A Reproduction and Further Exploration of “Flux balance analysis predicts essential genes in clear cell renal cell carcinoma metabolism”

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Background and Motivation

- Flux Balance Analysis
- In our case we want to maximize the formation of biomass
- The authors conducted single gene knockouts of more than a one-thousand genes
- They then conducted a flux balance analysis to see how biomass production was affected
- If biomass production was ablated or if there was a significantly negative flux in biomass formation then the gene was deemed to be essential
- The authors then compared the results from their flux balance analysis to an in-vitro siRNA screen.
- They were then able to determine the numbers of true and false positives in their experiments.

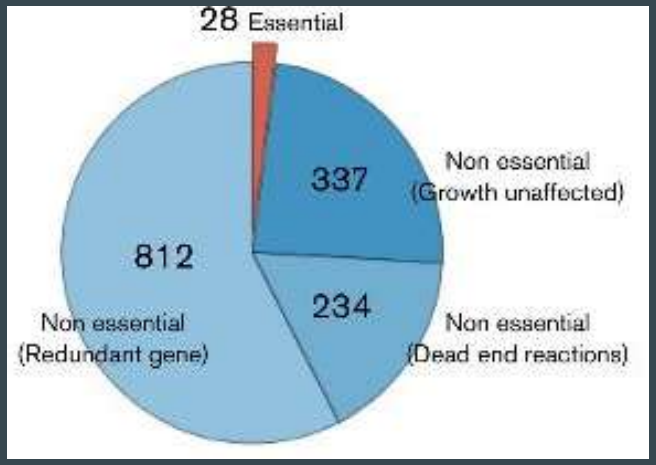
Models Used By Authors and Goals For Further Directions

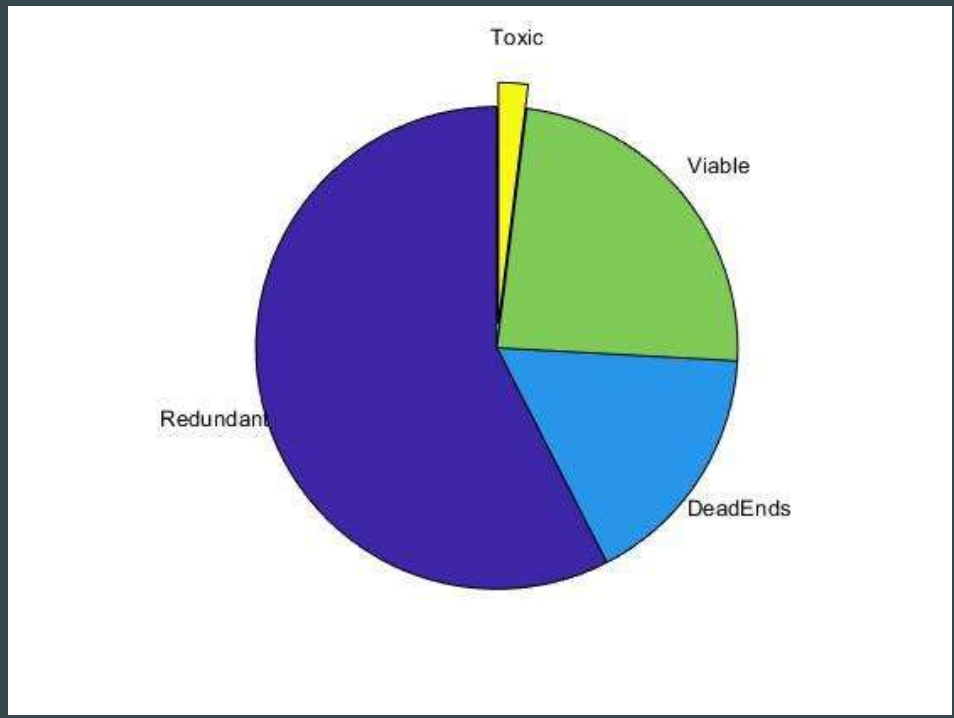
- The authors conducted flux balance analysis on models from both clear cell Renal Cell Carcinoma (ccRCC) and prostate adenocarcinoma (PC).
- We replicated the results that the author's used when they were examining metabolic network topology only
- Then used a more easily implemented model to perform flux balance analyses to do our own gene essentiality predictions on other cell lines
- We tested the robustness of the FBA and applied it to models of other types of cancer
- After comparing to a known database we believe that we may have found evidence that certain genes may play a greater role than previously thought in certain types of cancer

Methods

- Metabolic Atlas - ccRCC and prostate cancer models.
- RAVEN Toolbox - third-party MATLAB extension
- Mosek linear optimizer tool - third party set of linear differential equations solver

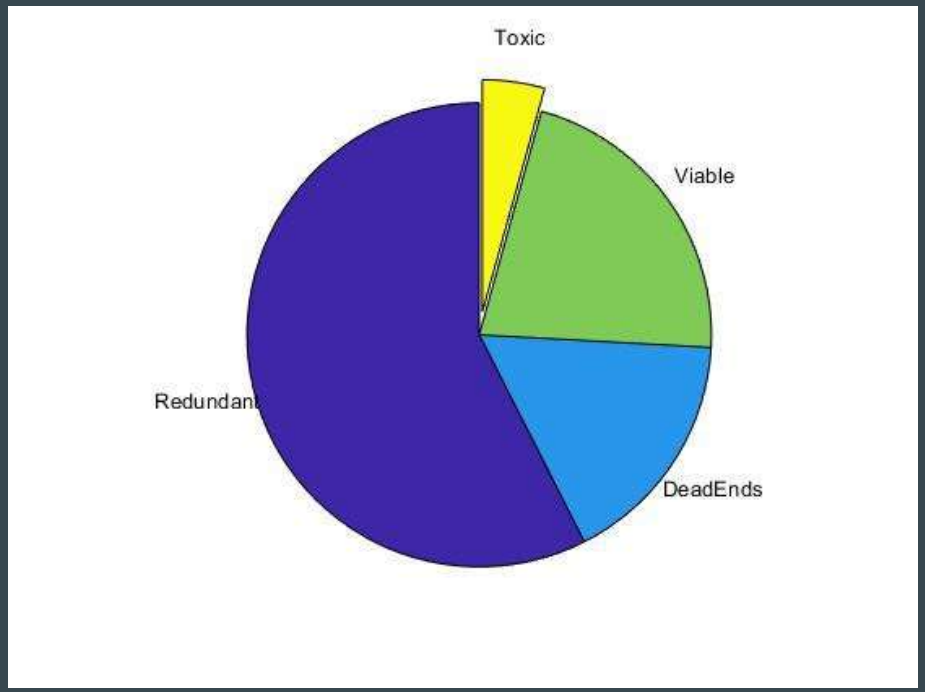
Replication of Results Using Flux Balance Analysis For Clear Cell Renal Cell Carcinoma Metabolism



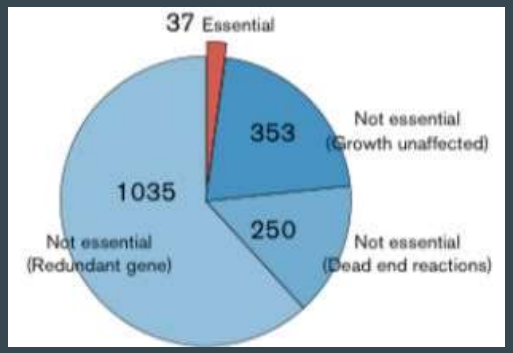


Replication of Results Using Flux Balance Analysis For Clear Cell Renal Cell Carcinoma Metabolism Using HAM's Medium

Cancer type	FBA constraints	Medium	TP	FN	FP	TN	Fisher exact test *p*-value	MCC
Clear cell renal cell carcinoma	Topology	FBS	2	18	1	135	0.043	0.226
		HAM	5	15	12	124	0.046	0.174
	Topology + Exchange fluxes	FBS	6	14	11	125	0.010	0.235
		HAM	6	14	15	121	0.032	0.186
	Exchange fluxes	FBS	1	19	2	134	0.339	0.086
Prostate adenocarcinoma	Topology	FBS	2	12	12	186	0.233	0.082
		HAM	2	12	14	184	0.285	0.068
	Topology + Exchange fluxes	FBS	2	12	19	179	0.635	0.039
		HAM	2	12	27	171	1	0.005



Replication of Results Using Flux Balance Analysis For Prostate Adenocarcinoma Metabolism Using HAM's Medium



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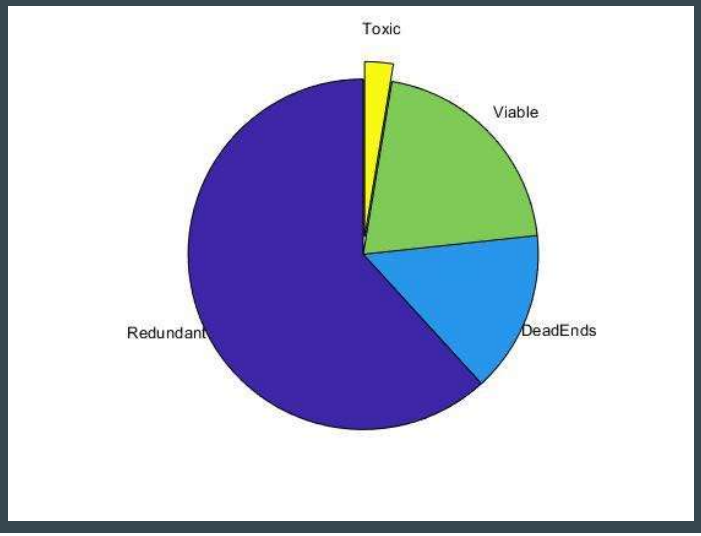
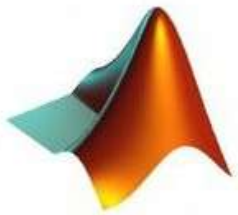


Using Dr. Segrè's More Easily Reproducible FBA In Predicting Genes Involved In Cancer Metabolism In Other Cell Lines

-82% of results from Gatto

-able to apply to other cell lines

Matlab 

RCCmodel

1x1 struct with 30 fields

Field	Value
description	'Automatically gener...
id	'iRenalCancer1410'
rxns	3914x1 cell
mets	3684x1 cell
S	3684x3914 sparse dou...
lb	3914x1 double
ub	3914x1 double
rev	3914x1 double
c	3914x1 double
b	3684x2 double
comps	9x1 cell
compNames	9x1 cell
compOutside	9x1 cell
rxnNames	3914x1 cell
metNames	3684x1 cell
metComps	3684x1 double
genes	1411x1 cell
rxnGeneMat	3914x1411 sparse dou...
grRules	3914x1 cell
metFormulas	3684x1 cell
geneShortNames	1411x1 cell
compMiriams	9x1 cell
metMiriams	3684x1 cell
subSystems	3914x1 cell
eccodes	3914x1 cell
geneMiriams	1411x1 cell
unconstrained	3684x1 double
rxnFrom	3914x1 cell
metFrom	3684x1 cell
geneFrom	1411x1 cell

Discussion

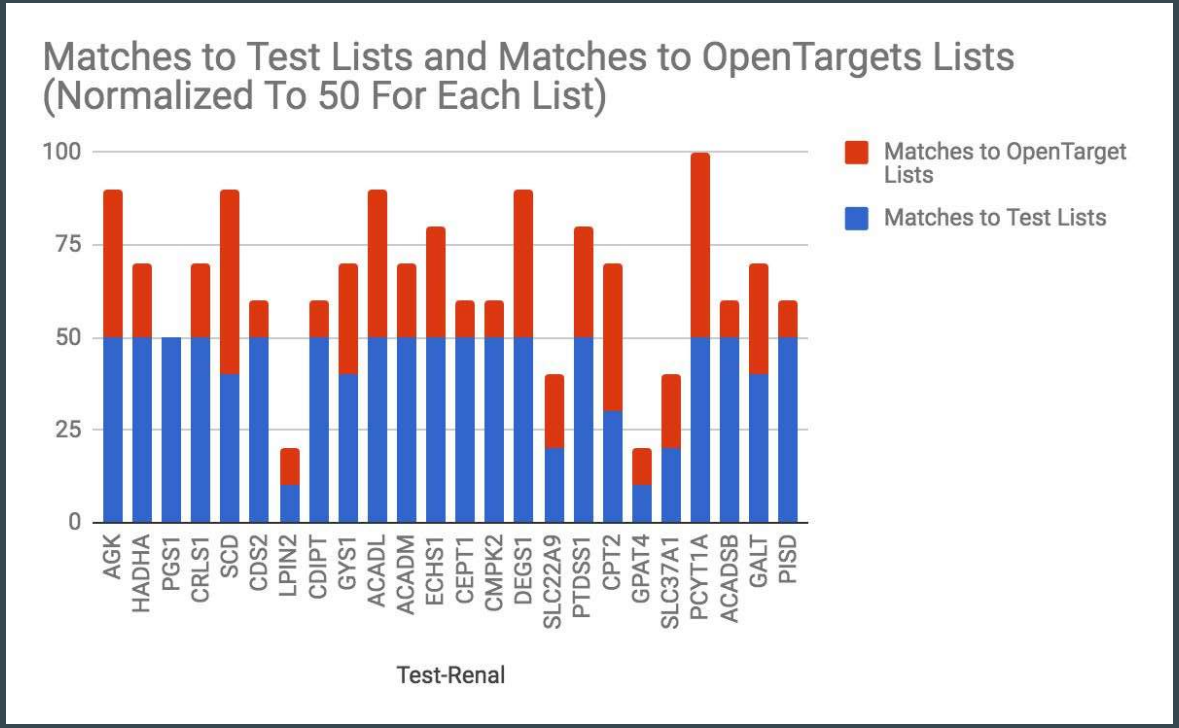
- Successfully reproduced some of the results from the Gatto et al. paper
- Using Dr. Segrè's model we were not able to fully replicate the results from Gatto's model, likely because Gatto's algorithm used alternative methods to initially exclude certain genes from the deletion process
- However, that model's ability to be implemented in the MATLAB environment made it possible for us to apply FBA to other models obtained from the Metabolic Atlas for further analysis

Further Analysis

- We tested Dr. Segrè's model on the Metabolic Atlas data for different cell lines
- We then compared our output to a list of known genes involved in cancer from the Open Targets database.

GALT - essential in our FBA models, also found in other cell lines; may be useful to perform in-vitro analyses on more cell lines

SPTLC1 - also essential in our FBA models, but not found in OpenTargets lists

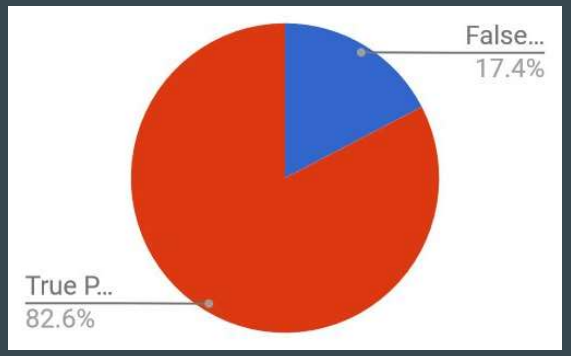


Further Analysis

- We also noted some proportionality between cancer research funding and genes from our FBA model found in the Open Targets database

	Funding for Cancer	True Positive %
Breast Cancer	**$519,880,371.00**	82.6%
Lung Cancer	**$283,755,731.00**	37.0%
Liver Cancer	**$75,693,176.00**	45.0%
Renal	**$41,837,803.00**	50.0%
Bladder	**$33,995,868.00**	22.2%

Breast Cancer



Urothelial Cancer