Galaxy in Plant Pathology: Not everything is NGS data

Peter Cock & Leighton Pritchard
Galaxy Community Conference
Lunteren, The Netherlands
25 May 2011



JHI Plant Pathology



- We work on a range of organisms
 - Plant Viruses
 - Bacteria
 - Oomycetes
 - Fungi
 - Nematodes
 - Aphids (as virus vectors)
- Many genome sequences now available



JHI Dundee site, formerly SCRI (Scottish Crop Research Institute)

Common themes – e.g. Effectors



- I will use "effector" to mean a pathogen produced protein which in some way manipulates the host plant
- The details depend on the type of organism, but we want to identify effector genes, e.g.
 - Similarity to known effectors (e.g. with BLAST)
 - Signal peptides
 - Localization signals
 - Possible horizontal gene transfer (e.g. different GC%)
- Part of larger task of automated gene annotation, e.g.
 - HMMER or RPS-BLAST domain searches

Why Galaxy?



- Hi Peter, could you run a big BLAST job for me?
 - Everyone using standalone BLAST is not practical
 - Want local BLAST web interface with multiple-query support
- Group XXX have just published the YYY genome could you look for ZZZ proteins please?
 - With a suitable interface, lots of analyses are simple enough for non-bioinformaticians to run and interpret
- You remember that analysis we did last year? I want to do it again on this new genome
 - Running old scripts on new data is tedious
 - Workflows should be reproducible

Why Galaxy?



- Could you run tool XXXX on this data please?
 - Getting the tool:
 - Many tools are Unix/Linux only (mostly Windows at JHI)
 - Running the tool:
 - Most tools lack any GUI or web interface
 - Using the results:
 - Many tools produce their own output formats
- So, we run it via Galaxy instead

Why Galaxy?



- Plus points for us:
 - Don't have to worry about local software installation
 - Uniform web based GUI for wrapped tools
 - Coupling tools together as sharable repeatable workflows
 - Sharing the same data version (better than email/shared drives)
 - Open Source (extendable, free)
 - Almost any tool can be added

Downsides:

- Missing tools (have to invest time wrapping them)
- Bugs in Galaxy (both for end users and tool wrapping)
- Investment in training users

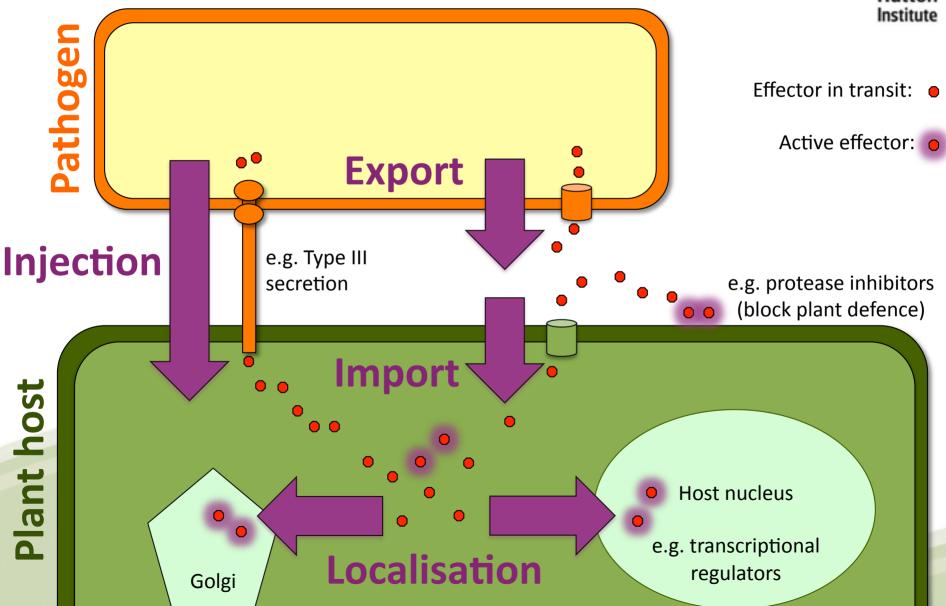
JHI Plant Pathology Server



- Dedicated Linux server (16 core, 32 GB RAM)
- Wiki (general and Lab specific)
- GBrowse (Genome browser)
- Local BLAST databases (wwwblast)
- Galaxy
 - PostgreSQL
 - Python 2.6 (not CentOS provided Python 2.4)
- Compute cluster (not used yet due to firewall issues)

Effector Protein Analysis





Protein Analysis Tools in our Galaxy



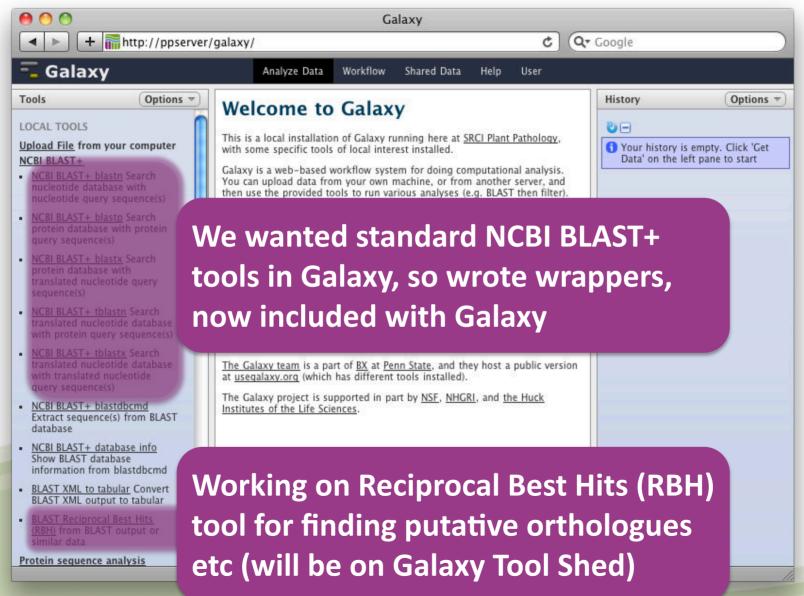
All take a FASTA protein file as input, return a tabular file.

- Sequence similarity
 - BLAST
- Transmembrane domains
 - TMHMM
- Signal Peptides/Motifs
 - SignalP
 - EffectiveT3
 - RXLR

- Nuclear Localisation
 - PredictNLS
 - NLStradamus
- Nucleolus Localisation
 - NoD
- Sub-cellular Localisation
 - PSORTB
 - WoLF PSORT

NCBI BLAST+





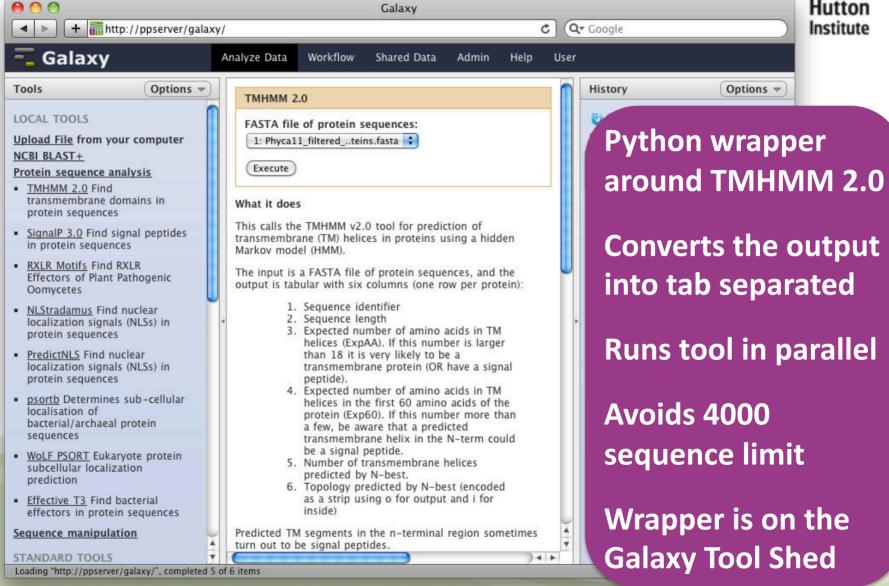
Protein Sequence analysis tools





Transmembrane Domains (TMHMM)

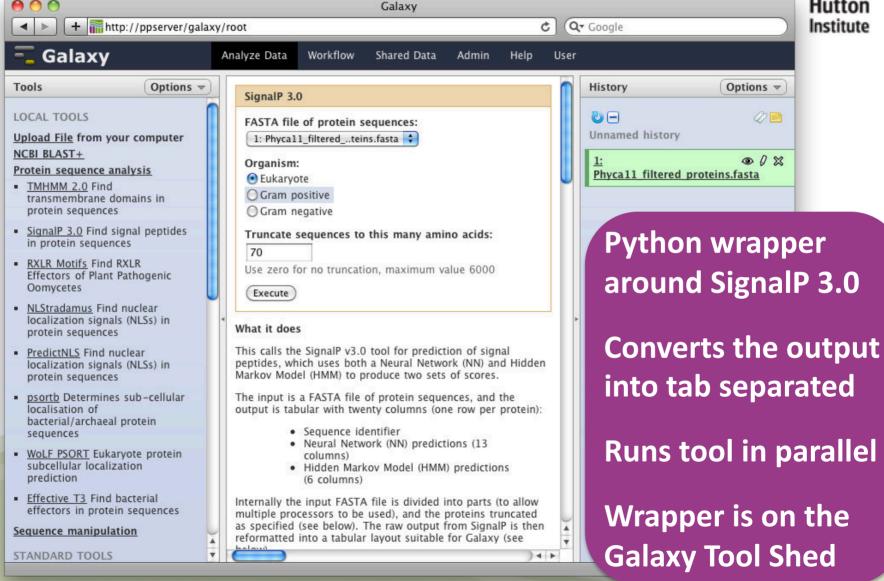




http://www.cbs.dtu.dk/services/TMHMM/ - Sonnhammer et al. (1998), Krogh et al. (2001)

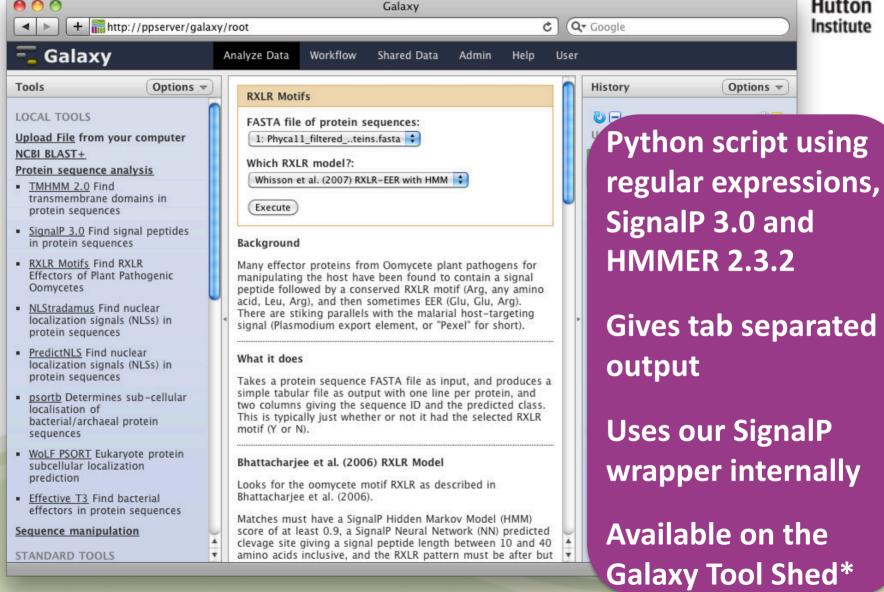
Signal Peptides (SignalP)





Oomycete RXLR Motifs

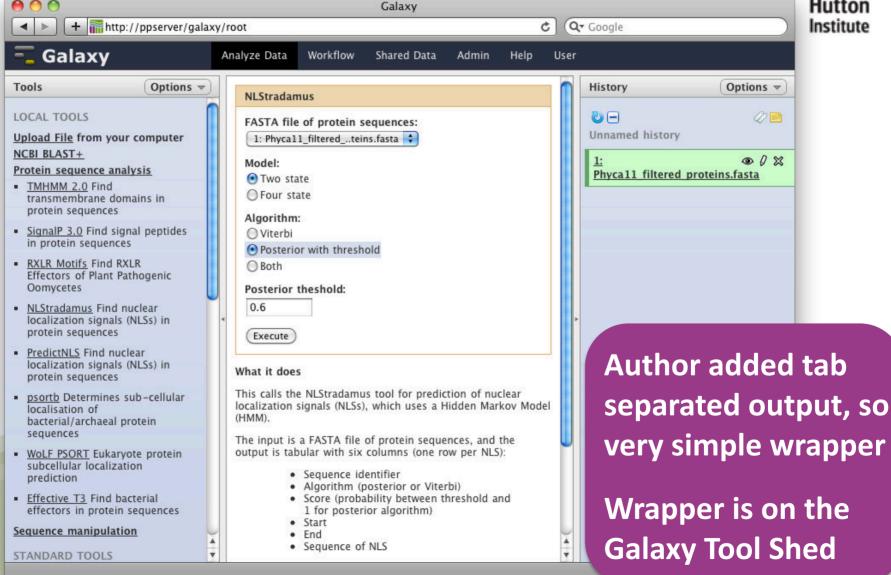




^{*} Submitted 20 May 2011

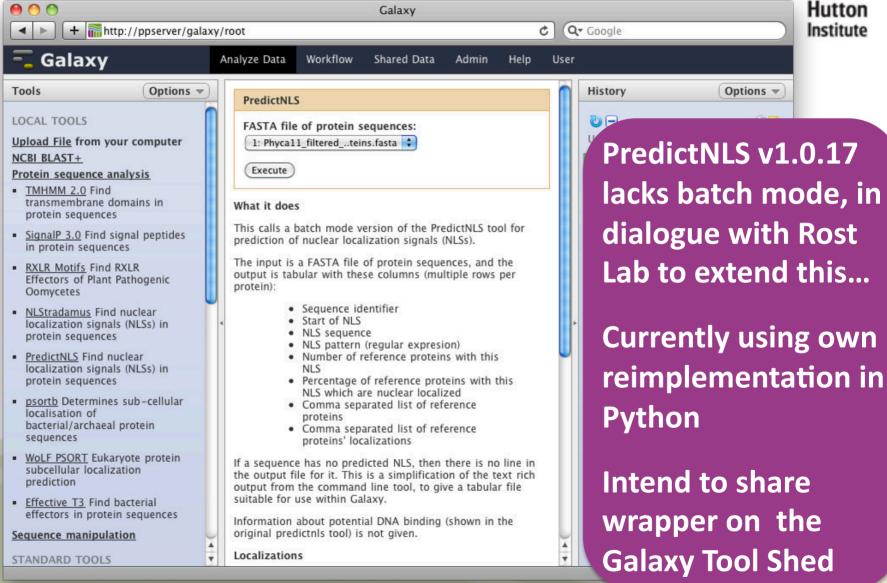
Nuclear Localisation Signals: NLStradamus





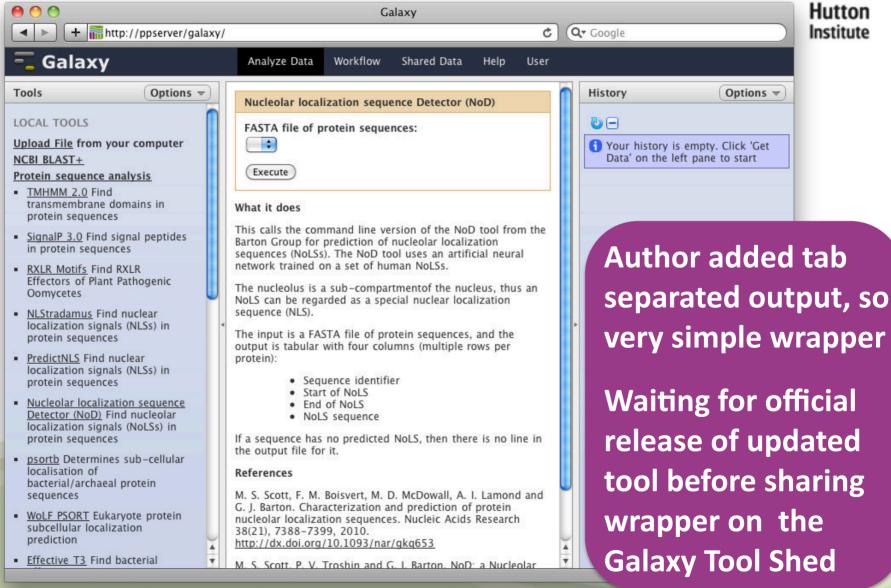
Nuclear Localisation Signals: PredictNLS





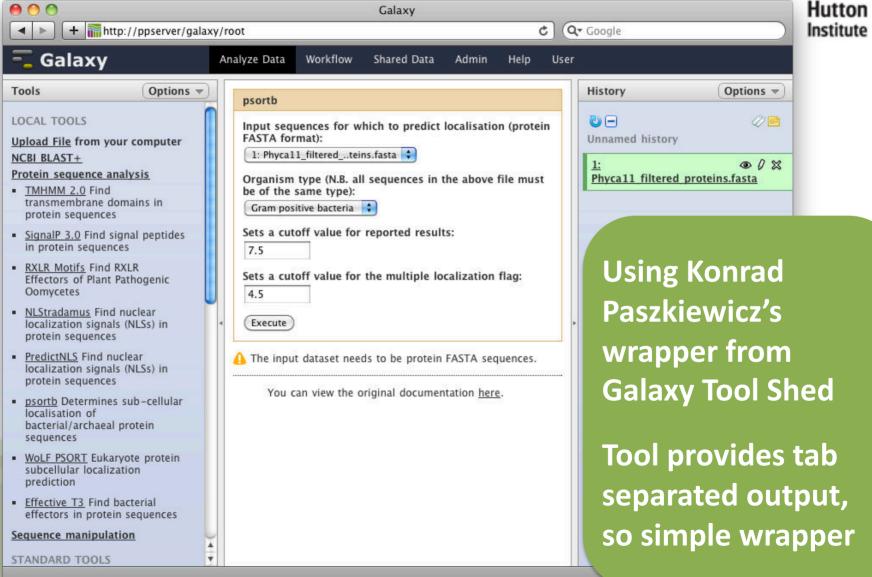
Nucleolar Localisation Signals: NoD





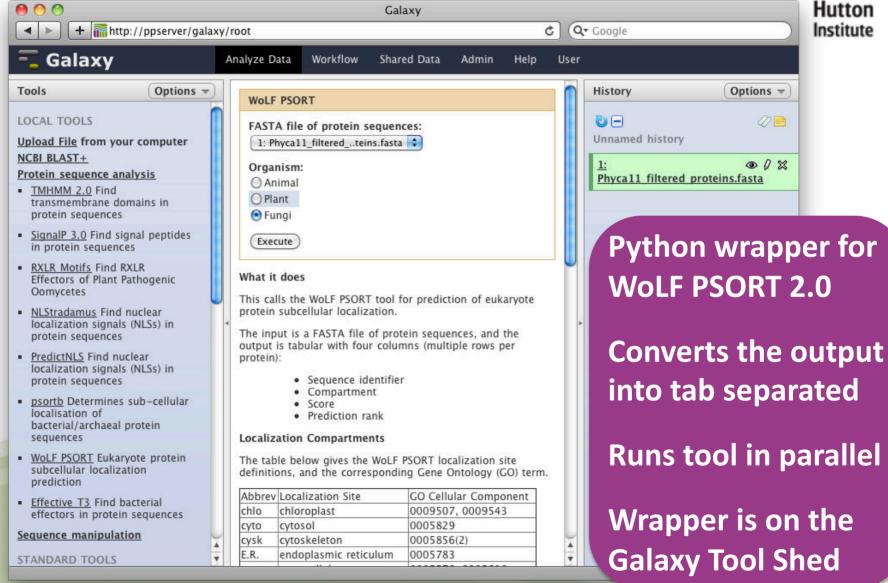
Prokaryotic Sub-cellular Localisation: PSORTb





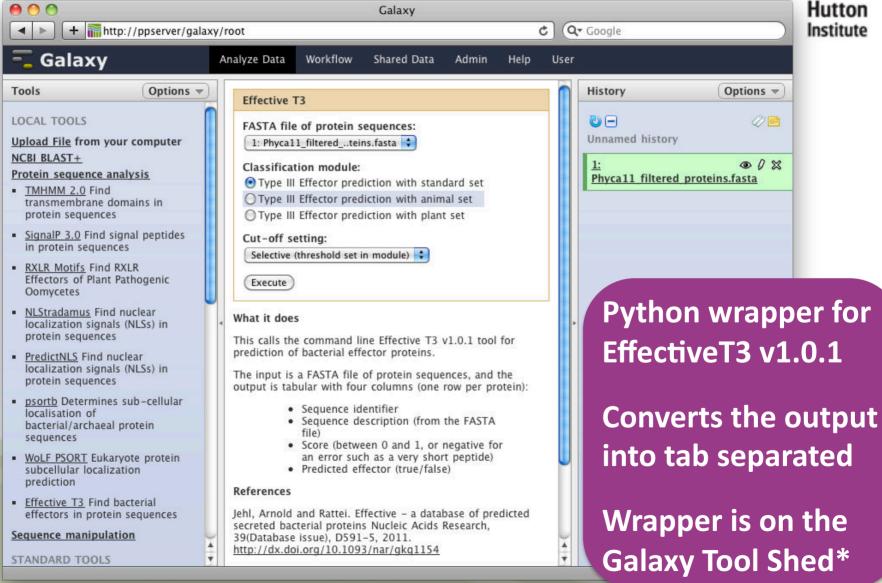
Eukaryotic Sub-cellular Localisation: Wolf PSORT





Type III Secretion Signals: EffectiveT3





Observations from Wrapping Tools

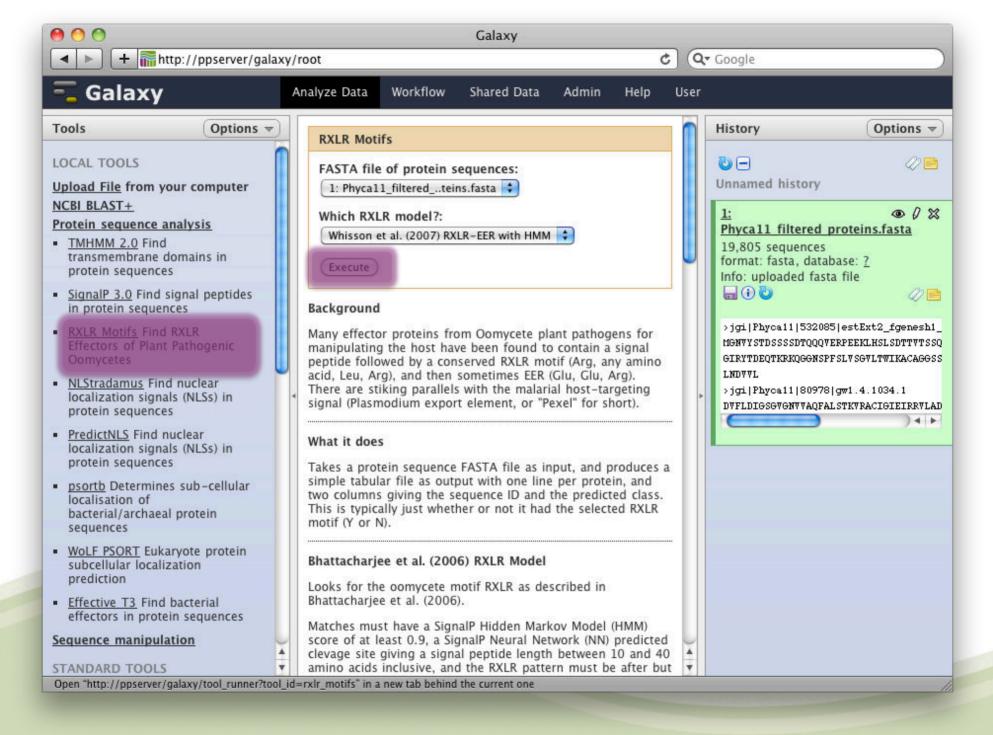


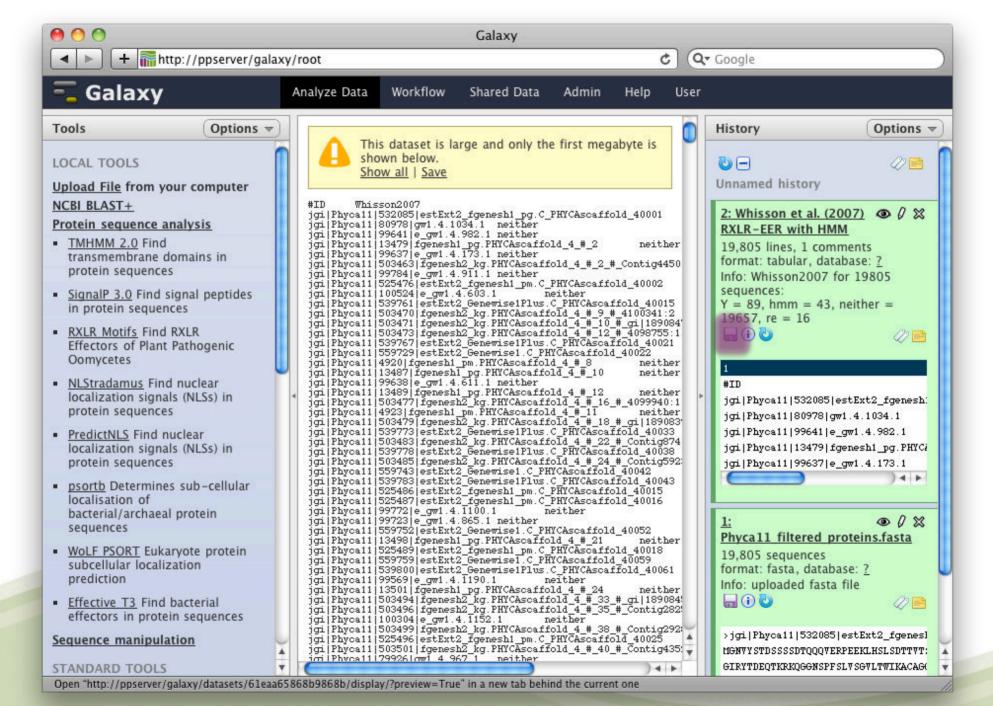
- Tabular output for Galaxy
 - Most tools' output needed reformatting
- Some tools are not threaded
 - I use a Python wrapper script to divide the input (using subprocess rather than multiprocessing module for Python 2.4 compatibility)
- Interaction with tool authors can be productive and informative, and improve their tools
- To tool authors
 - Offer tabular output (if appropriate)
 - Better error handling (e.g. zero length sequences)

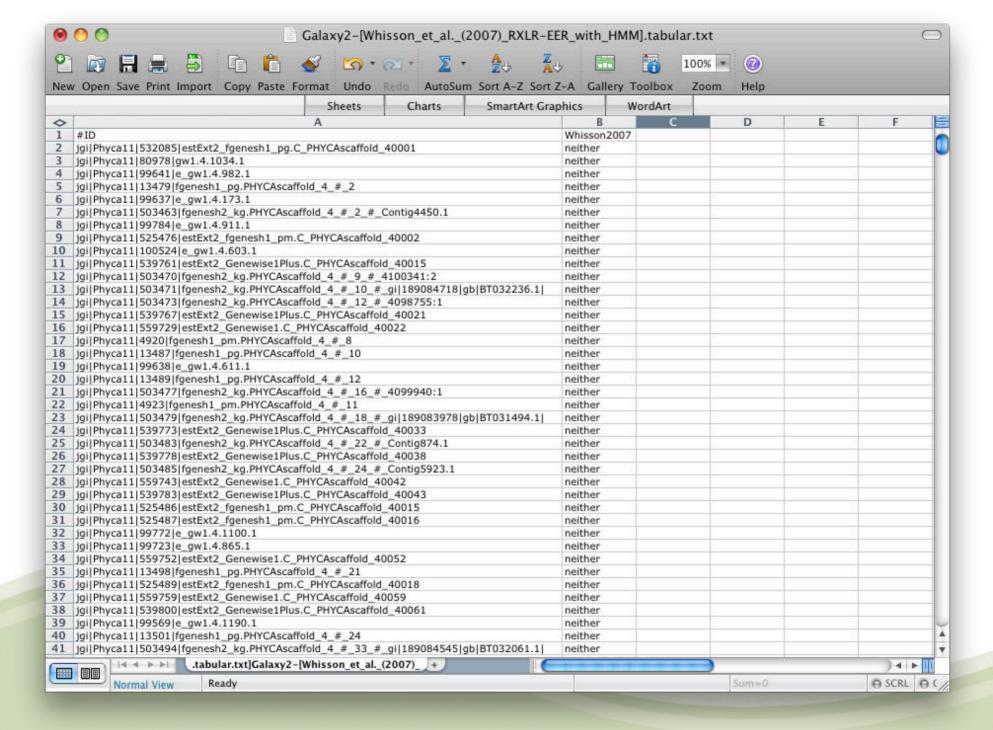
Workflow example - RXLR motifs

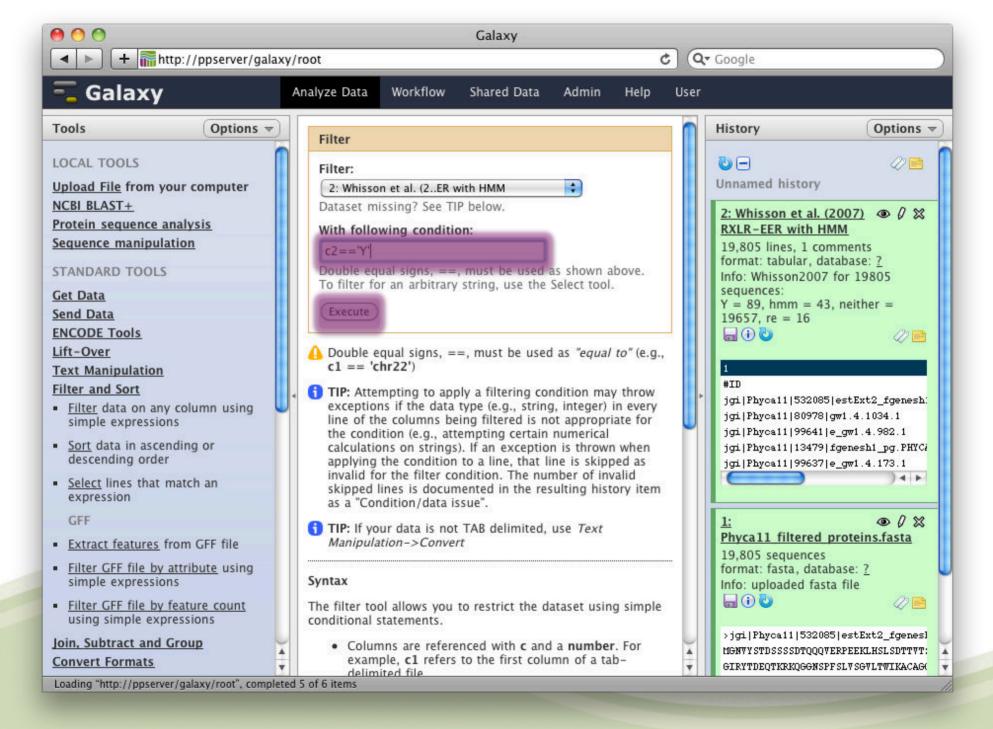


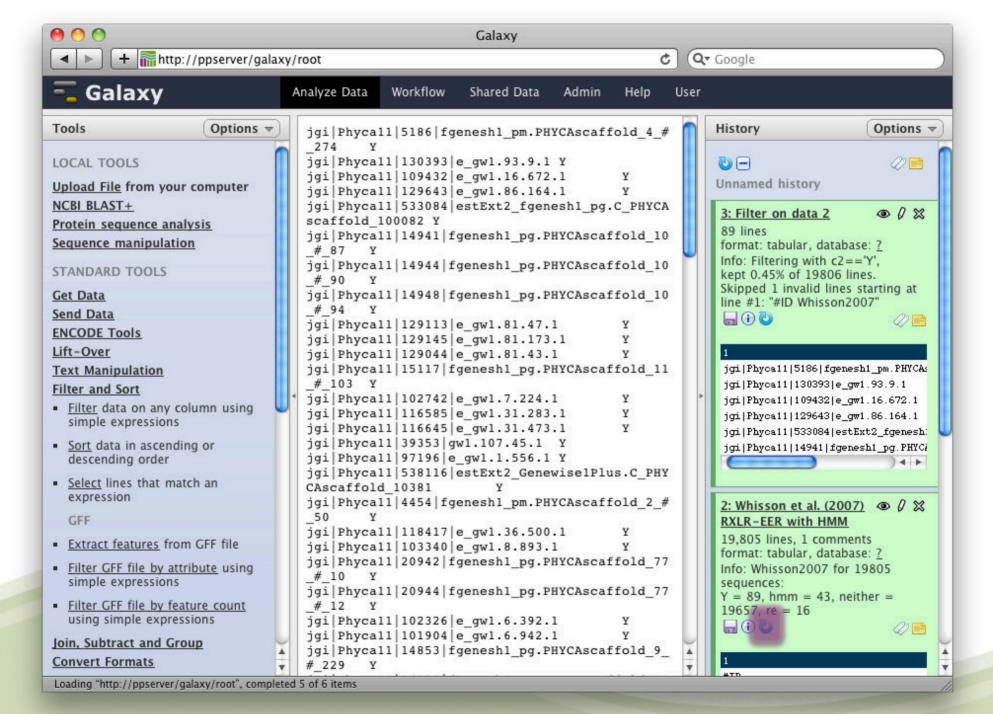
- Important translocation motif in oomycetes
- We have implemented three methods in Galaxy:
 - Bhattacharjee et al. (2006)
 - Win et al. (2007)
 - Whisson et al. (2007)
- Venn Diagram comparing the three methods



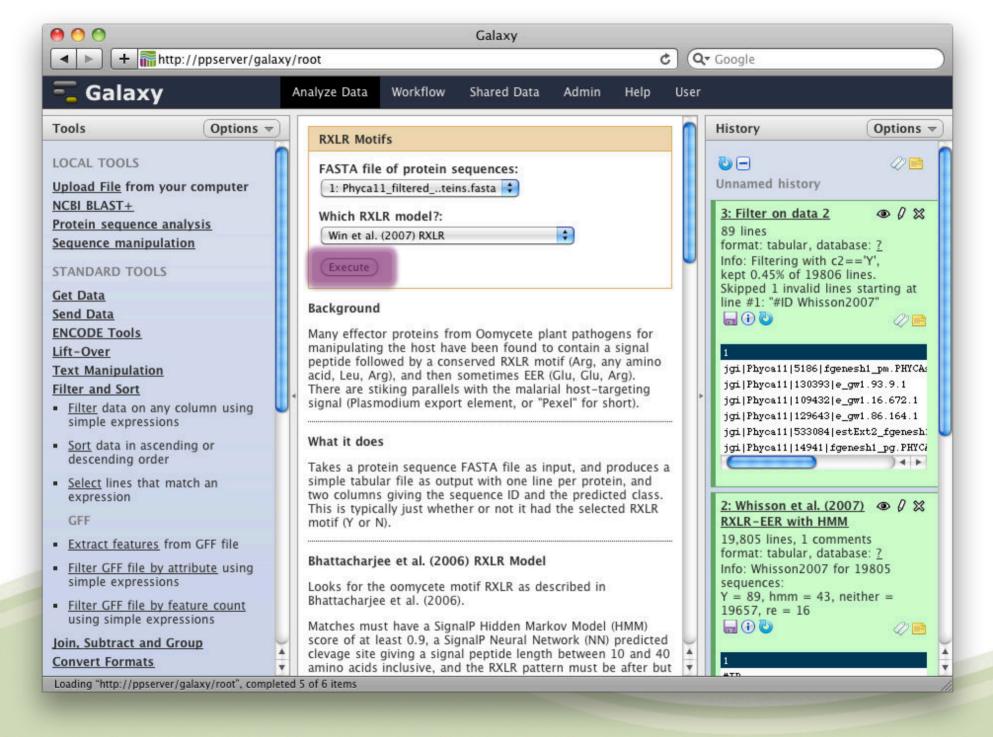








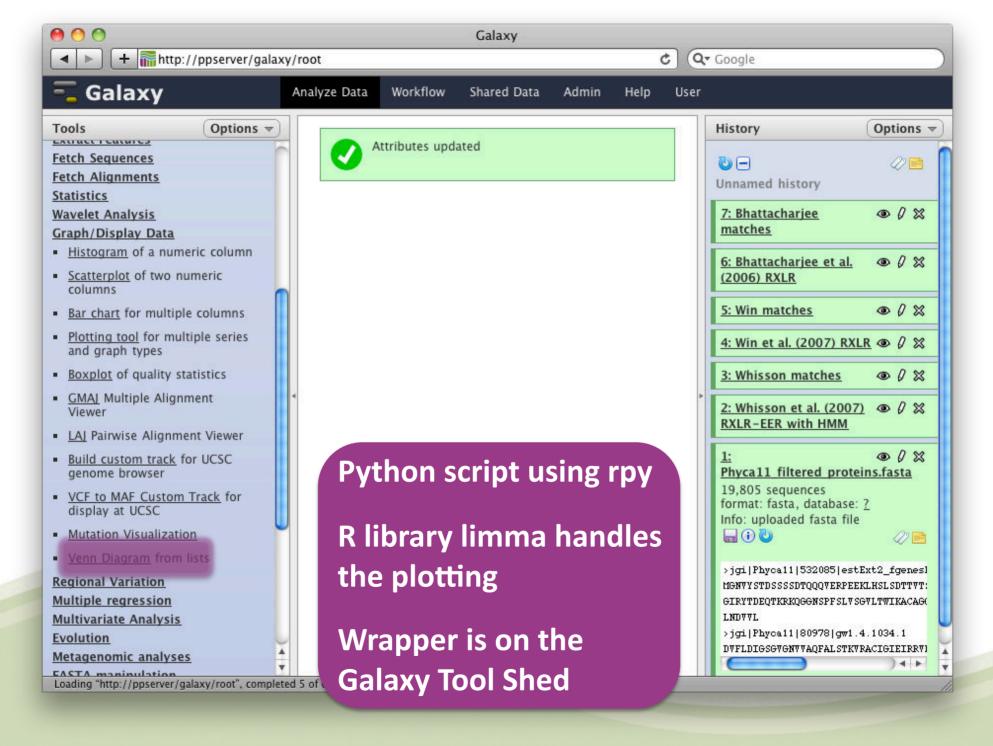


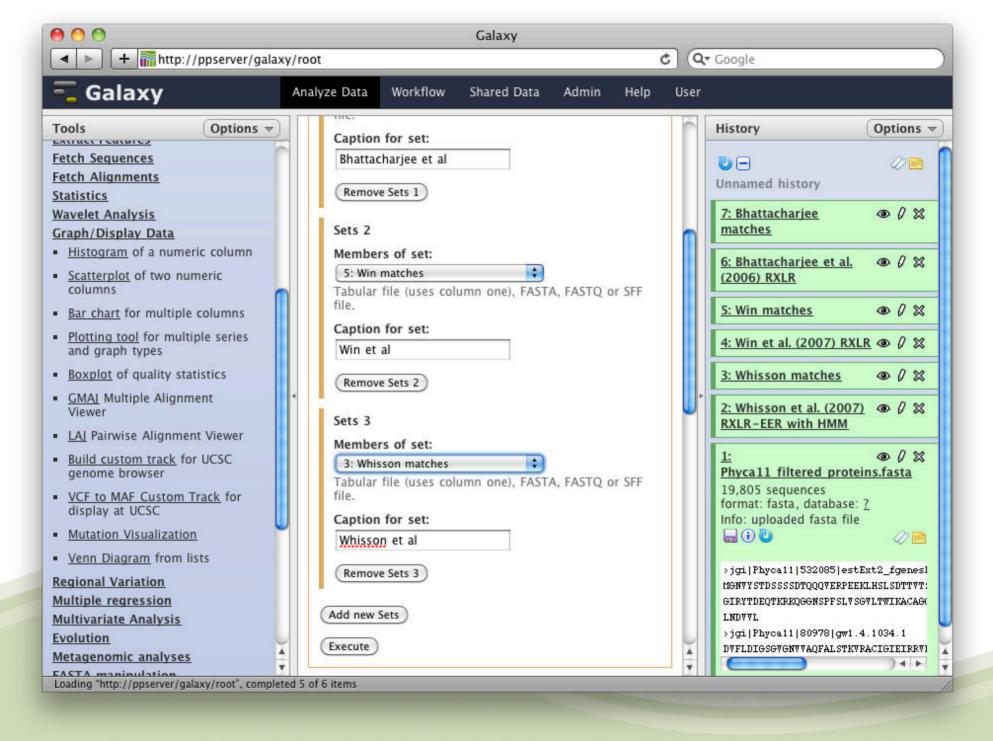


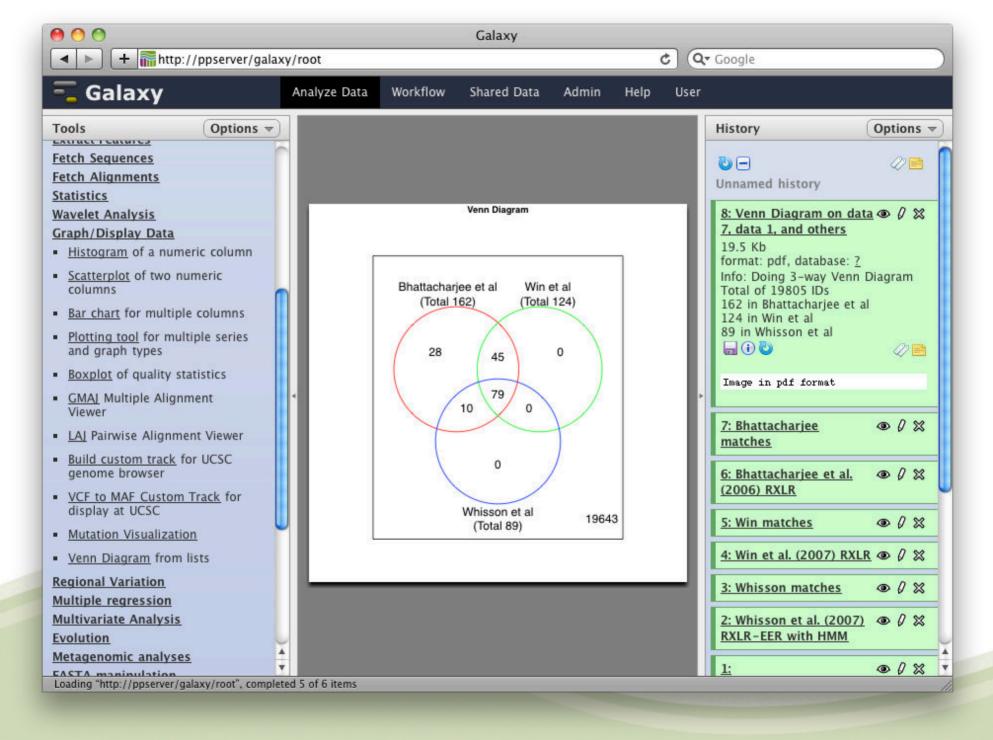
Next few steps omitted...



- Repeated RXLR search & filter using other two models
- Labelled some history entries

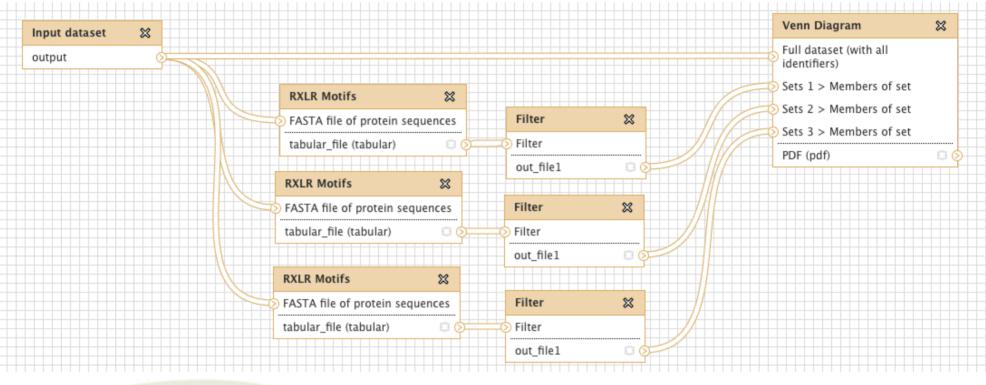






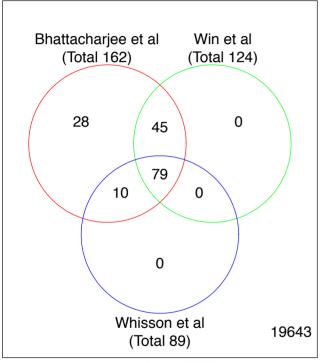
Repeating analysis as a Workflow



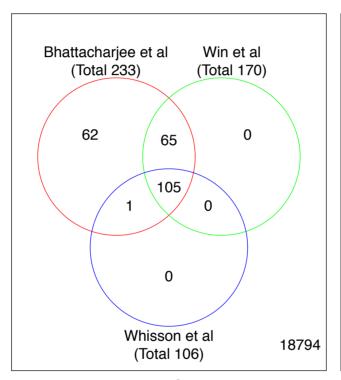


RXLRs in *Phytophthora* draft genomes

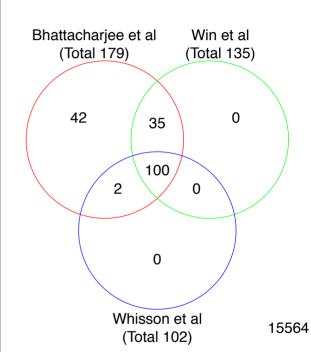




P. capsici (19,805 proteins)



P. sojae (19,027 proteins)



P. ramorum (15,743 proteins)

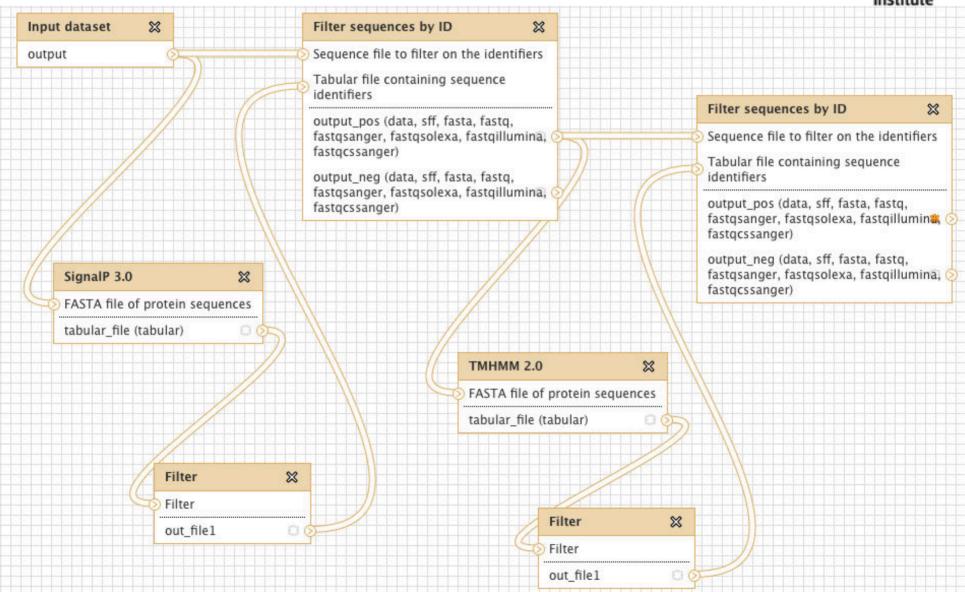
Example – Finding effector proteins



- Start with a FASTA file of proteins
- Run signal peptide prediction
- Select proteins with signal peptide
- Run transmembrane prediction
- Select proteins without transmembrane (TM) domains
- Get FASTA file of proteins with signal peptide but not TM

Workflow Editor – Effector finding





Acknowledgements

The James Hutton Institute

- Helpful tool authors:
 - Alex Nguyen (NLStradamus)
 - Laszlo Kajan (PredictNLS)
 - Peter Troshin, Michelle Scott (NoD)
- JHI Testers:
 - John Jones, Remco Stam, Julietta Jupe
- The Galaxy Developers & mailing list community