



Newton's ideas and methods are preserved forever: how about yours?

Marco Roos, Kristina Hettne, Jun Zhao, Mark Thompson

Cloud and Workflows for Reproducible
Bioinformatics

Shenzhen, December 19, 2012

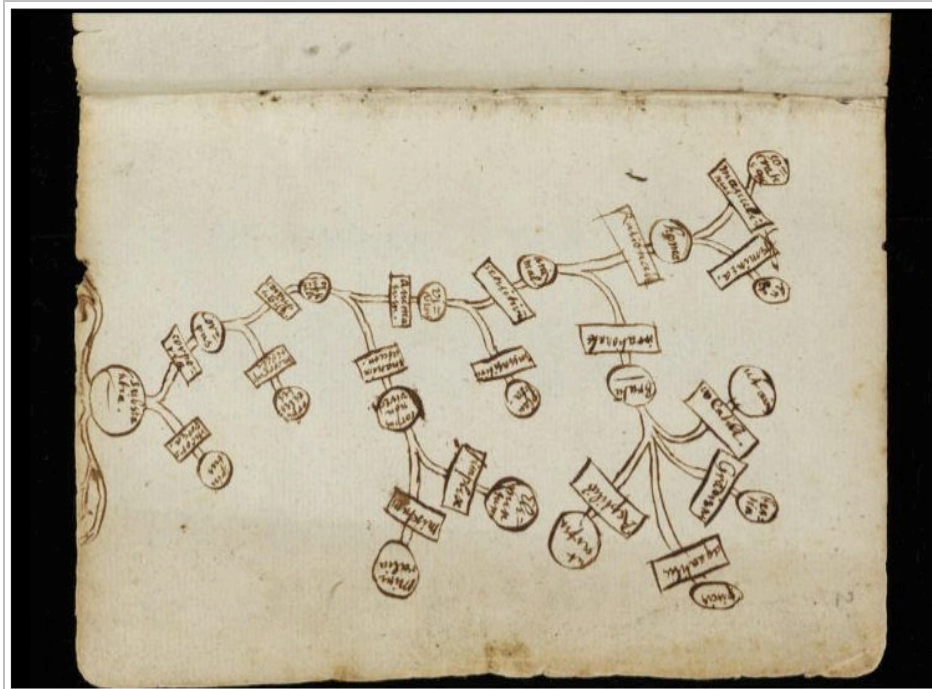


Newton's Books Scanned, Posted Online

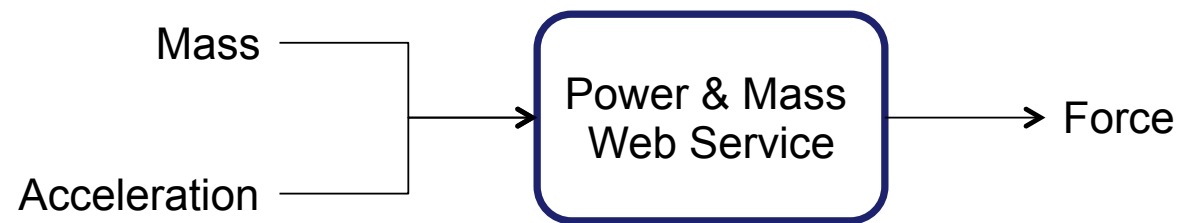
Officials at the Cambridge University, in the United Kingdom, announce that they recently made most of the books written by Sir Isaac Newton available online. These works, some of the most important scientific documents ever, are now available to the general public here. Newton was the Lucasian Ch... [[read more >>](#)]



Image comment: This book includes many notes from Isaac Newton's studies and, increasingly, his own explorations into mathematics, physics and metaphysics
Image credits: © Cambridge University Library

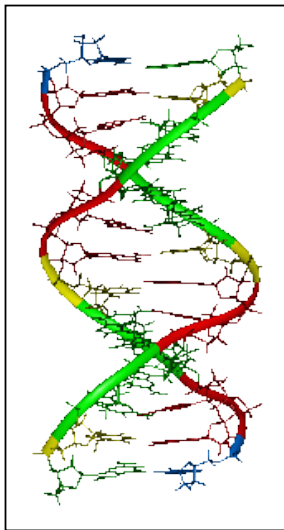
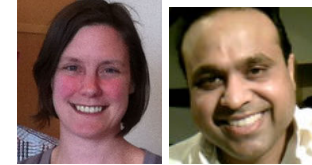


Reproduced workflows



Case study

Bioinformatics analysis of Metabolic Syndrome
Kristina Hettne, Harish Dharuri



Genome Wide Association Studies

What is the genetic basis for the diseases associated with Metabolic Syndrome?



Research article

Open Access

Mutant huntingtin activates Nrf2-responsive genes and impairs dopamine synthesis in a PC12 model of Huntington's disease

Abstract

Background: Huntington's disease is a progressive autosomal dominant neurodegenerative disorder that is caused by a CAG repeat expansion in the HD or Huntington's disease gene. Although micro array studies on patient and animal tissue provide valuable information, the primary effect of mutant huntingtin will inevitably be masked by secondary processes in advanced stages of the disease. Thus, cell models are instrumental to study early, direct effects of mutant huntingtin. mRNA changes were studied in an inducible PC12 model of Huntington's disease, before and after aggregates became visible, to identify groups of genes that could play a role in the early pathology of Huntington's disease.

Results: Before aggregation, up-regulation of gene expression predominated, while after aggregates became visible, down-regulation and up-regulation occurred to the same extent. After aggregates became visible there was a down-regulation of dopamine biosynthesis genes accompanied by down-regulation of dopamine levels in culture, indicating the utility of this model to identify functionally relevant pathways. Furthermore, genes of the anti-oxidant Nrf2-ARE pathway were up-regulated, possibly as a protective mechanism. In parallel, we discovered alterations in genes which may result in increased oxidative stress and damage.

Conclusion: Up-regulation of gene expression may be more important in HD pathology than previously appreciated. In addition, given the pathogenic impact of oxidative stress and neuroinflammation, the Nrf2-ARE signaling pathway constitutes a new attractive therapeutic target for HD.

Methods

Cell culture

Inducible rat PC12 cell lines expressing an exon 1 fragment of huntingtin with 23 (Q23) or 74 (Q74) glutamine repeats fused to the Green Fluorescent Protein (GFP), [11,12] were cultured in standard high glucose Dulbecco's modified Eagle's medium (DMEM, Invitrogen Life Technologies, Carlsbad, USA) supplemented with 100 U/ml penicillin/streptomycin (Invitrogen Life Technologies), 2 mM L-glutamine (Invitrogen Life Technologies), 10% heat-inactivated horse serum (Invitrogen Life Technologies), 5% Tet-approved heat inactivated fetal bovine serum (Clontech, Palo Alto, USA), 100 µg/ml G418 (Invitrogen Life Technologies) and 75 µg/ml hygromycin (Invitrogen Life Technologies) at 37°C and 10% CO₂. Cells were induced with 1 µg/ml doxycycline (dox, Clontech) and harvested on day 0 (uninduced cells), 1 day (when only a few cells expressing mutant huntingtin contain aggregates) and 5 days (when nearly all cells expressing mutant huntingtin contain aggregates) [12]. The same culture conditions were used for PC12 cells without a construct, to eliminate the effect of doxycycline treatment on gene expression.

Hybridization design

For each construct, we performed duplicate experiments with 2 independent cell lines for each construct (biological replicates). Furthermore, from each cell line, two separate RNA isolations were performed (technical replicates).

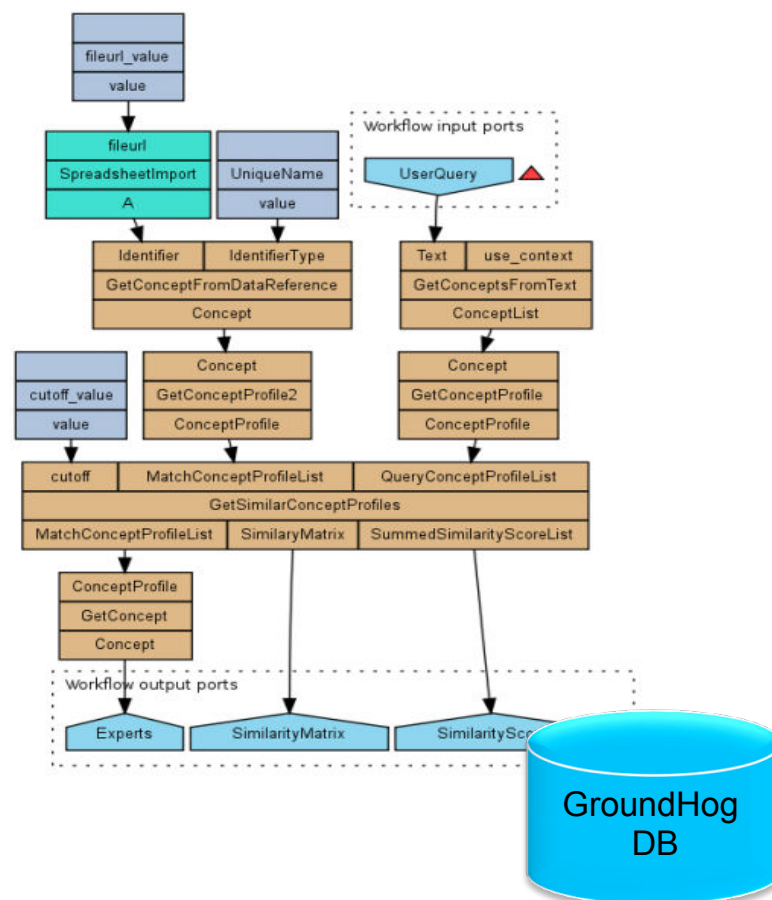
Preservation for the
wet laboratory
scientist

From Van Roon-Mom *et al.*, BMC Molecular Biology 2008
doi: 10.1186/1471-2199-9-84.

What is the digital equivalent?

Is it equally good?

Can we do better?
- or worse?



Reproduced from Jelier *et al.*, Schuemie *et al.*, Hettne *et al.*, Haagen *et al.*,
<http://biosemantics.org>, myExperiment.org/workflows/2197

What is our incentive?

Nobility

Good Reproducible Science



Greater Good

Serve the public



What is our incentive?

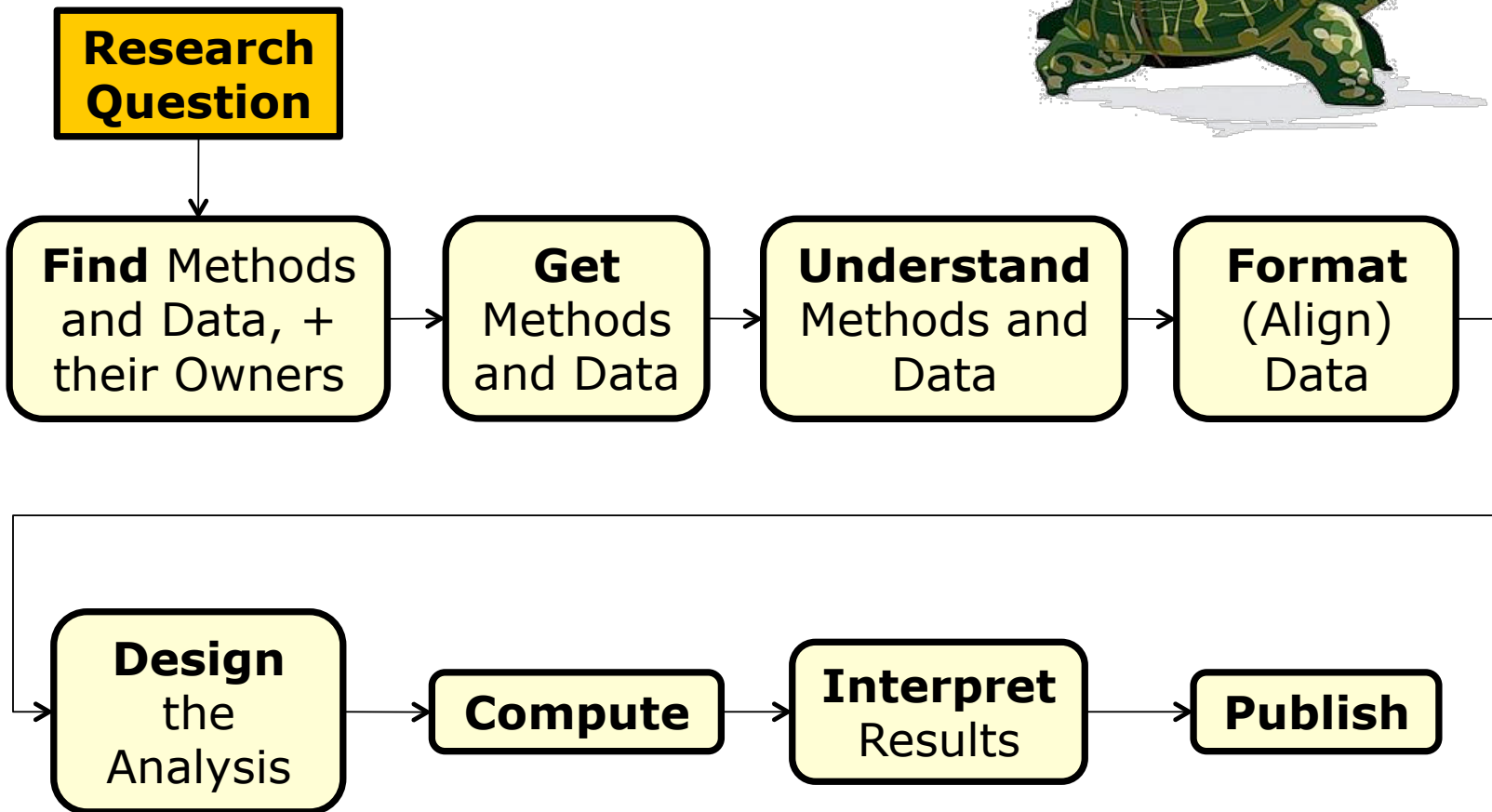
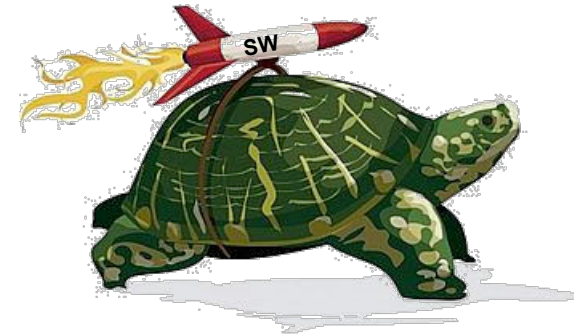
Fame and Glory
Getting on with it...



CHALLENGE

Stimulate preservation and
reproducibility while speeding up
the research process

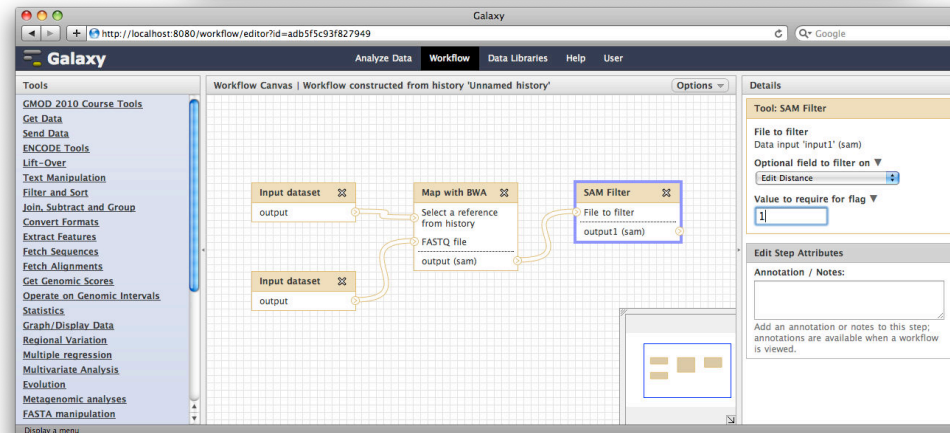
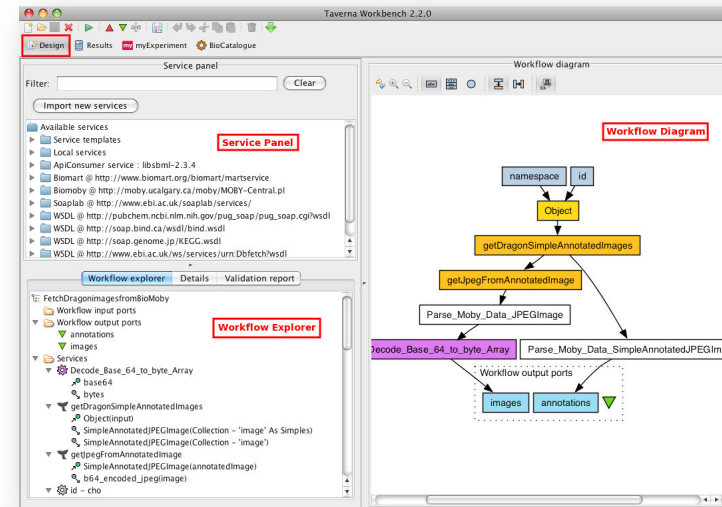
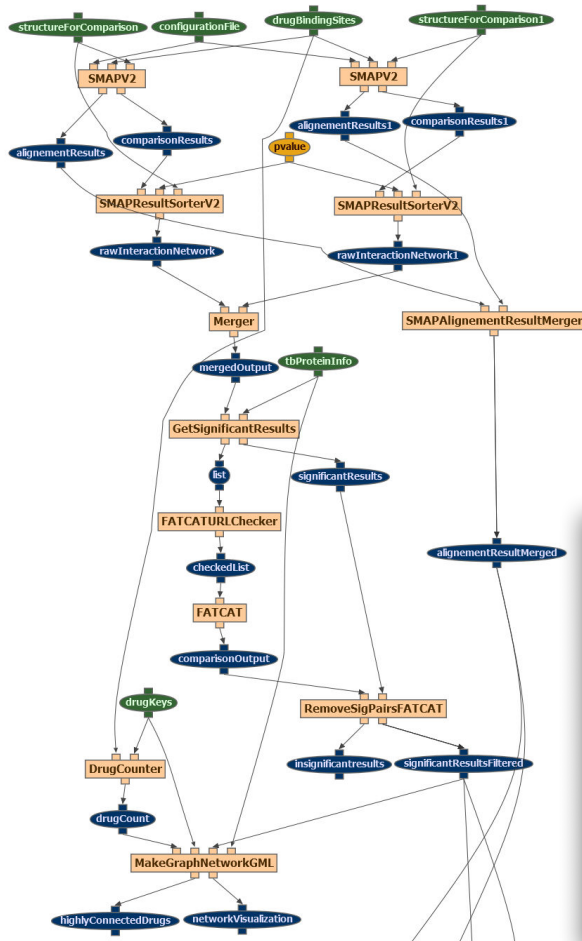
Enhance the research cycle What slows us down?



- Loosing track of what you did
- Messy storage
- Preparing material for a publication
- Understanding the computational procedure
- Communication with (non-technical) colleagues
- Keeping tools working
- Getting credit for digital results outside of traditional publications



Getting on with workflows



Wednesday, December 19, 2012

Towards preserving bioinformatics experiments

12





~~Monolithic Tool~~ →
Web Services → Workflows → (Web) Tool
Example: Anni 2.0 → Anni workflows

Galaxy / Netherlands | Analyze Data | Workflow | Shared Data | Visualization | Help | User | Using the

Tools | Options ▾

- NGS: Simulation
- SNP/WGA: Data: Filters
- SNP/WGA: QC: LD: Plots
- SNP/WGA: Statistical Models
- Human Genome Variation
- Genome Diversity
- VCF Tools
- NGS Taskforce: Hubrecht - Alignment tool benchmarking
- NGS Taskforce: WUR denovo benchmarking
- NGS Taskforce: LUMC - GAPSS v2
- NGS Taskforce: LUMC -

Options ▾

NCBI GI to Kegg Pathway Descriptions (version 1.0.0)

Select source for GI_numbers:

Type manually ▾

Enter GI_numbers:

215422380
1201101048

Would you also like the raw results as a zip file:

No ▴

Execute

History | Options ▾

0 bytes

Your history is empty. Click 'Get Data' on the left pane to start.

nbic | netherlands bioinformatics centre | **Workflow: AnniWF** | LUMC | Leiden University Medical Center | workflow by Reinout van Schouwen

Configure Workflow Inputs

Enter Concept IDs:

79822, 25362

Upload file? ☐

Execute

Workflow Description

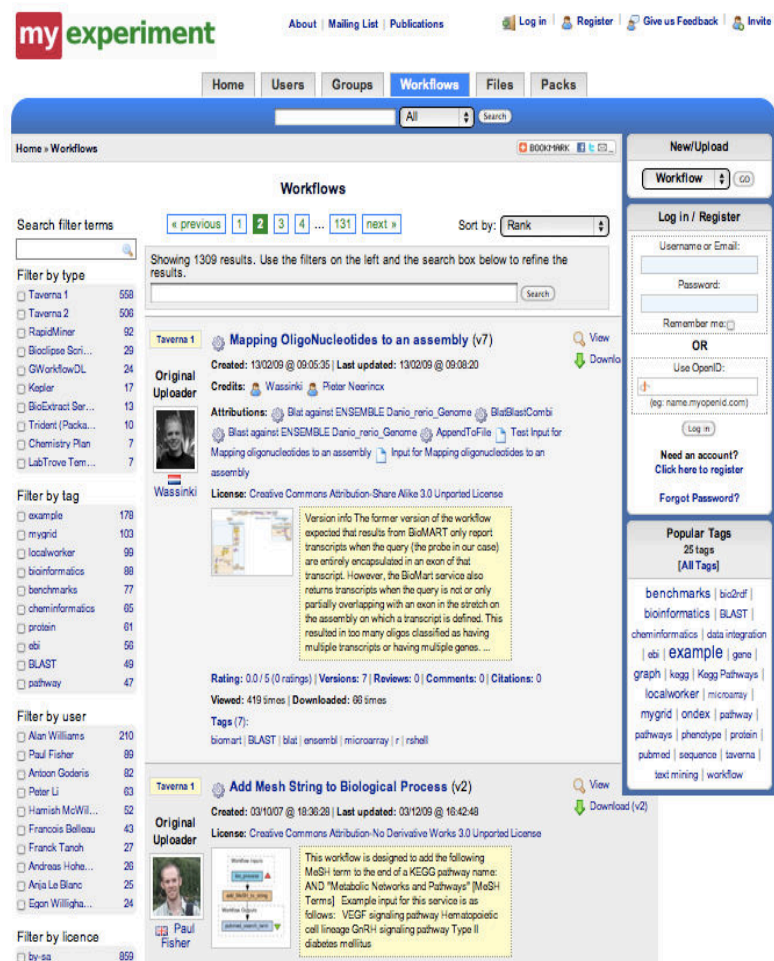
This workflow demonstrates the 'explain scores' functionality from Anni 2.1, made available as a web service.

<http://workflow.biosemantics.org/t2web/workflow/2725>



The recipes store

- Find workflows
- Share workflows & files
- Find people
- Build communities
- Publish packages
- Tag workflows
- Score, rate, comment



The screenshot shows the myExperiment website interface. At the top, there's a navigation bar with links like 'Home', 'Users', 'Groups', 'Workflows', 'Files', and 'Packs'. Below this, a search bar and a 'New/Upload' button are visible. The main content area is titled 'Workflows' and shows a list of workflows. On the left, there are filters for 'Filter by type' and 'Filter by tag'. The central part of the page displays a workflow titled 'Mapping OligoNucleotides to an assembly (v7)' by Wassinki. This workflow includes details such as its creation date (13/02/09), last update (13/02/09), and a description of its purpose: mapping oligonucleotides to an assembly. It also shows a version history, a license (Creative Commons Attribution-Share Alike 3.0 Unported License), and a list of tags (biomart, BLAST, blat, ensemble, microarray, r, rshall). Below this, another workflow titled 'Add Mesh String to Biological Process (v2)' by Paul Fisher is partially visible. On the right side, there's a sidebar with a 'Log in / Register' section and a 'Popular Tags' section listing various tags like 'benchmarks', 'bioinformatics', 'BLAST', etc.

Instructions for workflow authors

10 Best Practices for creating workflows

1. Make a sketch workflow
2. Use modules
3. Think about the output
4. Provide example inputs and outputs
5. Annotate
6. Test execution from outside local environment
7. Choose services carefully
8. Reuse existing workflows
9. Advertise
10. Maintain

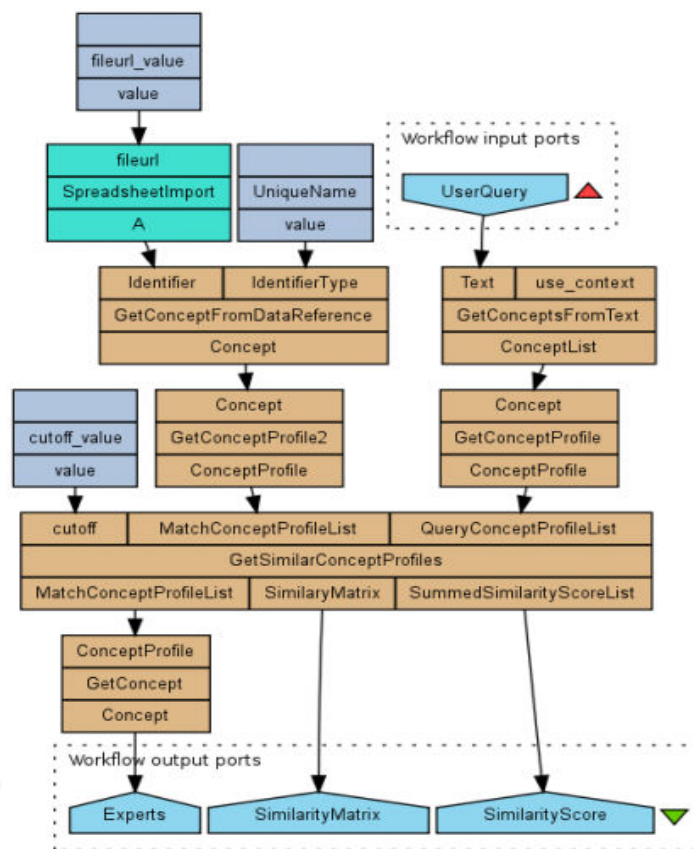


Reproducible Science Is a workflow sufficient?

Useful Preservation
=
Understandable Objects

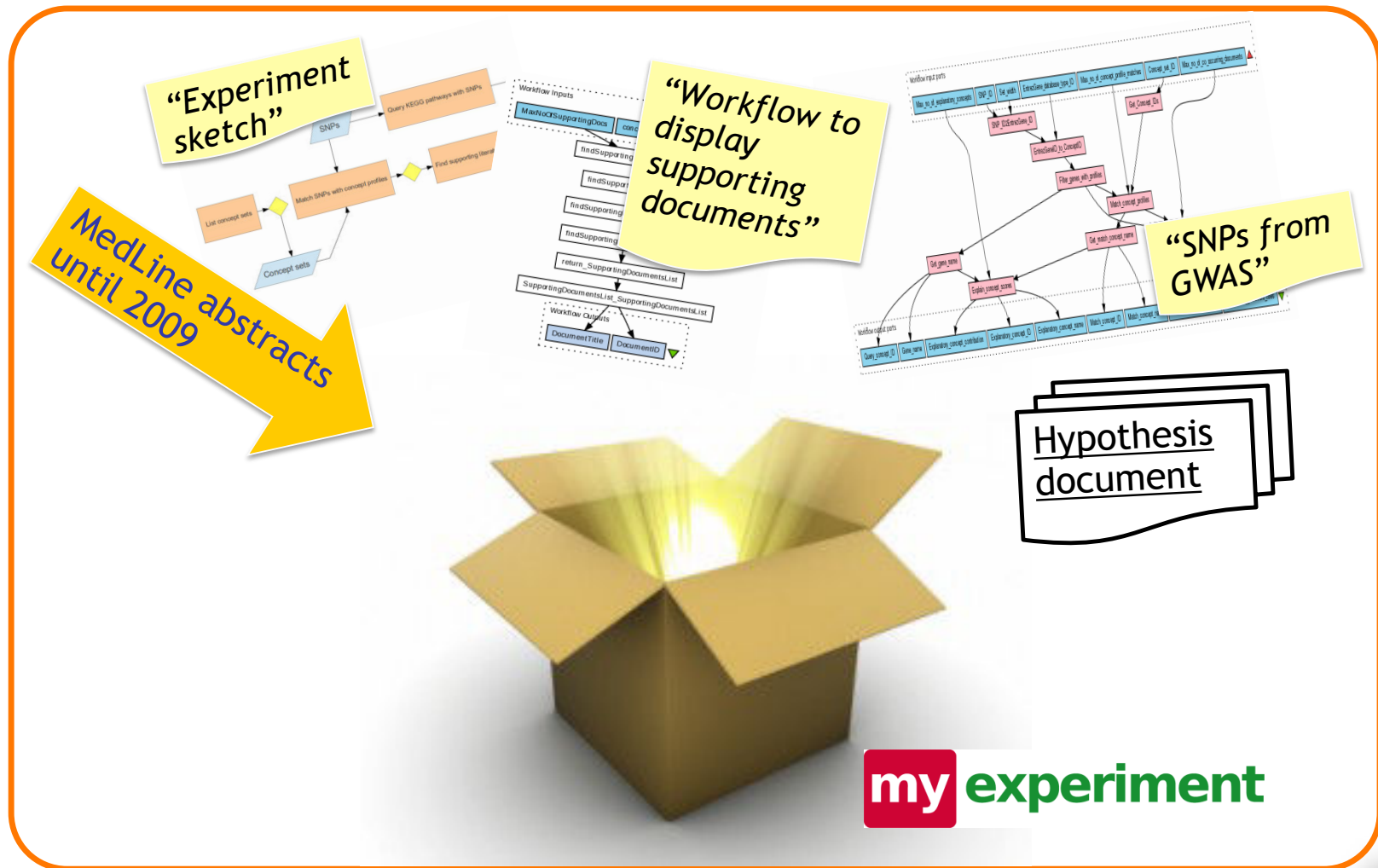
Reproduce, Reuse, Repurpose,
Repair, ...

What is this
doing?



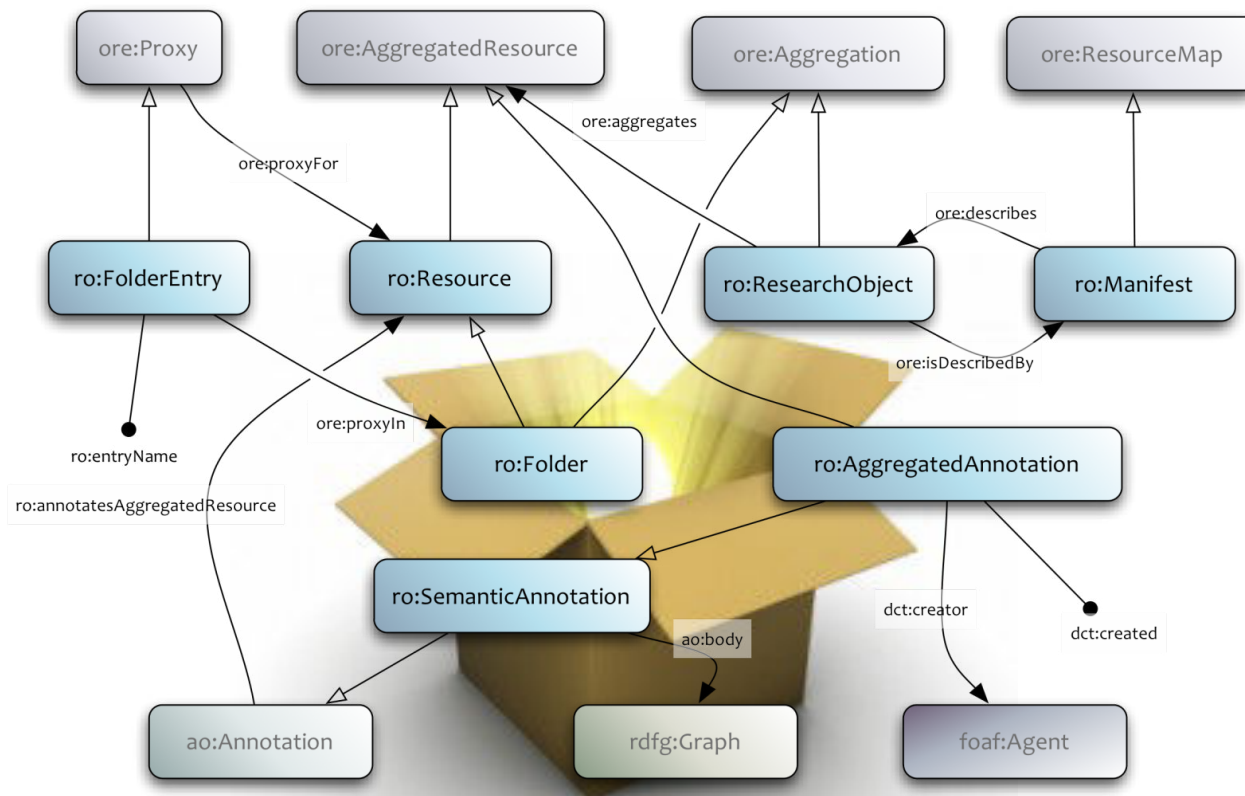
Reproduced from Jellier *et al.*, Schuemie *et al.*, Hettne *et al.*, Haagen *et al.*,
<http://biosemantics.org>, myExperiment.org/workflows/2197

Useful preservation 1 myExperiment Packs



Useful preservation Research Object Model

Research Object Model Aggregation and Annotation Model for Digital Methods



<http://wf4ever.github.com/ro/>

Research Object (RO) Model

RO = ORE + AO + vocabularies

Object Re-use and Exchange (OAI-ORE)

Describes aggregations of resources:
data, metadata, papers, *etc.*

Annotation Ontology (AO)

Associates RDF metadata descriptions with resources

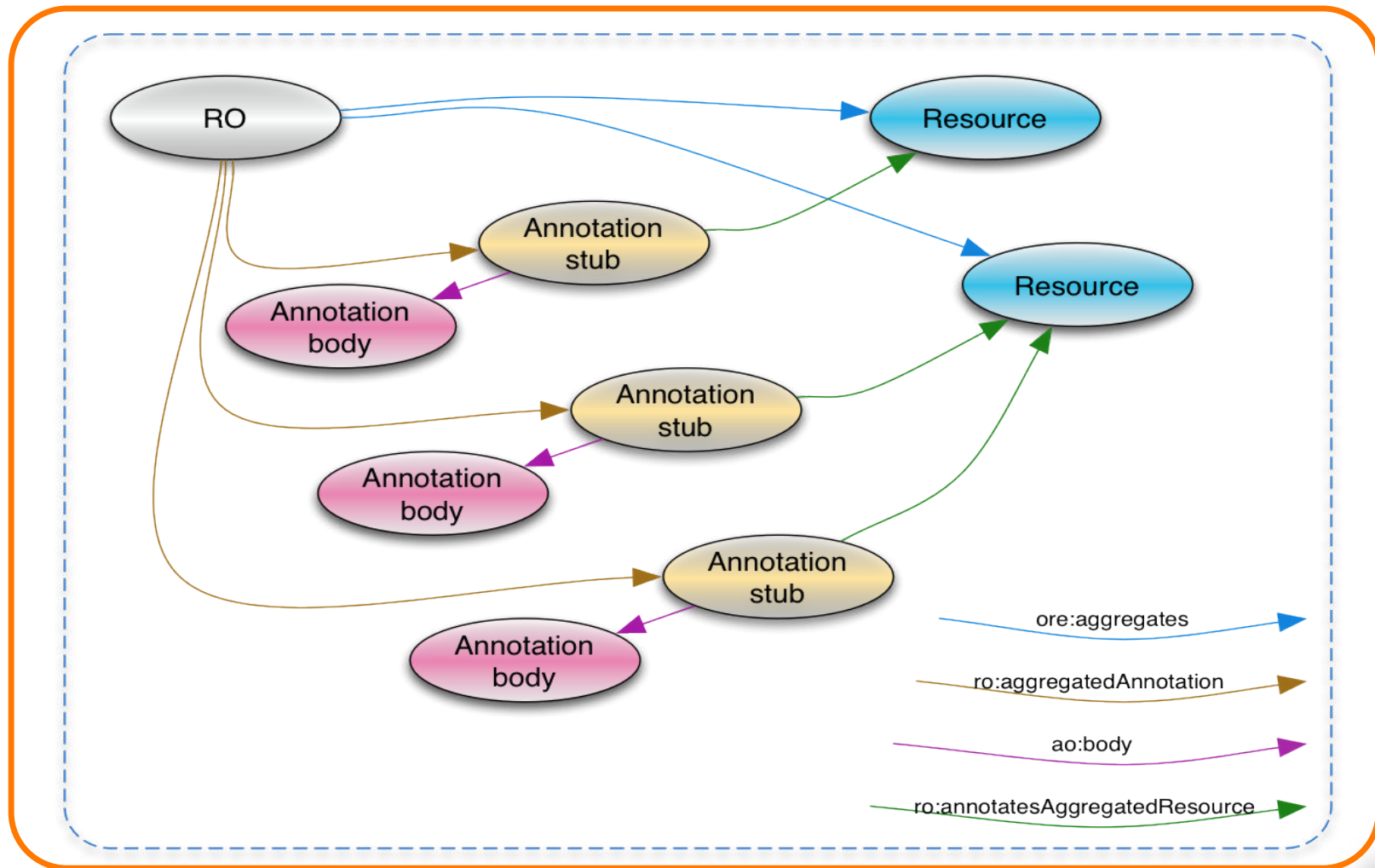
Generic and domain-specific vocabularies

Used in annotation bodies to provide information about
resources (types, dependencies, descriptions, *etc.*)

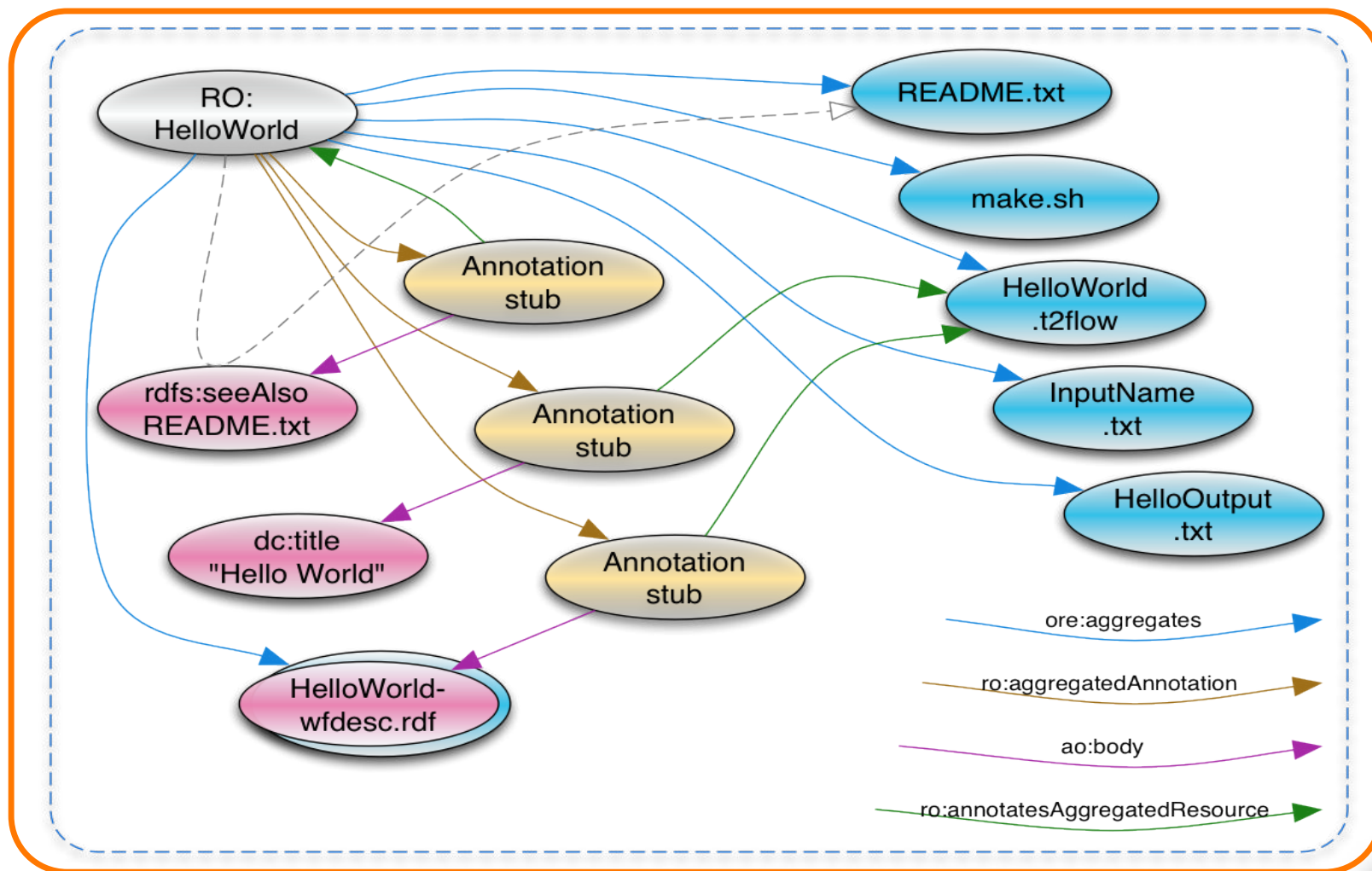
Builds on RDF, leading to RDF as a natural implementation choice

Model specification: <http://wf4ever.github.com/ro/>

Research Object Model



Research Object: "Hello World"

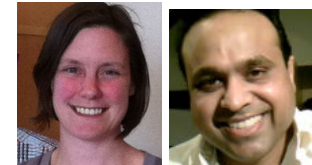


<https://github.com/wf4ever/ro-catalogue/tree/master/v0.1/HelloWorld>

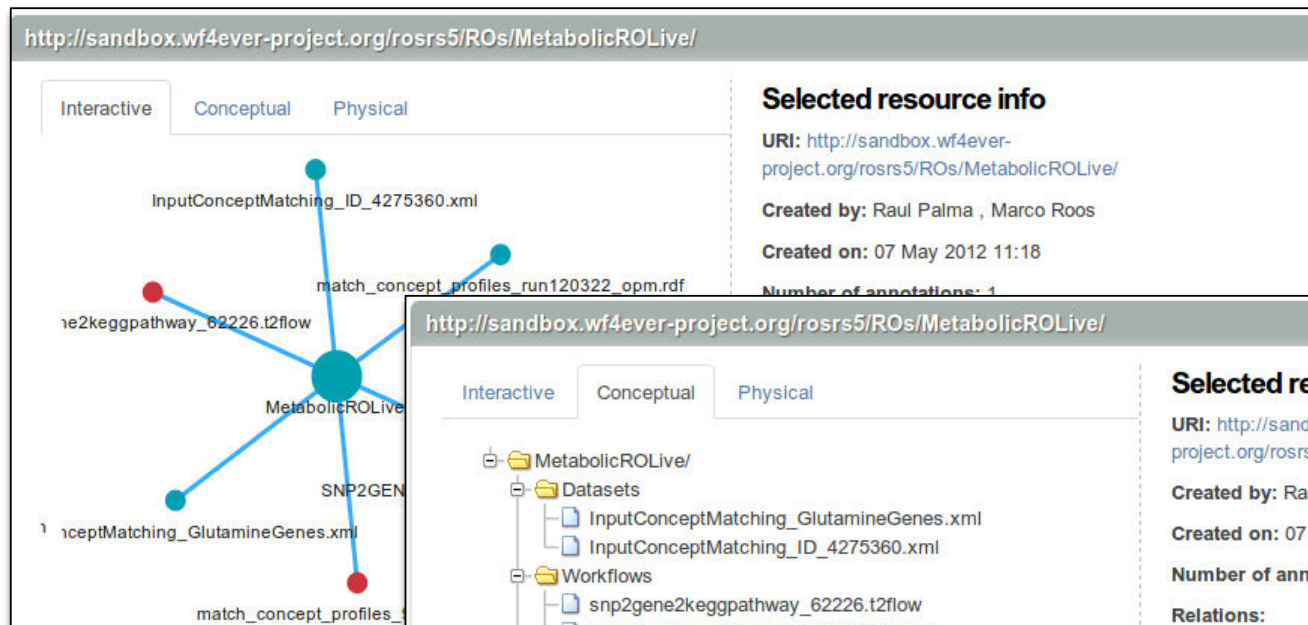


Help organize the materials and methods of computational analysis

Research Object Portal



*Materials & Methods of
Metabolic Syndrome
Analysis*
Kristina Hettne
Harish Dharuri



This screenshot shows the Research Object Portal interface with a file tree view. The tree structure is as follows:

- MetabolicROLive/
 - Datasets
 - InputConceptMatching_GlutamineGenes.xml
 - InputConceptMatching_ID_4275360.xml
 - Workflows
 - snp2gene2keggpathway_62226.t2flow
 - match_concept_profiles_986283.t2flow
 - SNP2GENE2KEGGPathway_run120322_opm.rdf
 - match_concept_profiles_run120322_opm.rdf

The right panel, titled 'Selected resource info', provides details for the selected resource: URI: <http://sandbox.wf4ever-project.org/rosrs5/ROs/MetabolicROLive/>, Created by: Raul Palma, Marco Roos, Created on: 07 May 2012 11:18, and Number of annotations: 1. Below this, the 'Relations' section shows that the resource aggregates 'InputConceptMatching_ID_4275360.xml', 'SNP2GENE2KEGGPathway_run120322_opm.rdf', and 4 more.

The 'Resource' section includes buttons for Add, Edit, Delete, and Download. The 'Research Object' section includes buttons for Download ZIP, Metadata, and Publish.

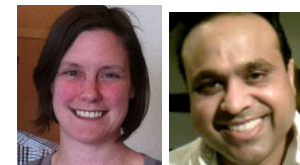
<http://sandbox.wf4ever-project.org/portal>

Research Objects inside!

- Packs more prominent
- Start a pack when you upload a workflow
- Upload wizards, pack management, export
- Checklists, automated star ratings
- Add workflow runs and example data
- Sticky annotations



RO-enabled myExperiment mockup



It was
me, me,
me!

What
I
found

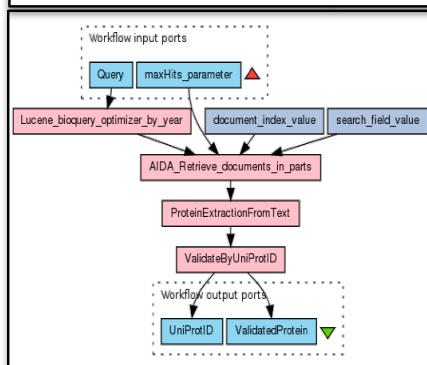
HDAC1 interacts with Parvb

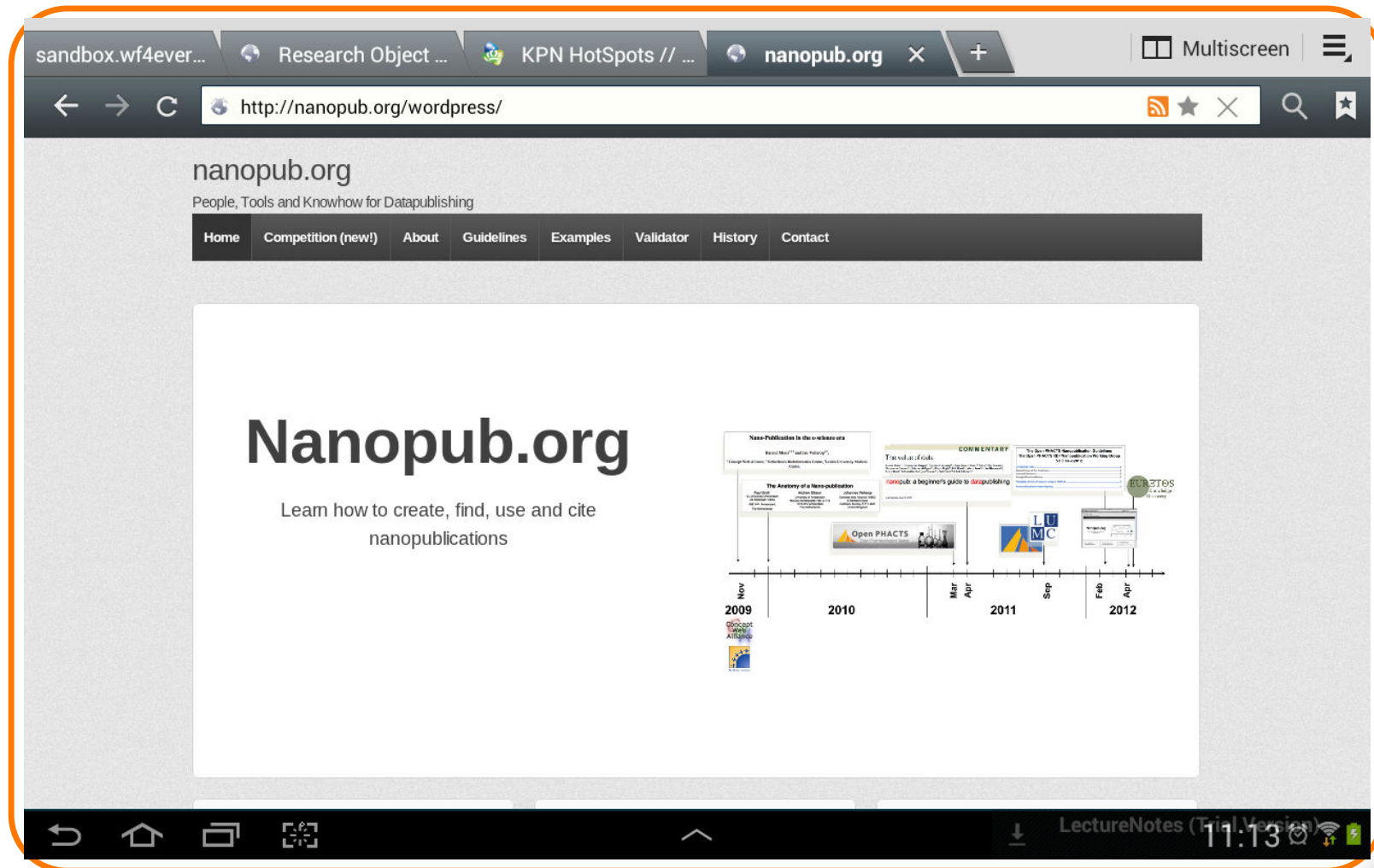
Discovered by: me
Published by: me



How I
found
it

Research Object





Examples

sandbox.wf4ever... Research Object ... KPN HotSpots // ... DNA varian... Multiscreen

← → ↻ http://nanopub.org/wordpress/?page_id=59

nanopub.org
People, Tools and Knowhow for Datapublishing

Home Competition (new!) About Guidelines Examples Validator History Contact

DNA variants of the dystrophin gene

Background

We plan to expose 2563 curated DNA variants of the dystrophin gene as nanopublications from the Leiden Open Variation Database.

Assertion

We are presently modeling the Assertion.

Attribution

Following the Open PHACTS Guidelines we plan to use a combination of dcterms and pav ontologies for assigning attribution. We will also list the nanopublication version, rights information and a link using the DOI to a research article that is the traditional method for citing LOVD data.

Following the OPS Guidelines, authors are listed using a Research ID and a Concept Wiki URI.

Supporting Evidence

We are presently modeling Supporting Provenance.

Details for this example

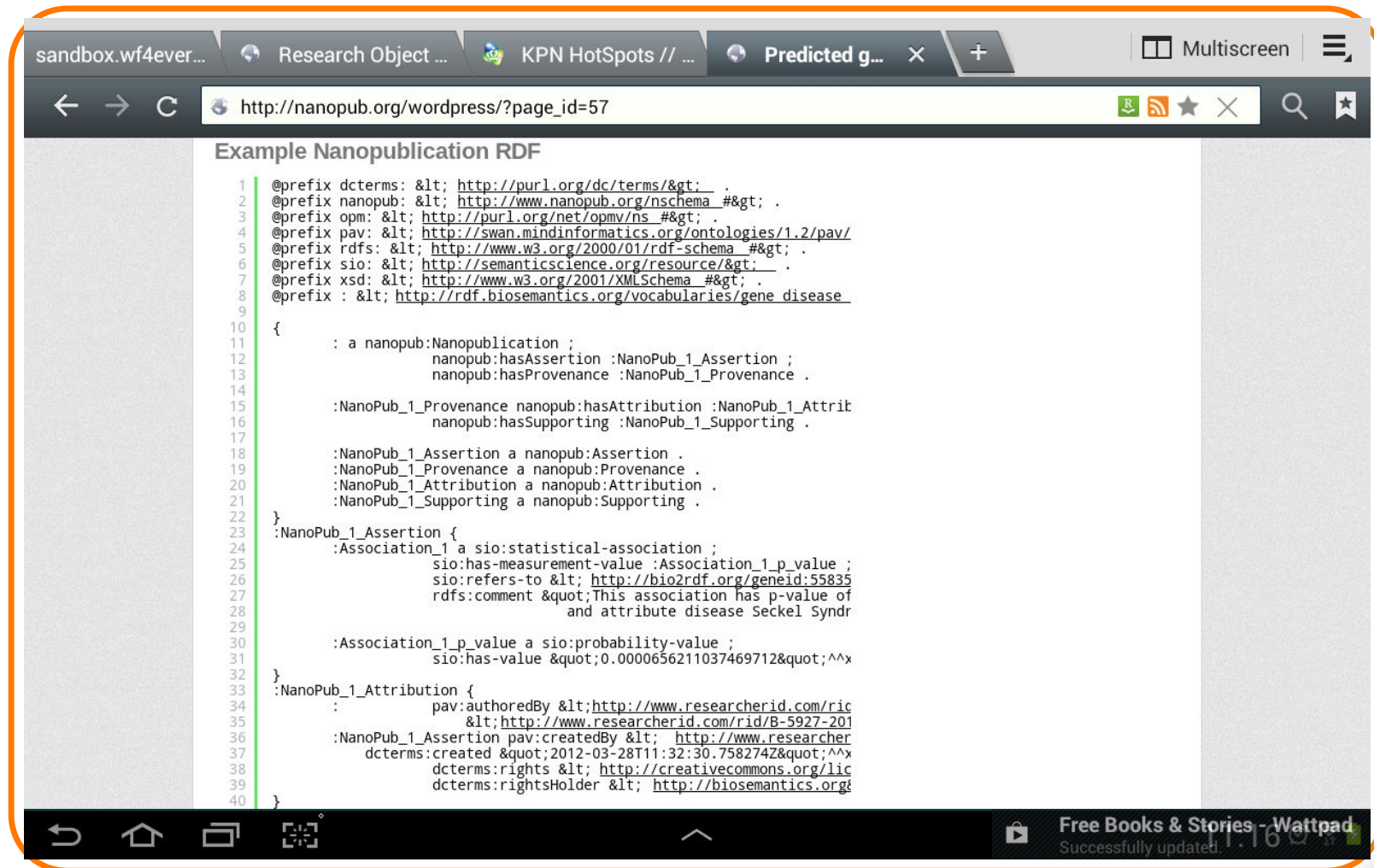
Creator: Zuotian Tatum
Author: Johan den Dunnen
Profile status: Coming soon
Acknowledgements:
http://www.dmd.nl/nmdb2/home.php?select_db=DMD
Access point to data: [here](#)

References:

Aartsma-Rus et al. (2006). Entries in the Leiden Duchenne muscular dystrophy mutation database: an overview of mutation types and paradoxical cases that confirm the reading-frame rule. Muscle Nerve. 34:135-144

ING Bankieren
Successfully updated.

Examples in RDF format



The screenshot shows a web browser window with the address bar displaying `http://nanopub.org/wordpress/?page_id=57`. The page title is "Example Nanopublication RDF". The content is an RDF Turtle document. The browser interface includes tabs for "sandbox.wf4ever...", "Research Object ...", "KPN HotSpots // ...", and "Predicted g...". The bottom of the browser shows a status bar with "Free Books & Stories - Wattpad" and "Successfully updated."

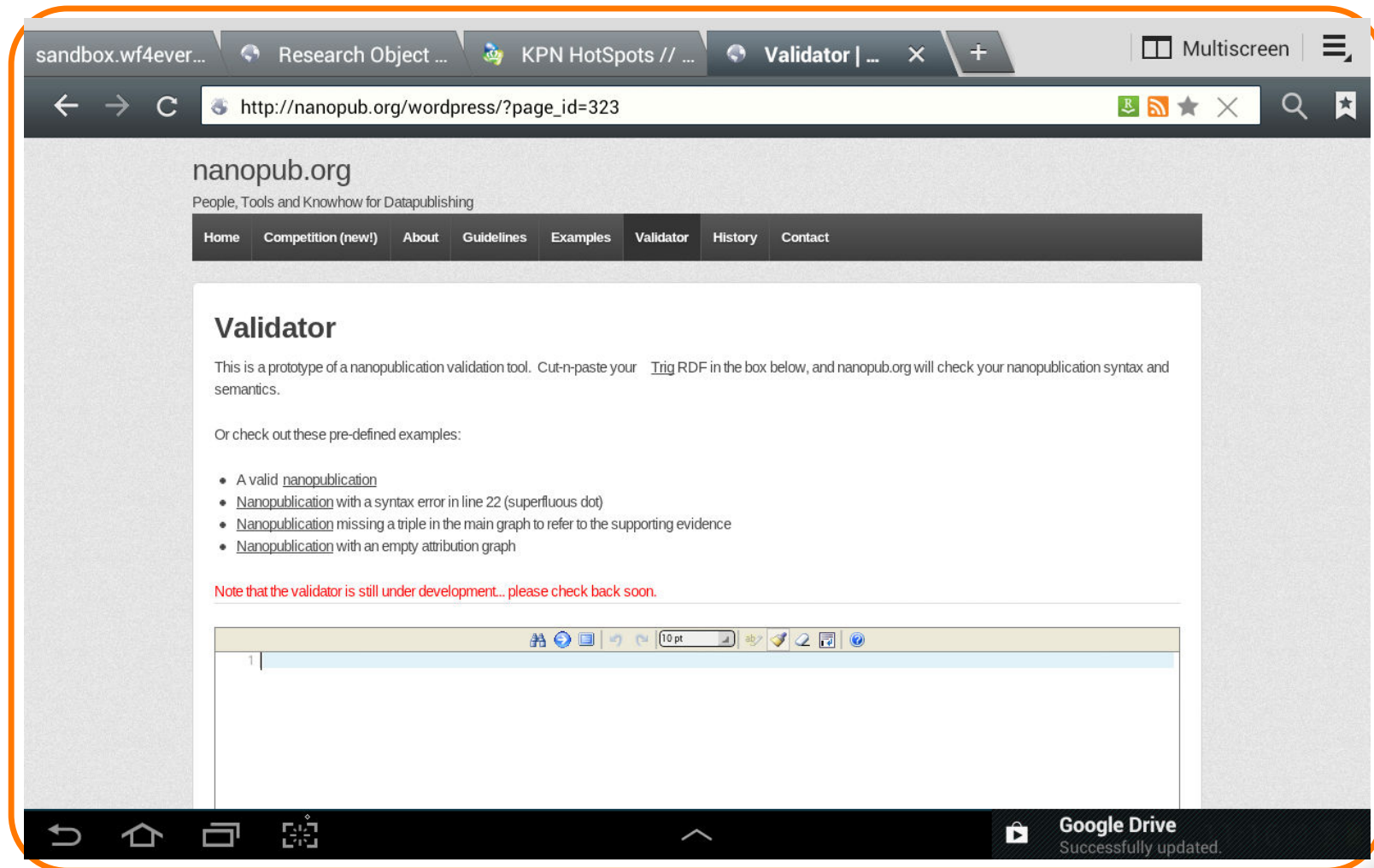
```

1  @prefix dcterms: <http://purl.org/dc/terms/> .
2  @prefix nanopub: <http://www.nanopub.org/nschema#> .
3  @prefix opm: <http://purl.org/net/opmv/ns#> .
4  @prefix pav: <http://swan.mindinformatics.org/ontologies/1.2/pav/> .
5  @prefix rdfs: <http://www.w3.org/2000/01/rdf-schema#> .
6  @prefix sio: <http://semanticscience.org/resource/> .
7  @prefix xsd: <http://www.w3.org/2001/XMLSchema#> .
8  @prefix : <http://rdf.biosemantics.org/vocabularies/gene_disease#> .
9
10 {
11   : a nanopub:Nanopublication ;
12     nanopub:hasAssertion :NanoPub_1_Assertion ;
13     nanopub:hasProvenance :NanoPub_1_Provenance .
14
15   :NanoPub_1_Provenance nanopub:hasAttribution :NanoPub_1_Attribution ;
16     nanopub:hasSupporting :NanoPub_1_Supporting .
17
18   :NanoPub_1_Assertion a nanopub:Assertion .
19   :NanoPub_1_Provenance a nanopub:Provenance .
20   :NanoPub_1_Attribution a nanopub:Attribution .
21   :NanoPub_1_Supporting a nanopub:Supporting .
22 }
23 :NanoPub_1_Assertion {
24   :Association_1 a sio:statistical-association ;
25     sio:has-measurement-value :Association_1_p_value ;
26     sio:refers-to <http://bio2rdf.org/geneid:55835> .
27     rdfs:comment "This association has p-value of
28                 and attribute disease Seckel Syndr
29
30   :Association_1_p_value a sio:probability-value ;
31     sio:has-value "0.0000656211037469712"^^xsd:float .
32 }
33 :NanoPub_1_Attribution {
34   : pav:authoredBy <http://www.researcherid.com/ric/>
35     <http://www.researcherid.com/rid/B-5927-201/> .
36   :NanoPub_1_Assertion pav:createdBy <http://www.researcherid.com/rid/B-5927-201/> .
37   dcterms:created "2012-03-28T11:32:30.758274Z"^^xsd:dateTime .
38   dcterms:rights <http://creativecommons.org/licenses/by/4.0/> .
39   dcterms:rightsHolder <http://biosemantics.org/> .
40 }


```




Validator



Example: LOVD


Leiden Muscular Dystrophy pages
Duchenne Muscular Dystrophy (DMD)
 Curator: [Johan den Dunnen](#)

[Home](#)
[Variants](#)
[Submitters](#)
[Submit](#)
[Documentation](#)

[View unique variants](#)
[Search unique variants](#)
[View all contents](#)
[Full database search](#)
[Variant listing based on patient origin](#)
[Database statistics](#)
[Switch gene](#)

When referring to this database please cite [Aartsma-Rus et al. \(2006\). Entries in the Leiden Duchenne muscular dystrophy mutation database: a](#)
NOTE: for deletions / duplications go to the [DMD database for whole exon changes](#).

LOVD - Variant listings

[Unhide all columns](#)
[Hide Specific Columns](#)
[Hide all columns](#)

[About this overview](#)
[\[Show\]](#)

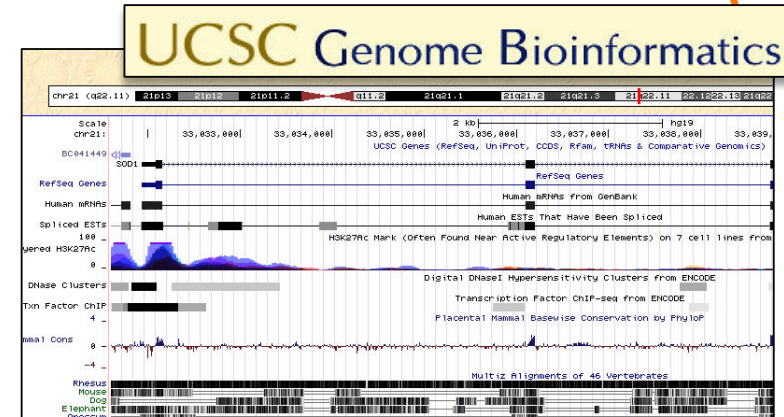
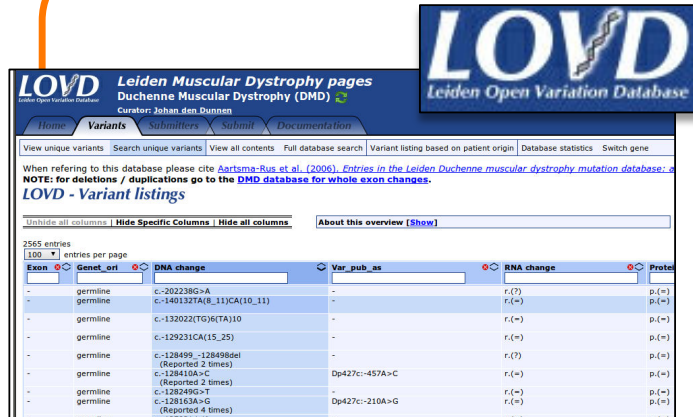
2565 entries
 100 entries per page

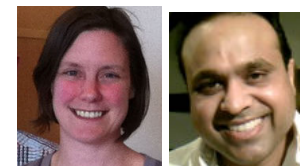
Exon	Genet_ori	DNA change	Var_pub_as	RNA change	Protein
-	germline	c.-202238G>A	-	r.(?)	p.(=)
-	germline	c.-140132TA(8_11)CA(10_11)	-	r.(=)	p.(=)
-	germline	c.-132022(TG)6(TA)10	-	r.(=)	p.(=)
-	germline	c.-129231CA(15_25)	-	r.(=)	p.(=)
-	germline	c.-128499_-128498del (Reported 2 times)	-	r.(?)	p.(=)
-	germline	c.-128410A>C (Reported 2 times)	Dp427c:-457A>C	r.(=)	p.(=)
-	germline	c.-128249G>T	-	r.(=)	p.(=)
-	germline	c.-128163A>G (Reported 4 times)	Dp427c:-210A>G	r.(=)	p.(=)

Nanopublications of Genetic Variations visualized on the genome



Zuotian Tatum, Jesse van Dam





It was
me, me,
me!

What
I
found

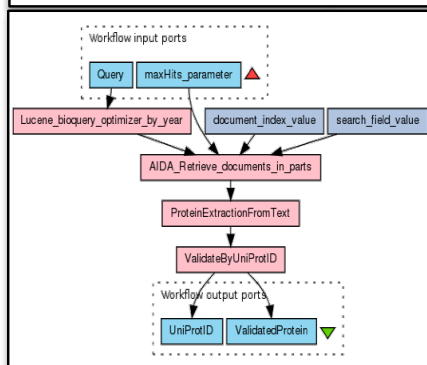


Nanopublication

<CS7183> <associatedWith> <MetS>

Discovered by: **me**
Published by: **me**

Research Object



How I
found
it

References

1. Knudson AG (1971) Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci USA 68: 820-823.
2. Varmus HE (1984) The molecular genetics of cellular oncogenes. Ann Rev Genet 18: 553-612.
3. Ruivenkamp CA, van Wezel T, Zanoni C, Stassen AP, Vlcek C, et al. (2002) Ptpn22 is a candidate for the mouse colon-cancer susceptibility locus Sccl and is frequently deleted in human cancers. Nat Genet 31: 295-298.
4. Nagase H, Mao JH, Balmain A (2003) The mouse colon-cancer susceptibility locus Sccl and is frequently deleted in human cancers. Nat Genet 31: 295-298.

<http://purl.org/nanopub/123>
<http://purl.org/ResObj/345>

Summary (1/2)

- Preservation under the hood of digital research tools
 - Research Object Model: annotated aggregates
 - Nanopublication: fine-grained digital credit
- Check Nanopub.org to stay updated**

Summary (2/2)

- Semantic Web for exchange and interoperability
- In progress: RO-enabling myExperiment
Watch myExperiment.org in 2013!
- Plans to RO-enable Taverna, Galaxy, GenomeSpace



Acknowledgements



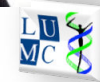
EU Wf4Ever project (270129)
funded under EU FP7 (ICT- 2009.4.1).
(<http://www.wf4ever-project.org>)



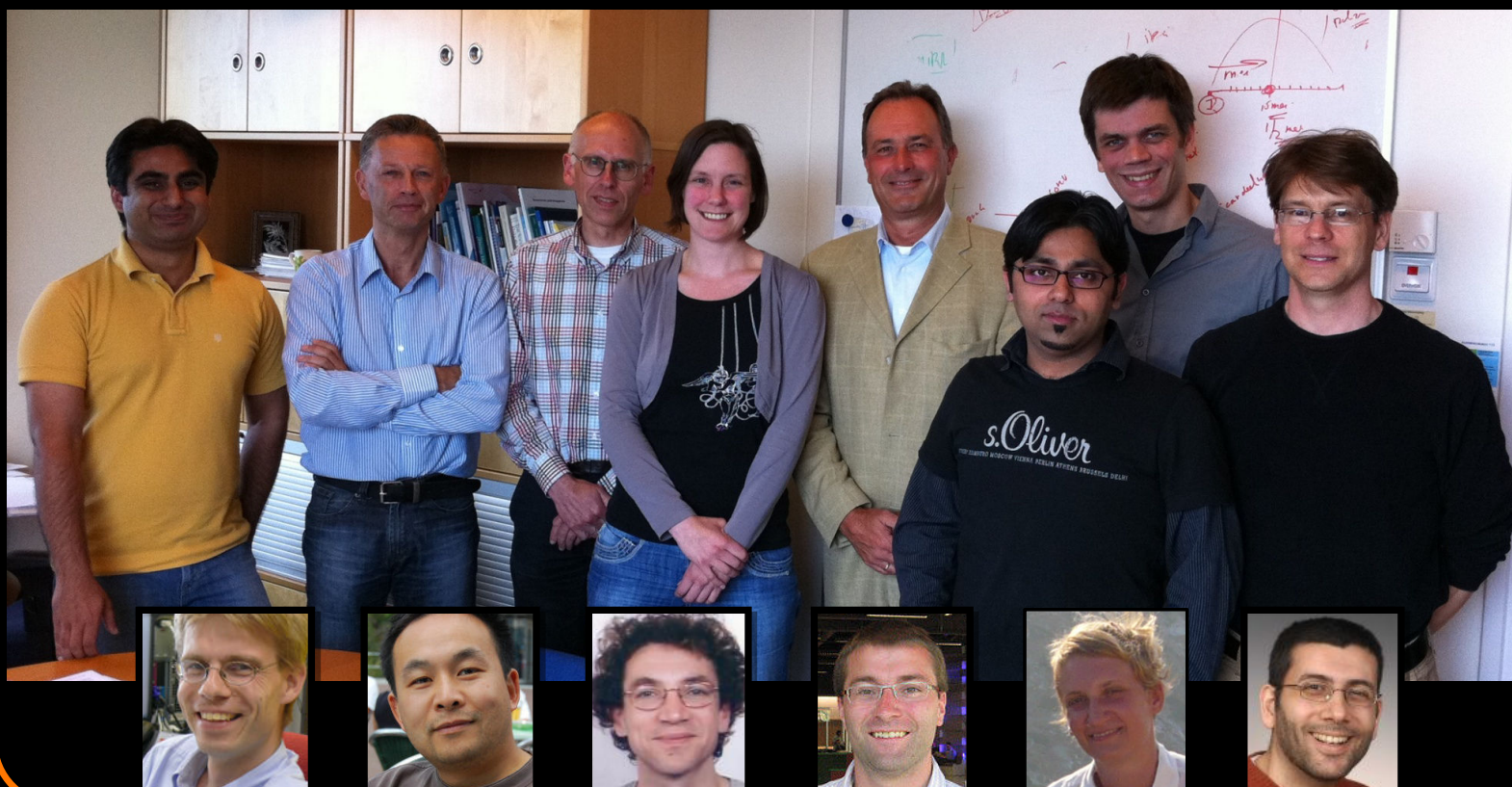
(GIGA)ⁿ
SCIENCE

35

nbic



Thank you for your attention



<http://biosemantics.org>

Preserved materials and methods for the 'wet laboratory' scientist

Research article

Open Access

Mutant huntingtin activates Nrf2-responsive genes and impairs dopamine synthesis in a PC12 model of Huntington's disease

Willeke MC van Roon-Mom^{*1}, Barry A Pepers^{1,2}, Peter AC 't Hoen¹, Carola ACM Verwijmeren¹, Johan T den Dunnen^{1,3}, Josephine C Dorschner¹ and GertJan B van Ommen¹

Methods

Cell culture

Inducible rat PC12 cell lines expressing an exon 1 fragment of huntingtin with 23 (Q23) or 74 (Q74) glutamine repeats fused to the Green Fluorescent Protein (GFP), [11,12] were cultured in standard high glucose Dulbecco's modified Eagle's medium (DMEM, Invitrogen Life Technologies, Carlsbad, USA) supplemented with 100 U/ml penicillin/streptomycin (Invitrogen Life Technologies), 2 mM L-glutamine (Invitrogen Life Technologies), 10% heat-inactivated horse serum (Invitrogen Life Technologies), 5% Tet-approved heat inactivated fetal bovine serum (Clontech, Palo Alto, USA), 100 µg/ml G418 (Invitrogen Life Technologies) and 75 µg/ml hygromycin (Invitrogen Life Technologies) at 37°C and 10% CO₂. Cells were induced with 1 µg/ml doxycycline (dox, Clontech) and harvested on day 0 (uninduced cells), 1 day (when only a few cells expressing mutant huntingtin contain aggregates) and 5 days (when nearly all cells expressing mutant huntingtin contain aggregates) [12]. The same culture conditions were used for PC12 cells without a construct, to eliminate the effect of doxycycline treatment on gene expression.

Hybridization design

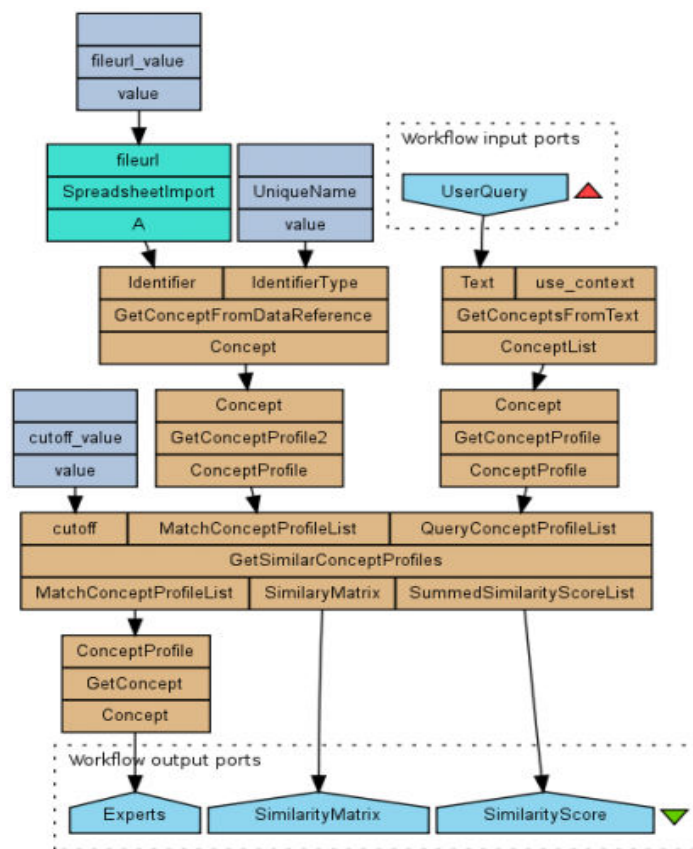
For each construct, we performed duplicate experiments with 2 independent cell lines for each construct (biological replicates). Furthermore, from each cell line, two separate RNA isolations were performed (technical replicates).

From Van Roon-Mom *et al.*, BMC Molecular Biology 2008
doi: 10.1186/1471-2199-9-84.

What is the digital equivalent?

Is it equally good?

Can we do better?
- or worse?



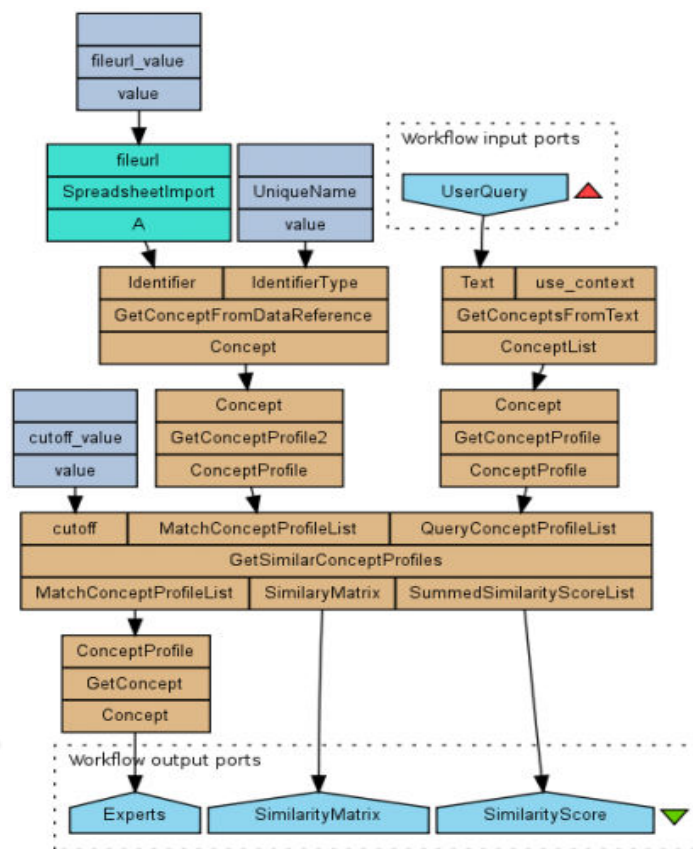
Reproduced from Jelier *et al.*, Schuemie *et al.*, Hettne *et al.*, Haagen *et al.*,
<http://biosemantics.org>, myExperiment.org/workflows/2197

What is the digital equivalent?

Is it equally good?

Can we do better?
- or worse?

Can you tell what this is doing?



Reproduced from Jelier *et al.*, Schuemie *et al.*, Hettne *et al.*, Haagen *et al.*,
<http://biosemantics.org>, myExperiment.org/workflows/2197

What is our incentive?

Nobility

Good Reproducible Science



Greater Good

Serve the public



What is our incentive?

Fame and Glory
Getting on with it...

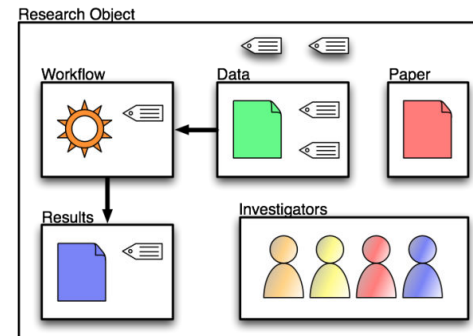
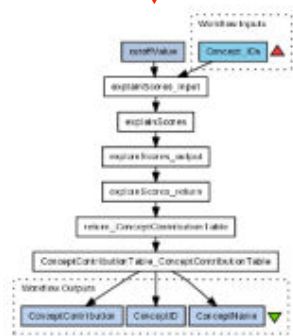


‘Useful’ preservation

**Support reproducibility
in tools and by guidelines that
speed up your research
get you acknowledgement**

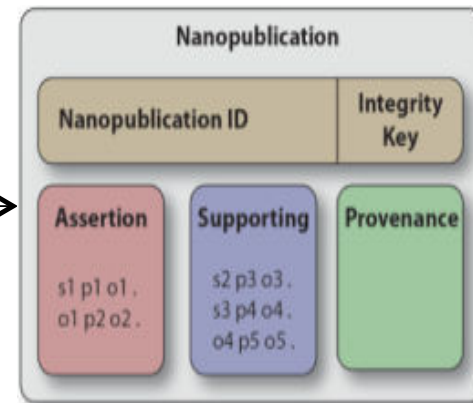


Preservation



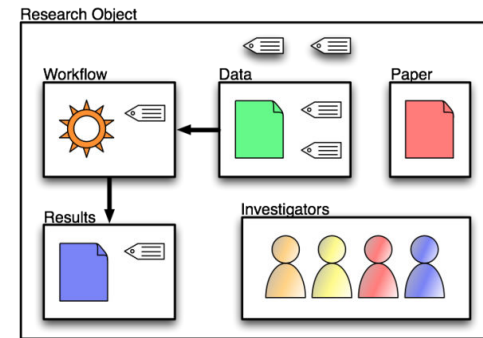
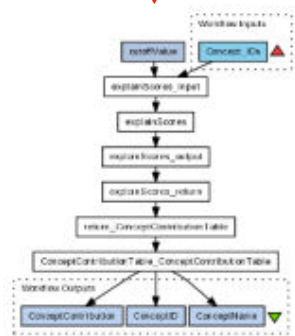
What?

How?





Preservation

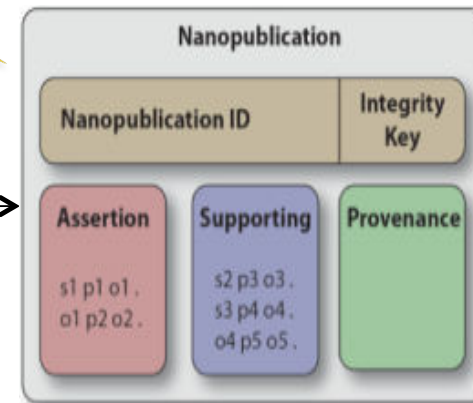
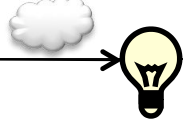


**Valuable
for
scientists**

**Digital
Value**

What?

How?



Acknowledgements

<http://biosemantics.org/>



- Erik Schultes
- Andrew Gibson
- Reinout van Schouwen
- Kostas Karasavvas
- Kristina Hettne
- Harish Dharuri
- Eleni Mina
- Jesse van Dam
- Herman van Haagen
- Zuotian Tatum
- Johan den Dunnen
- Peter-Bram 't Hoen
- Barend Mons
- Gert-Jan van Ommen



- Paul Groth
 - Frank van Harmelen
- 
- Erik van Mulligen
 - Bharat Singh
 - Jan Kors



- Christine Chichester
- Kees Burger - NBIC
- Spyros Kotoulas - VU
- Antonis Loizou - VU
- Valery Tkachenko - RSC
- Andra Waagmeester - Maastricht
- Sune Askjaer - Lundbeck
- Steve Pettifer - Manchester
- Lee Harland - Pfizer/CD
- Carina Haupt - Fraunhofer
- Colin Batchelor - RSC
- Miguel Vazquez - CNIO
- José María Fernández - CNIO
- Jahn Saito - Maastricht
- Andrew Gibson (Outside Expert) - Amsterdam
- Louis Wich - DTU



Netherlands
Bioinformatics
Centre



Melton
Foundation

