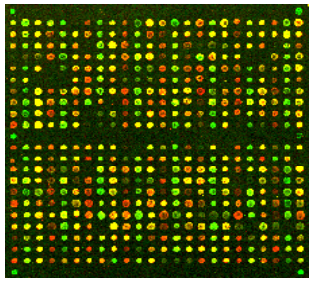


Metabolic pathways

?



Microarray + QTL

Genes captured in microarray experiment and present in QTL (**Quantitative Trait Loci**) region

Phenotypic response investigated using microarray in form of expressed genes or evidence provided through QTL mapping

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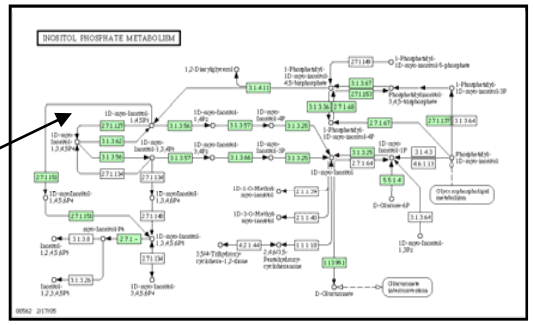
Phenotype

Pathway A



literature

Pathway linked to phenotype – high priority

CHR

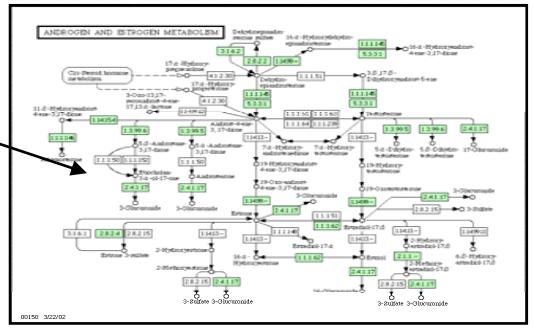
QTL

Gene A

Gene B

Gene C

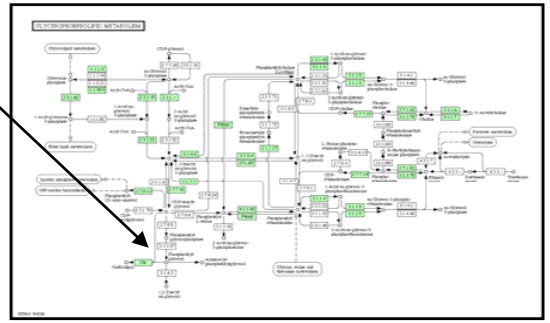
Pathway B



literature

Pathway not linked to phenotype – medium priority

Pathway C



literature

Pathway not linked to OTL – low priority

Genotype

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Issues with current approaches

- Scale of analysis task
- User bias and premature filtering
- Hypothesis-Driven approach to data analysis
- Constant flux of data - problems with re-analysis of data
- Implicit methodologies (hyper-linking through web pages)
- Error proliferation from any of the listed issues

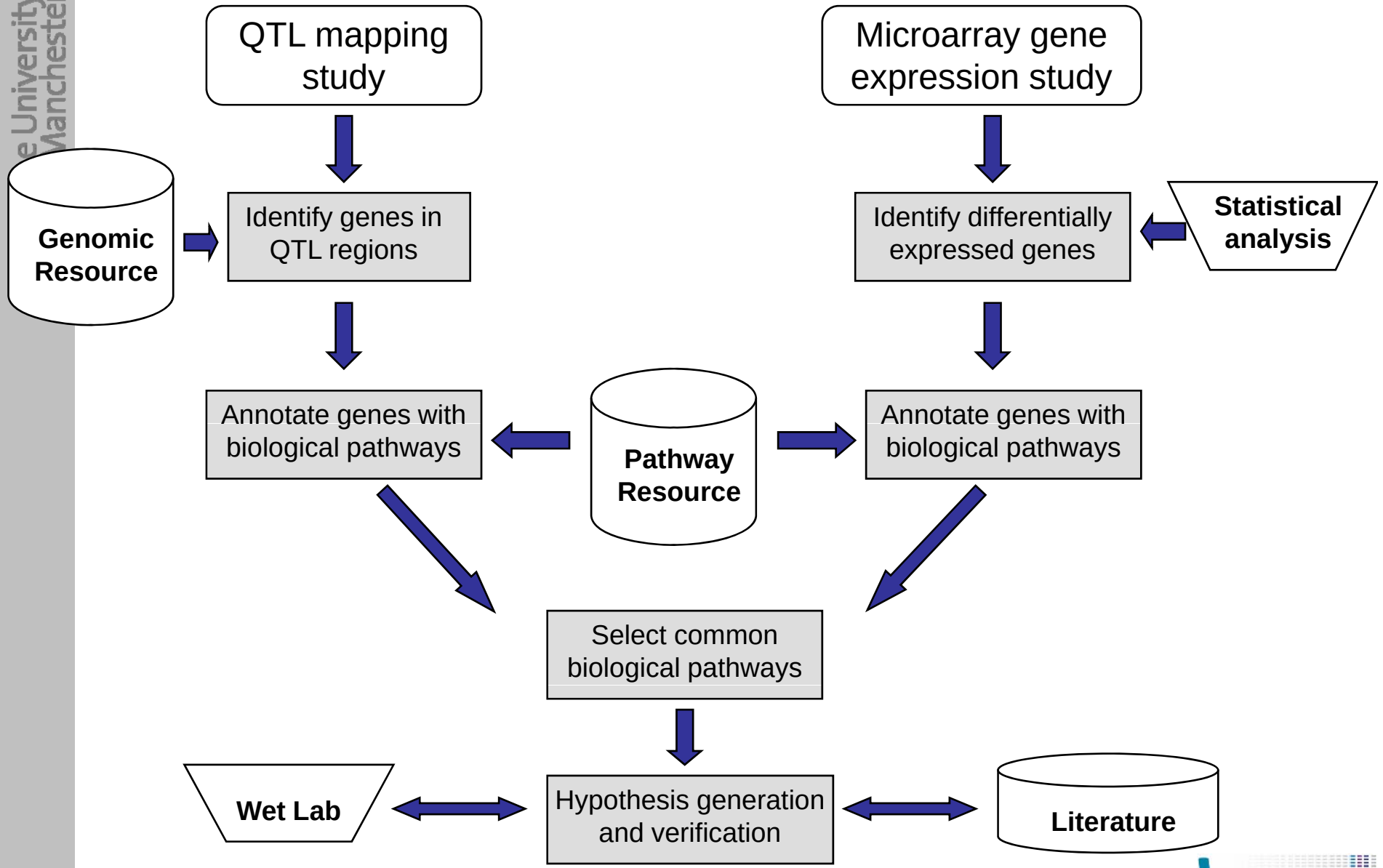
Solution – Automate through workflows

Hypothesis

Utilising the capabilities of workflows and the pathway-driven approach, we are able to provide a more:

- systematic
- explicit
- scalable
- un-biased

the benefit will be that **new biology** results will be derived, increasing community knowledge of genotype and phenotype interactions.


QTL mapping study
Microarray gene expression study
Genomic Resource
Identify genes in QTL regions
Identify differentially expressed genes
Statistical analysis
Annotate genes with biological pathways
Pathway Resource
Annotate genes with biological pathways
Select common biological pathways
Wet Lab
Hypothesis generation and verification
Literature

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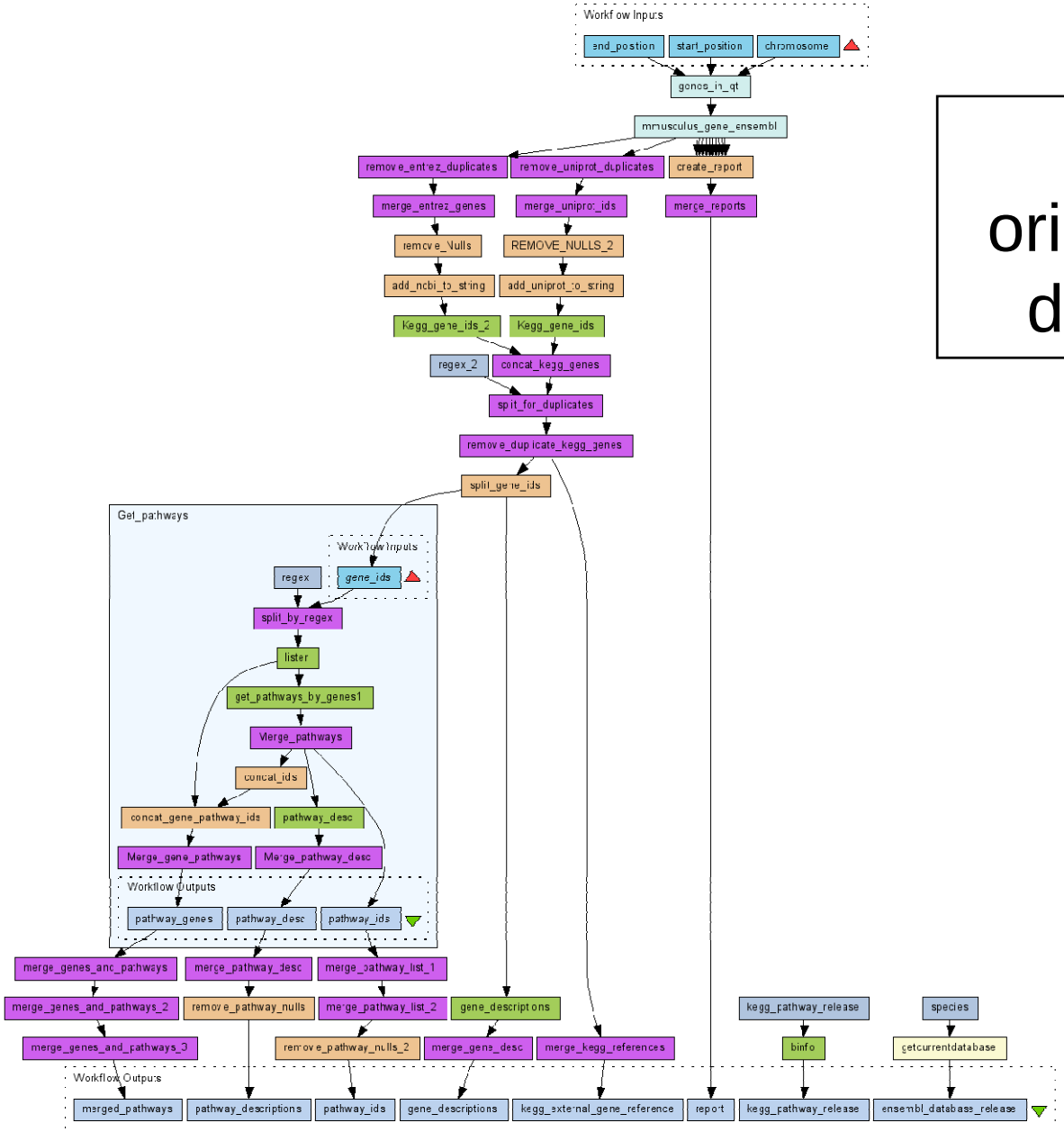

Replicated original chain of data analysis

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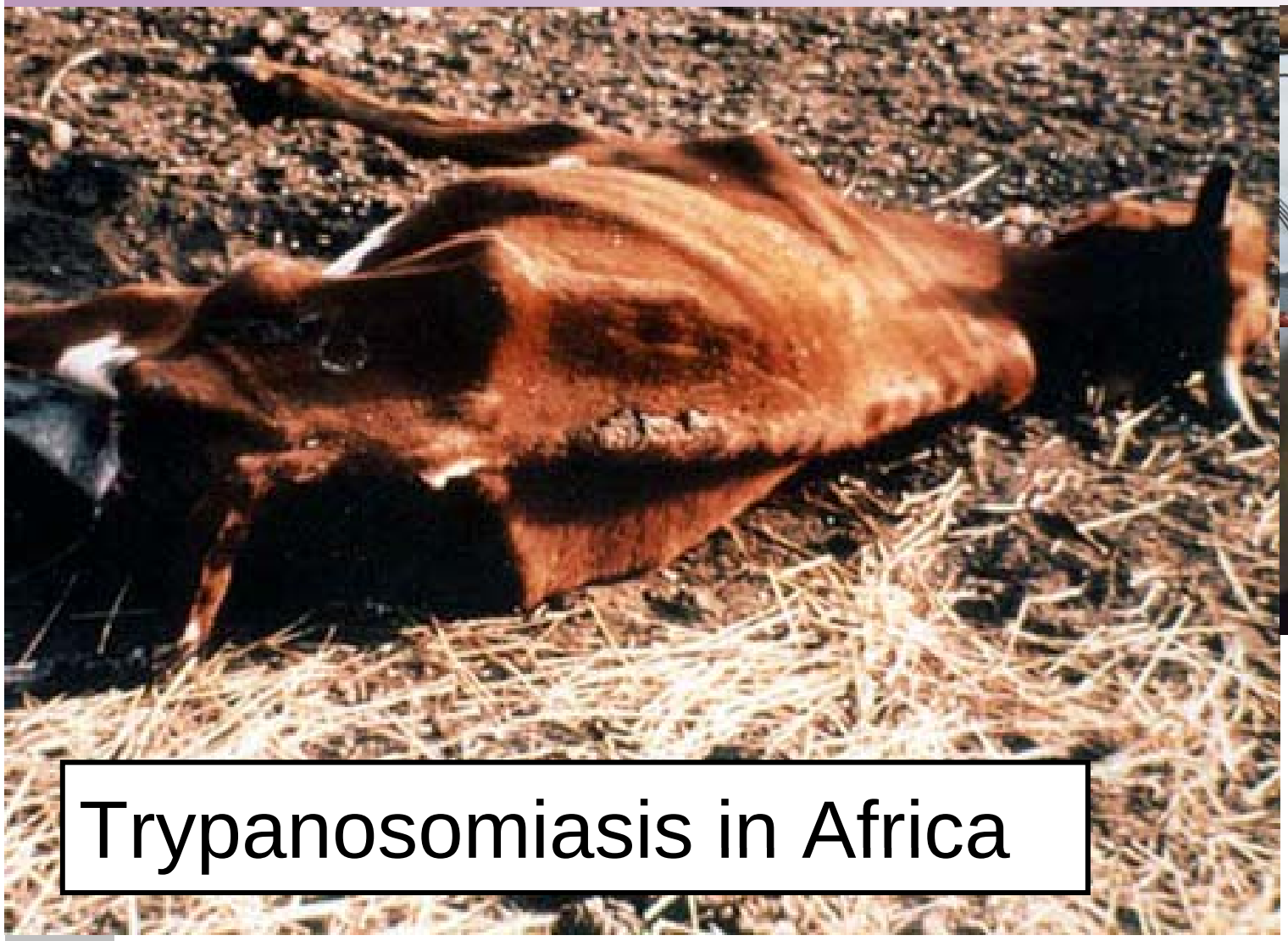
The Wellcome Trust Funded Host-Pathogen Project



Steve Kemp

Andy Brass

+ many Others

Trypanosomiasis in Africa

http://www.genomics.liv.ac.uk/tryps/trypsindex.html

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Preliminary Results

Trypanosomiasis resistance

A strong candidate gene was found

- **Daxx** gene not found using manual investigation methods
- The gene was identified from analysis of biological pathway information
- Possible candidate identified by Yan et al (2004): Daxx SNP info
- Sequencing of the Daxx gene in **Wet Lab** showed mutations that is thought to change the structure of the protein
- Mutation was published in scientific literature, noting its effect on the binding of Daxx protein to p53 protein – **p53 plays direct role in cell death and apoptosis, one of the Trypanosomiasis phenotypes**
- More genes to follow (hopefully) in publications being written

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Shameless Plug!

A Systematic Strategy for Large-Scale Analysis of Genotype-Phenotype Correlations: Identification of candidate genes involved in African Trypanosomiasis

Fisher *et al*., (2007) Nucleic Acids Research
doi:10.1093/nar/**gkm623**

- Explicitly discusses the methods we used for the Trypanosomiasis use case
- Discussion of the results for Daxx and shows mutation
- Sharing of workflows for re-use, re-purposing

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Recycling, Reuse, Repurposing

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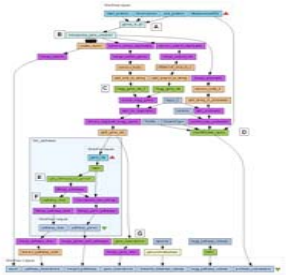
Here's the **Science**!

- Identified a candidate gene (Daxx) for Trypanosomiasis resistance.
- Manual analysis on the microarray and QTL data failed to identify this gene as a candidate.
- Unbiased analysis. Confirmed by the wet lab.



Here's the **e-Science**!

- Trypanosomiasis mouse workflow reused without change in *Trichuris muris* infection in mice
- Identified biological pathways involved in sex dependence
- Previous manual two year study of candidate genes had failed to do this.

Workflows now being run over Colitis/ Inflammatory Bowel Disease in Mice (without change)

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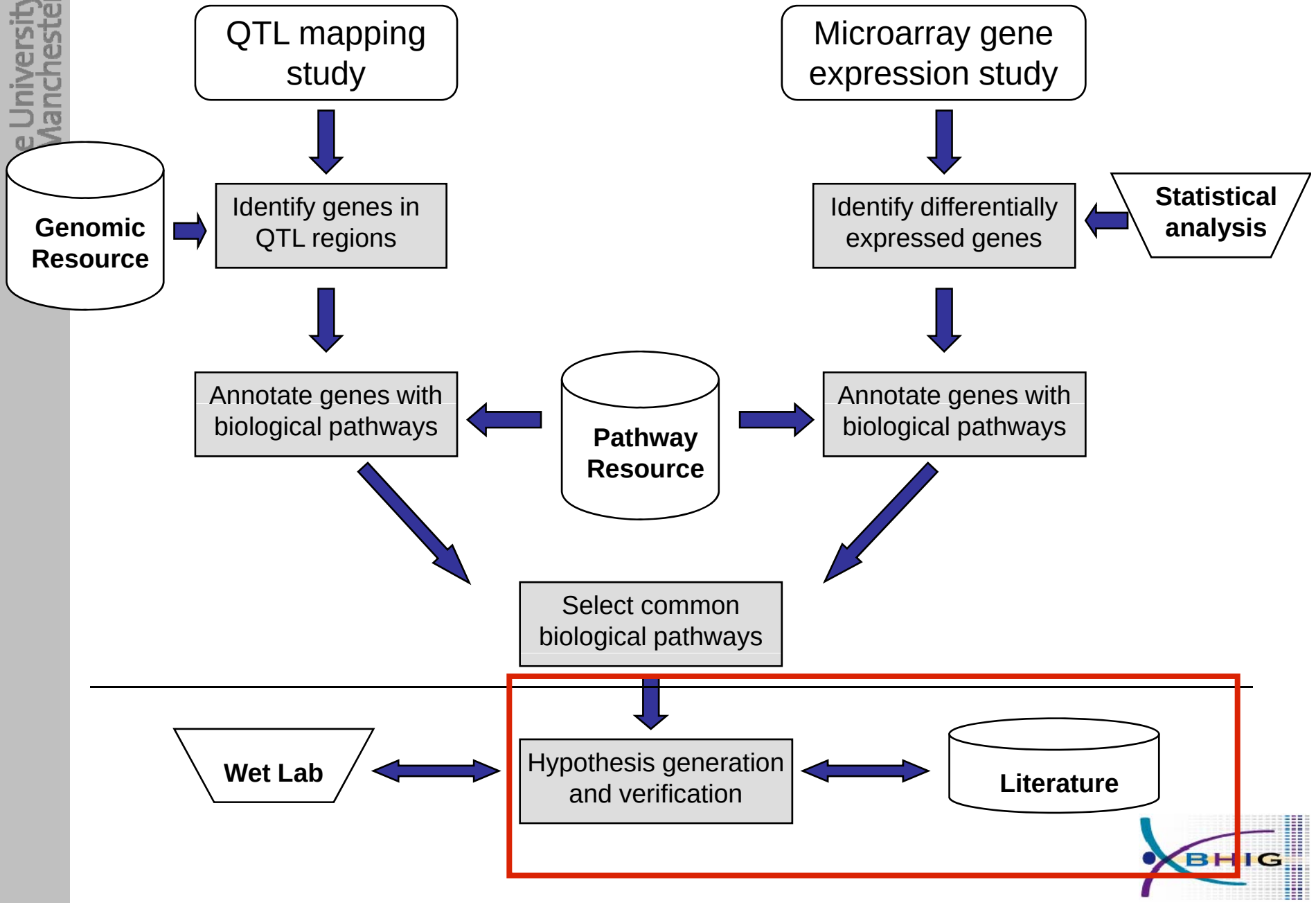
Recycling, Reuse, Repurposing

- Share
- Search
- Re-use
- Re-purpose
- Execute
- Communicate
- Record

http://www.myexperiment.org/

What next?

- More use cases??
  - Can be done, but not for my project
- Text Mining !!!
  - Aid biologists in identifying novel links between pathways
  - Link pathways to phenotype through literature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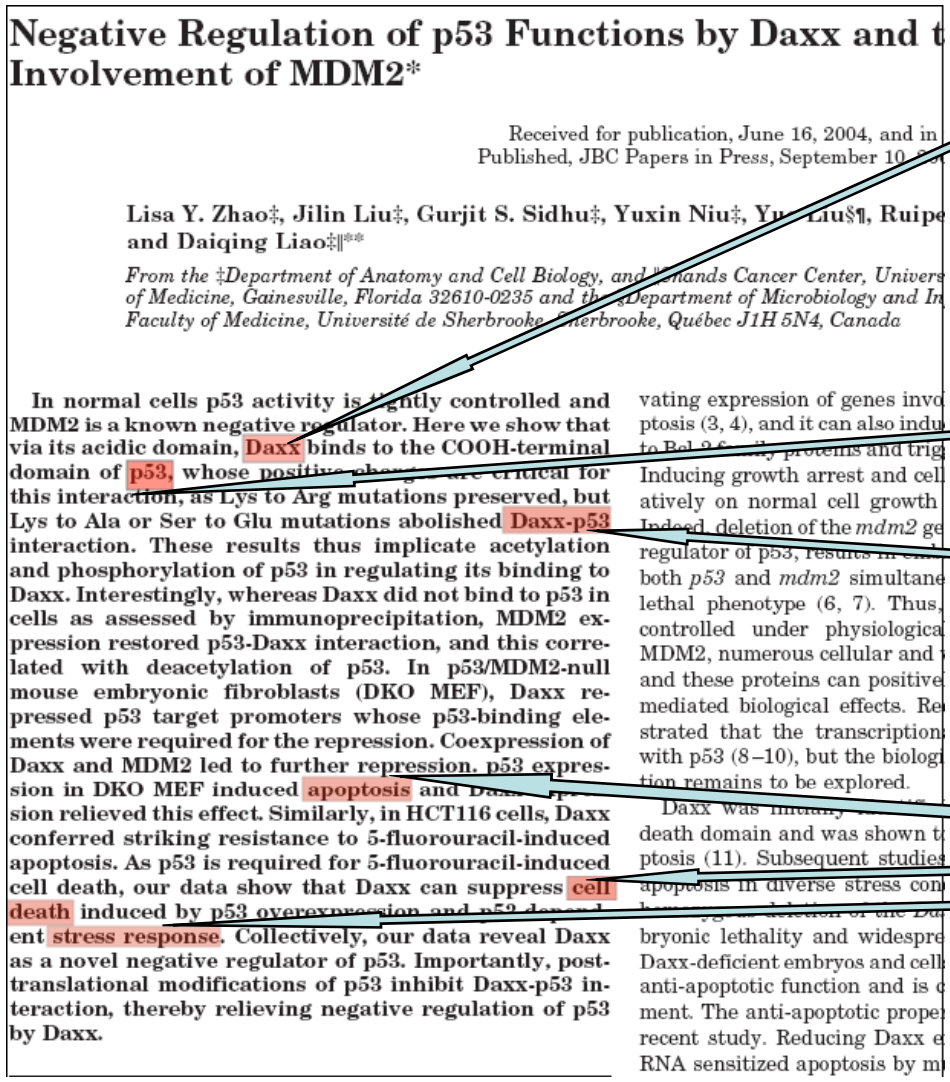
What Does the Text Hold?



Negative Regulation of p53 Functions by Daxx and t
Involvement of MDM2*

Received for publication, June 16, 2004, and in
Published, JBC Papers in Press, September 10,

Lisa Y. Zhao‡, Jilin Liu‡, Gurjit S. Sidhu‡, Yuxin Niu‡, Yu Liu§¶, Ruipe
and Daiqing Liao‡‖**

*From the ‡Department of Anatomy and Cell Biology, and Shands Cancer Center, Univers
of Medicine, Gainesville, Florida 32610-0235 and the Department of Microbiology and In
Faculty of Medicine, Université de Sherbrooke, Sherbrooke, Québec J1H 5N4, Canada*

In normal cells p53 activity is tightly controlled and MDM2 is a known negative regulator. Here we show that via its acidic domain, Daxx binds to the COOH-terminal domain of p53, whose positive charges are critical for this interaction, as Lys to Arg mutations preserved, but Lys to Ala or Ser to Glu mutations abolished Daxx-p53 interaction. These results thus implicate acetylation and phosphorylation of p53 in regulating its binding to Daxx. Interestingly, whereas Daxx did not bind to p53 in cells as assessed by immunoprecipitation, MDM2 expression restored p53-Daxx interaction, and this correlated with deacetylation of p53. In p53/MDM2-null mouse embryonic fibroblasts (DKO MEF), Daxx repressed p53 target promoters whose p53-binding elements were required for the repression. Coexpression of Daxx and MDM2 led to further repression. p53 expression in DKO MEF induced apoptosis and Daxx expression relieved this effect. Similarly, in HCT116 cells, Daxx conferred striking resistance to 5-fluorouracil-induced apoptosis. As p53 is required for 5-fluorouracil-induced cell death, our data show that Daxx can suppress cell death induced by p53 overexpression and p53-dependent stress response. Collectively, our data reveal Daxx as a novel negative regulator of p53. Importantly, post-translational modifications of p53 inhibit Daxx-p53 interaction, thereby relieving negative regulation of p53 by Daxx.

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Protein Info

Related Proteins

Protein-Protein Interactions

Pathways

Biological processes

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What Next ?

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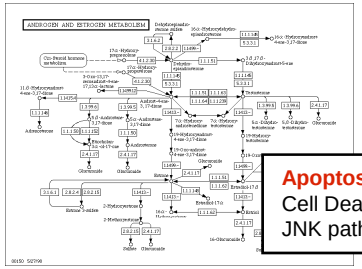
Biological processes

Generate a Profile for Pathway / Phenotype

Apoptosis
Cell Death
Stress response
……..

Score and Rank Terms



Apoptosis
Cell Death
JNK pathway

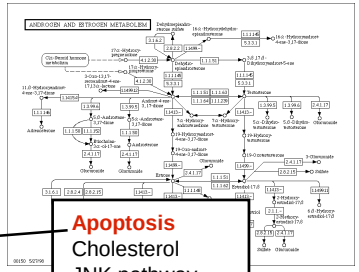
13.27

Common terms

Phenotype Terms

Apoptosis

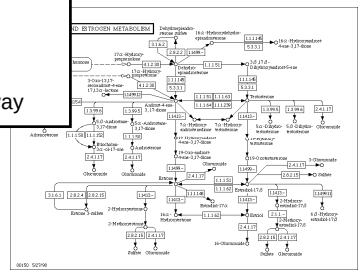
0.15



Apoptosis
Cholesterol
JNK pathway

28.35

Apoptosis
Cholesterol
Diabetes
Apoptosis
JNK pathway
Another pathway



Score pathway links based on occurrence of phenotype term in pathway abstracts

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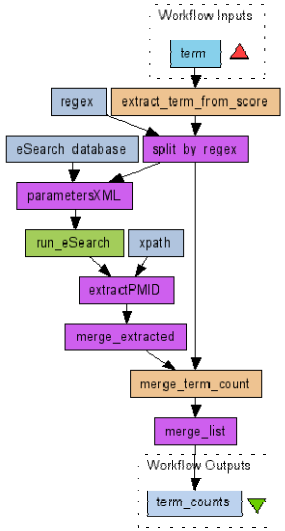
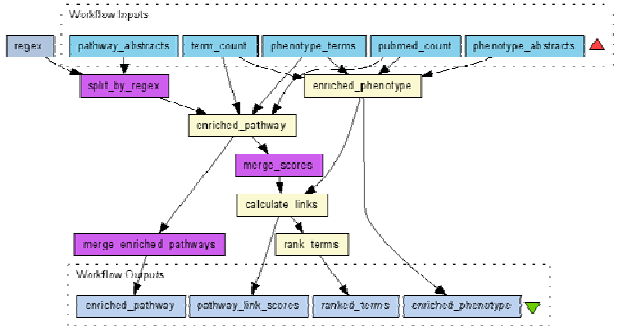
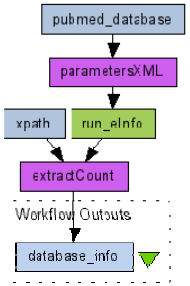
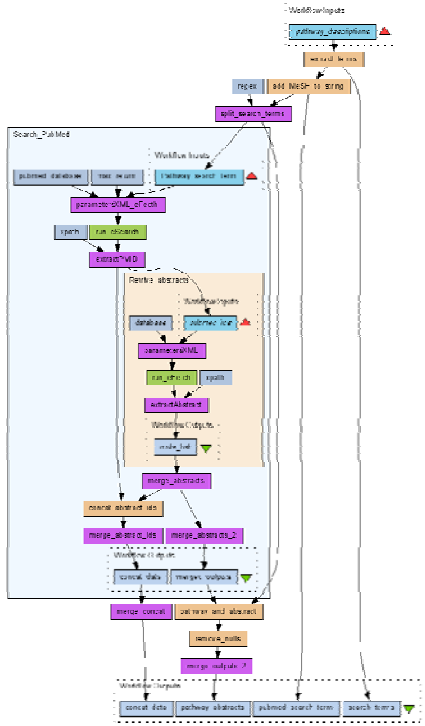
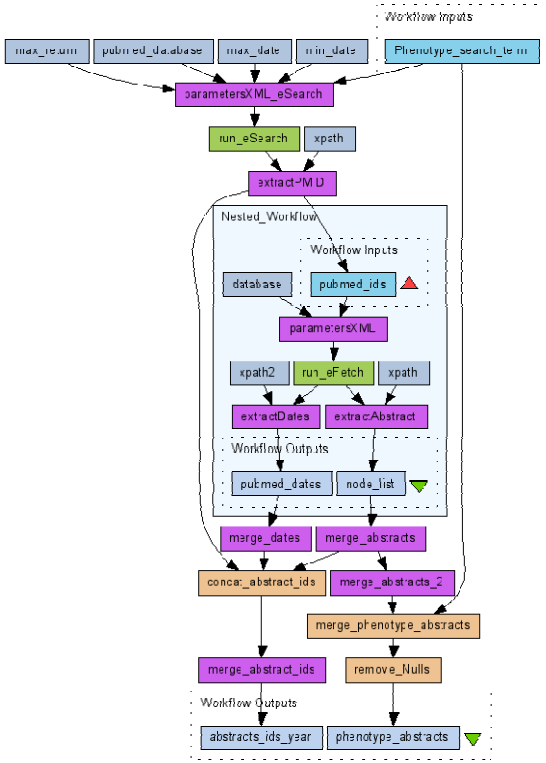
The Workflows

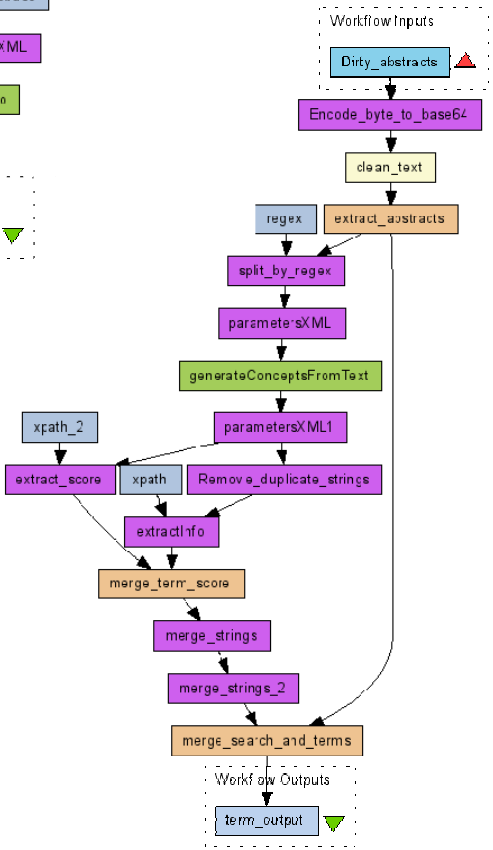












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To Sum Up ….

- Need for Genotype-Phenotype correlations with respect to disease control
- High-throughput data can provide links between Genotype and Phenotype
- Highlighted issues with manually conducted *in silico* experiments
- Improved the methods of current microarray and QTL based investigations through systematic nature
- Increased reproducibility of our methods
  - workflows stored in XML based schema
  - explicit declaration of services, parameters, and methods of data analysis
- Shown workflows are capable of deriving new biologically significant results
  - African Trypanosomiasis in the mouse
  - Infection of mice with *Trichuris muris*
- The workflows require expansion to accommodate new analysis techniques – text mining

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Many thanks to:











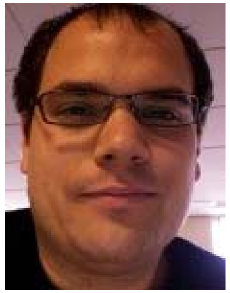

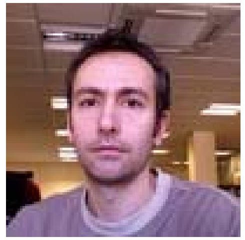
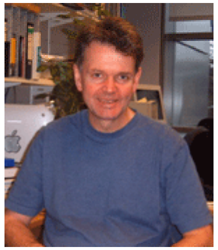






including: Joanne Pennock, EPSRC, OMII, myGrid, and lots more people