Computational Biology of PIP3 signalling

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Signaling ISP Seminar, September 2013

Background
System Biology Modeling
Gene Expression Analysis
Acknowledgments

PI3K signaling pathway
Phosphoinositides
Systems Biology

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General View

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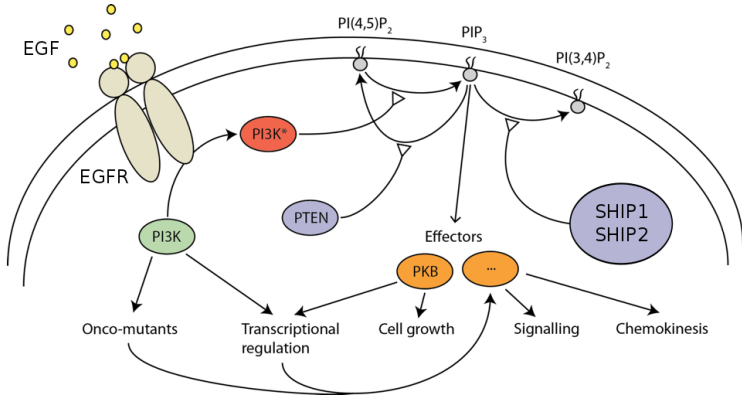
Properties

- Interactions both on the cell membrane (2D) and in the cytoplasm (3D)
- Kinase and phosphatase activities
- Phosphoinositide (PIP2, PIP3 etc.) complexity

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Isoform Complexity

Figure from www.biochemsoctrans.org
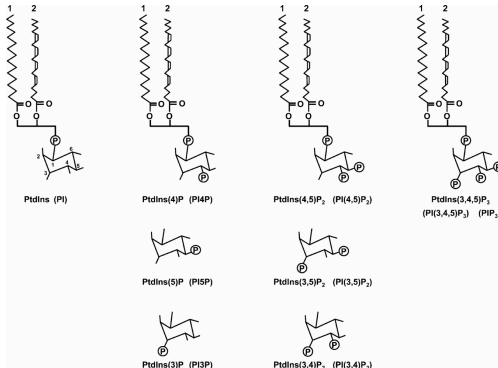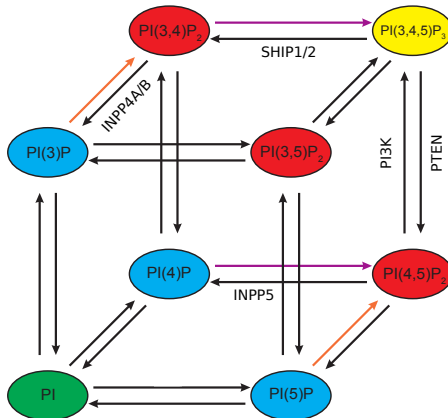
- Each PI species has 6 isoforms
- In total $8 * 6 = 48$ isoforms

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Conversion Complexity

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Computational Modeling

- Has been widely used in different biological disciplines
- Has been proved to be robust and reliable
- Helps tackling the complexity problem

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Kinetic Modeling

- Deterministic – use a set of ODE to describe evolution of the system
    - Assuming that the system is well stirred and spatially homogeneous
- Stochastic – more general approach directly taking into account system fluctuations
    - Is required when number of particles in the systems is small
- Others (e.g. Rule Based Modeling)

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Design Overview

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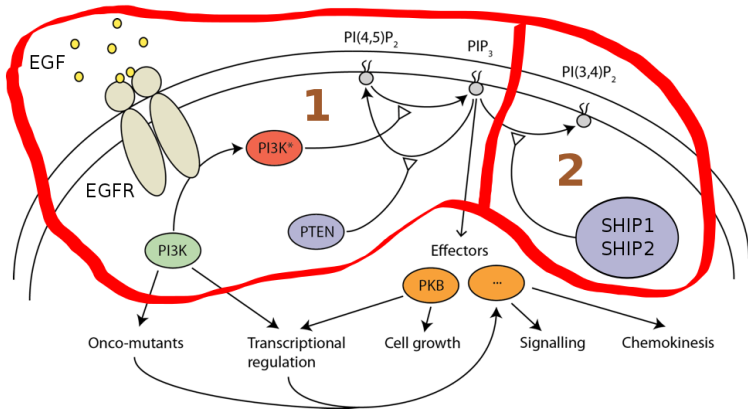
Mass-Spec Data

- PI, PIP, PIP2, PIP3 concentrations were measured using mass-spectrometry
- Impossible to distinguish between several species
- $[PI(4,5)P2] \gg [PI(3,4)P2]$
  $[PI(4,5)P2] \gg [PI(3,5)P2]$
- $[PI(4)P] \gg [PI(3)P]$
  $[PI(4)P] \gg [PI(5)P]$

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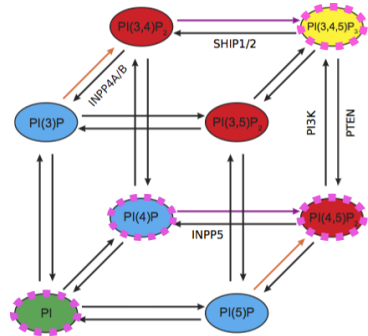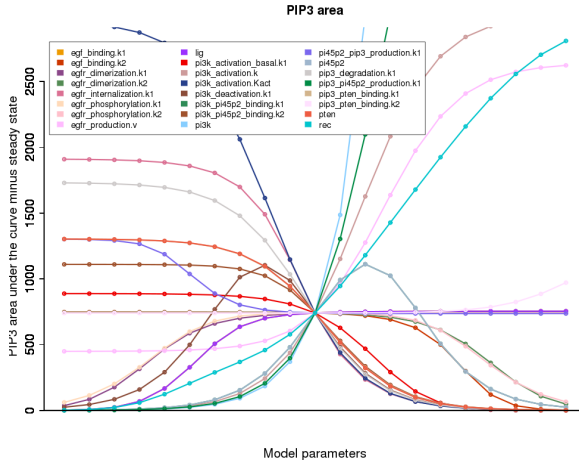Acknowledgments

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Design 1 – without PI(3,4)P2

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Analysis

PIP3 area

Legend:
- egf_binding.k1
- egf_binding.k2
- egfr_dimerization.k1
- egfr_dimerization.k2
- egfr_internalization.k1
- egfr_phosphorylation.k1
- egfr_phosphorylation.k2
- egfr_production.v
- lig
- pi3k_activation_basal.k1
- pi3k_activation.k
- pi3k_activation.Kact
- pi3k_deactivation.k1
- pi3k_pi45p2_binding.k1
- pi3k_pi45p2_binding.k2
- pi3k
- pi45p2_pip3_production.k1
- pi45p2
- pip3_degradation.k1
- pip3_pi45p2_production.k1
- pip3_pten_binding.k1
- pip3_pten_binding.k2
- pten
- rec

PIP3 area under the curve minus steady state

Model parameters

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Parameter Estimation

Parameter	Ranking value	From	To	Source	Value
pi3k	1+	-	-	literature	0.2
pip3_pi45p2_production.k1	1	0	1100	estimated	283.069
pi3k_activation.Kact	0.4092065232	0	1000	estimated	468.605
pi3k_activation.k	0.3528758625	0	10000	estimated	4510.44
rec	0.3332101512	-	-	literature	0.15
pip3_degradation.k1	0.3161319637	0	12	estimated	0.0638764
egfr_internalization.k1	0.2827946551	-	-	literature	0.0055
pi3k_deactivation.k1	0.2721791102	1	1000	estimated	203.269
pi3k_pi45p2_binding.k1	0.2721195867	0	100	estimated	8.44855
pten	0.2454299223	0	1	estimated	0.502768
pip3_pten_binding.k1	0.2454217573	0	10	estimated	0.103891
egfr_production.v	0.2136297575	0	0.0003	estimated	0.000181083
pi3k_pi45p2_binding.k2	0.1962720248	0	3000	estimated	632.48
pi3k_activation_basal.k1	0.1763170064	-	-	x	0.108887
egf_binding.k2	0.1549547047	-	-	literature	0.022
egf_binding.k1	0.1502826433	-	-	literature	29
egfr_dimerization.k2	0.1486302434	-	-	literature	0.3
egfr_phosphorylation.k2	0.1470041127	-	-	literature	0.08
egfr_dimerization.k1	0.1465098154	-	-	literature	10
egfr_phosphorylation.k1	0.1425294984	-	-	literature	1.33
pi45p2_pip3_production.k1	0.1240844428	-	-	x	7.14235
pip3_pten_binding.k2	0.0369564535	-	-	x	0.00941346
pi45p2	-	-	-	experimental	115
lig	-	-	-	experimental	0.00157
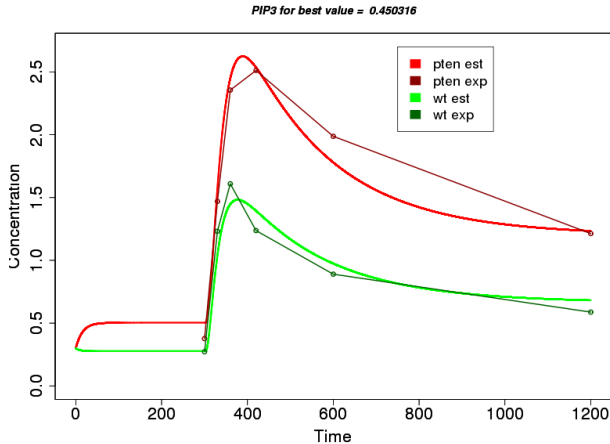
- 1000 runs
- Best 10 runs were considered

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Results

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SHIP1/SHIP2 Activity

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SHIP1/SHIP2 Activity

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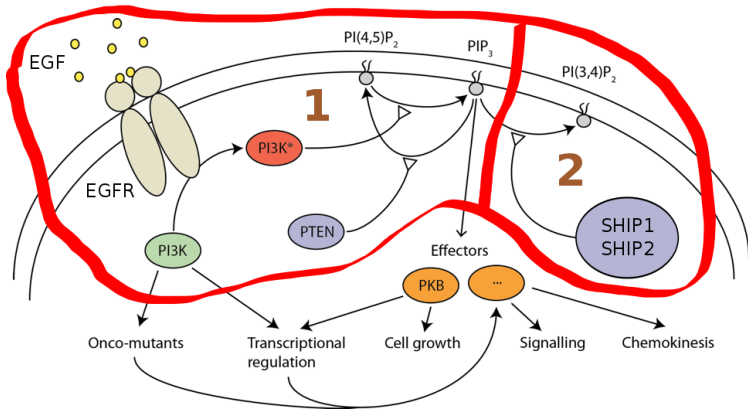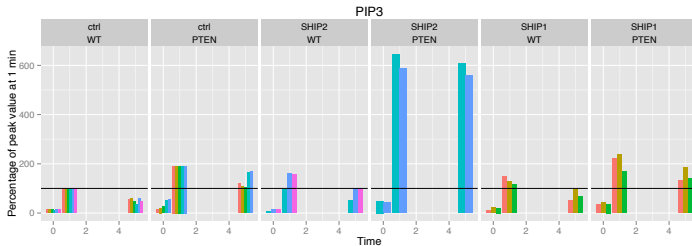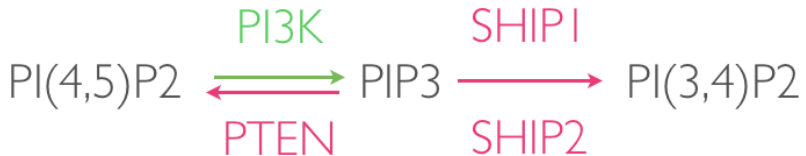
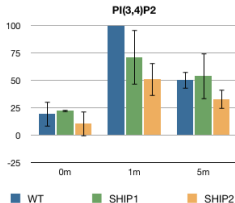# FRET Data

- PI(3,4)P2 concentration was identified at the University of Dundee

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SHIP1/SHIP2 Activity

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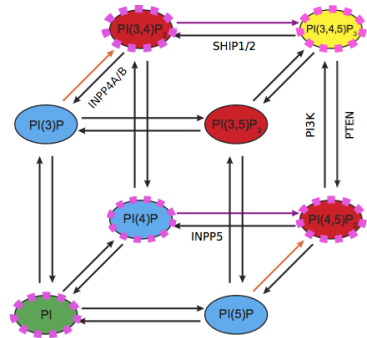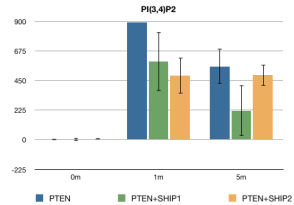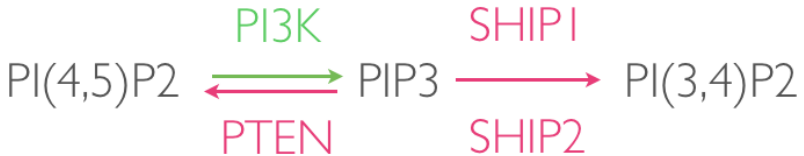
Adding SHIP1/SHIP2 – In Progress

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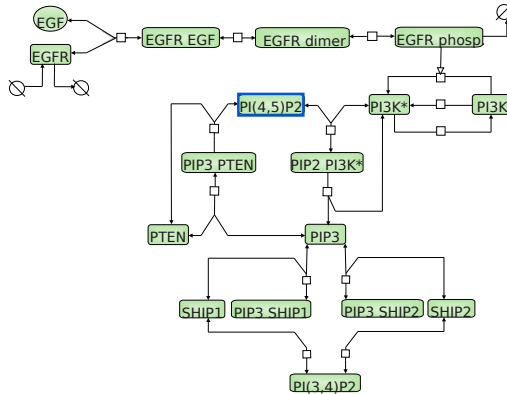
Future Plan

- Adding spatial dimension
- Stochastic reactions
- Single molecule representations rather than populations
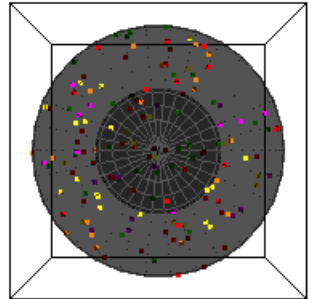
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RNA-seq
Our Data
Preliminary Analysis
Time-Course Analysis

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Brief Intro
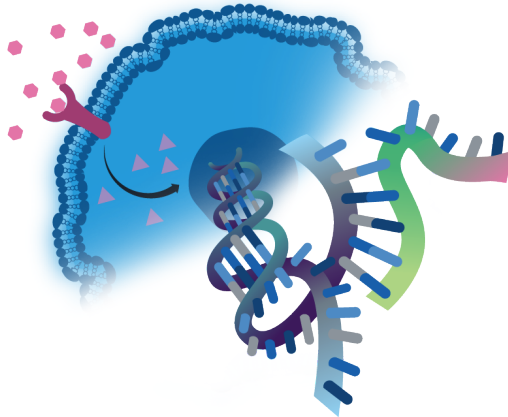
Figure from www.discovery.lifemapsc.com

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Method
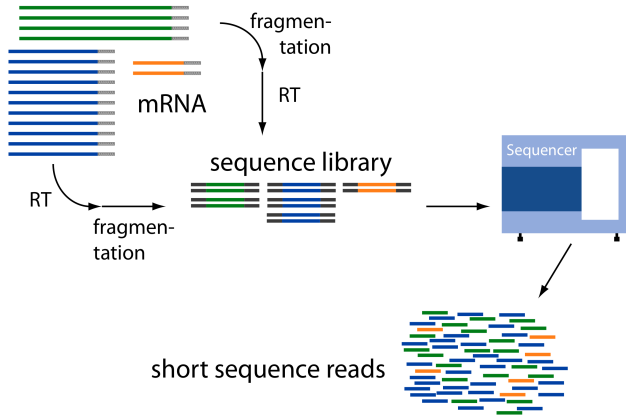
Figure from www.raetschlab.org

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Read Alignment
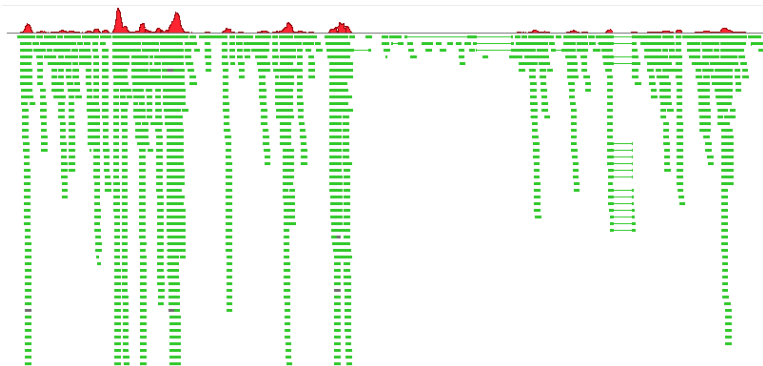
Figure from www.arrayserver.com

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Experiment Design

– Everything done by Veronique Juvin

- Human, breast-derived MCF10a cell line
- 4 conditions
    - WT
    - WT + p110α selective inhibitor (A66) – KO below
    - PTEN -/- (both mutant alleles) – PTEN below
    - p110α H1047R/+ (1 WT allele / 1 mutant allele) – KI below
- RNA-seq time course measurements at 0m-15m-40m-90m-180m-300m
- 3 replicates for each point

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Expression Data

3 replicates

	0	15	40	90	180	300
wt						
pten						
ko						
ki						

gene_id	read_num
ENSG00000002330	369
ENSG00000004779	1928
ENSG00000006015	525
ENSG00000026559	73
ENSG00000040608	304
ENSG00000050820	1862

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Correlation Matrix
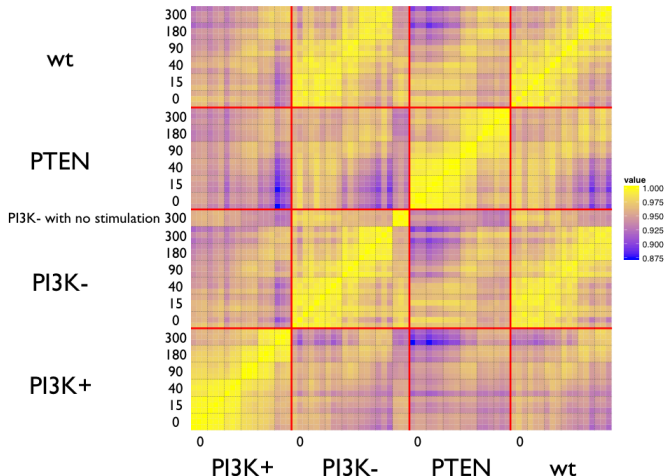
Principal Component Analysis
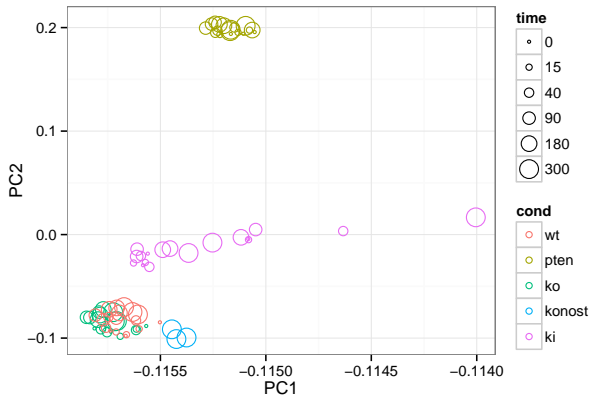
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DE Analysis Tool

- DESeq package (from Bioconductor) – takes into account all replicates
- FDR of 1%

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Time Direction

	0	15	40	90	180	300
wt						
pten						
ko						
ki						

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Time Direction

Condition 1

logFold change

	Δt_1	Δt_2	Δt_3	Δt_4	Δt_5	profile
gene1	2.16	0.1	3.18	1.01	-1.98	ususd
gene2	-3.26	0.57	-0.24	2.21	0.4	dssus
gene3	0.15	-0.64	0	-0.47	0.59	sssss

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Time Profiles

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Condition Direction

	0	15	40	90	180	300
wt						
pten						
ko						
ki						

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Condition Direction

Condition 1

| 0 | 15 | 40 | | 90 | | 180 | | 300 |

t_1 t_2 t_3 t_4 t_5 t_6

Condition 2

| 0 | 15 | 40 | | 90 | | 180 | | 300 |
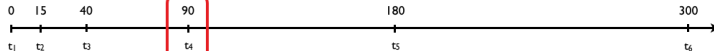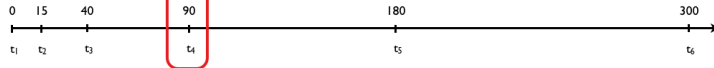
t_1 t_2 t_3 t_4 t_5 t_6

logFold change

	t_1	t_2	t_3	t_4	t_5	t6	profile
gene1	2.16	0.1	3.18	1.01	-1.98	-1.6	ususdd
gene2	-3.26	0.57	-0.24	2.21	0.4	1.1	dssuss
gene3	0.15	-0.64	0	-0.47	0.59	0.37	ssssss

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Condition Profiles

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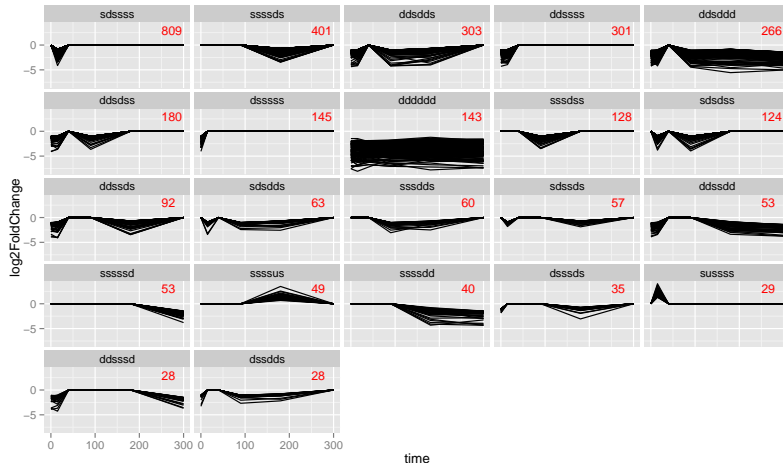
Future Plan

- Identify unique EGF stimulation related genes

Acknowledgments

- People
  - Mouhannad Malek, Veronique Juvin
  - Len Stephens, Phill Hawkins
  - Nicolas Le Novere and the group
  - Nicholas Luscombe and the LRI group
  - Marija Jankovic, Sven Bergmann, Anne Segonds-Pichon, Simon Andrews
- Money
  - BBSRC grant
  - Babraham Institute

Note: this presentation was made with LaTeX, Beamer + Wiki2Beamer

Example: http://github.com/gjuggler/talk-pdoc-seminar