Synthetic lethality in cancer research via genetic Minimal Cut Sets

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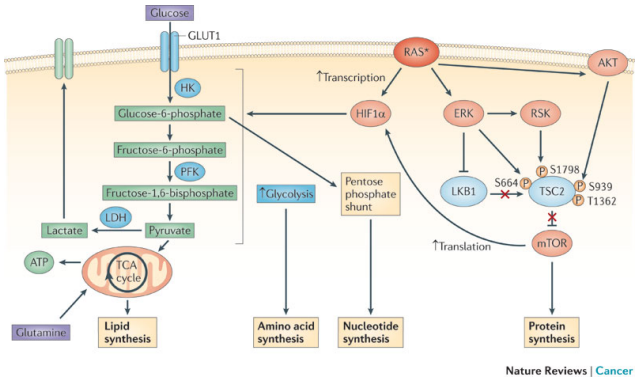
- Metabolism is a hot topic in cancer research.
- Signals and tumor microenvironment define different metabolic programs for enhancing proliferation, dissemination and invasion.
- Opportunity of identifying **biomarkers** and **drug targets for cancer cells** based on **metabolic networks** and **–omics data**.



Pylayeva-Gupta et al, 2011, Nature Reviews Cancer

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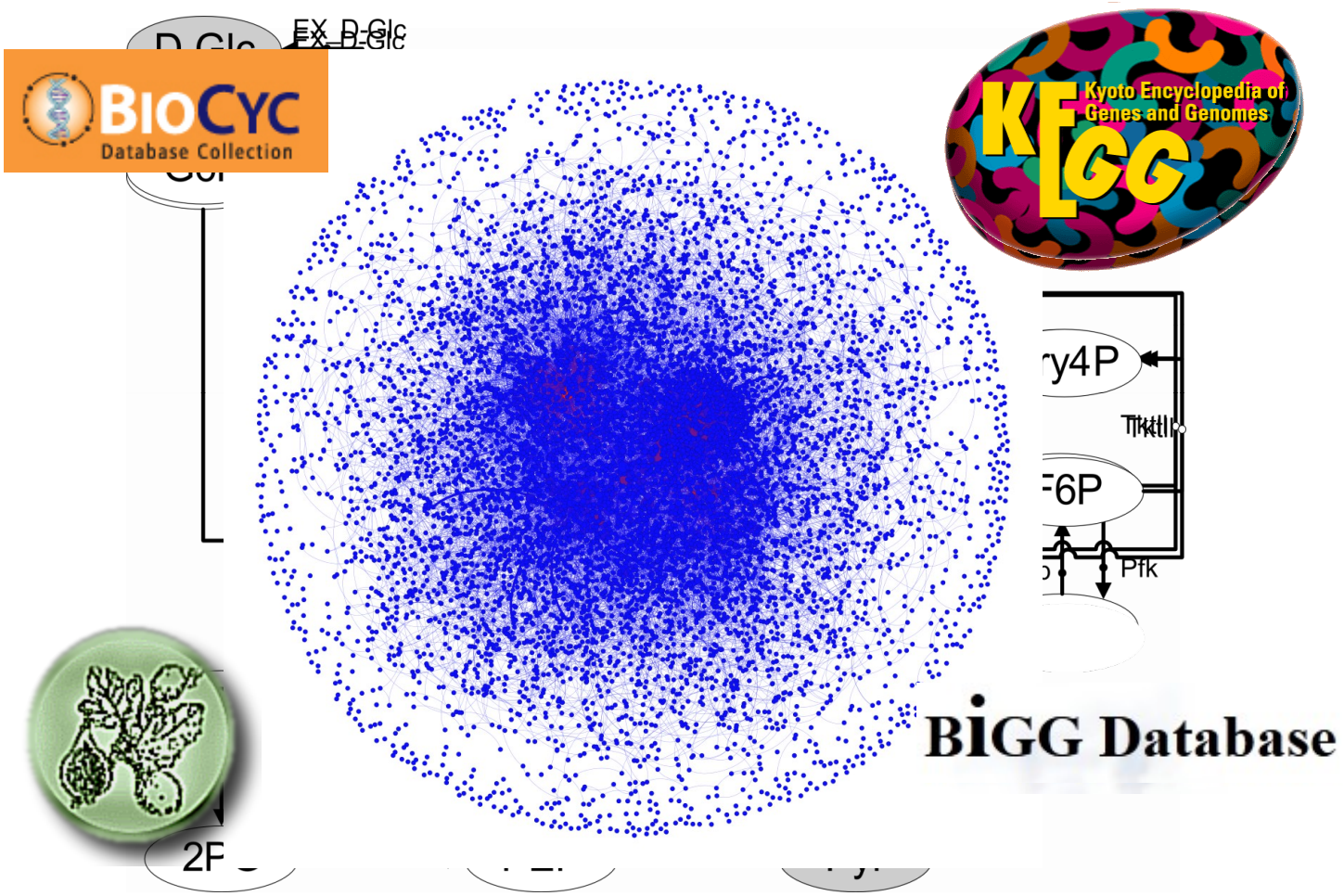
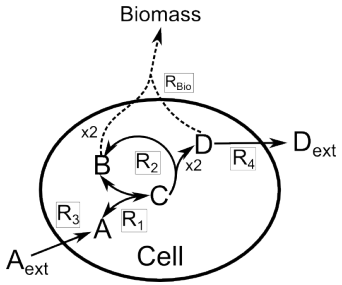
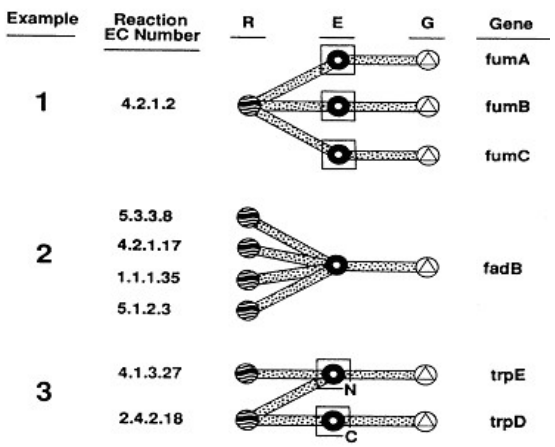
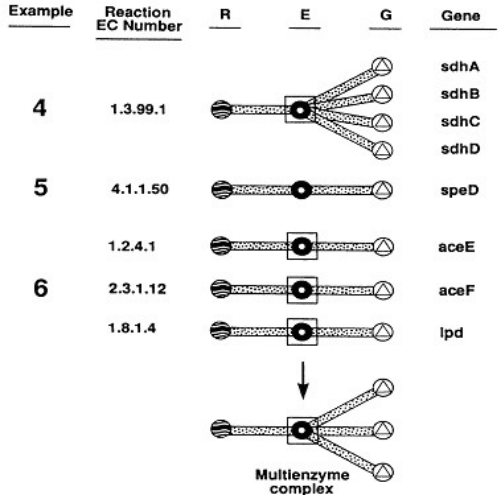


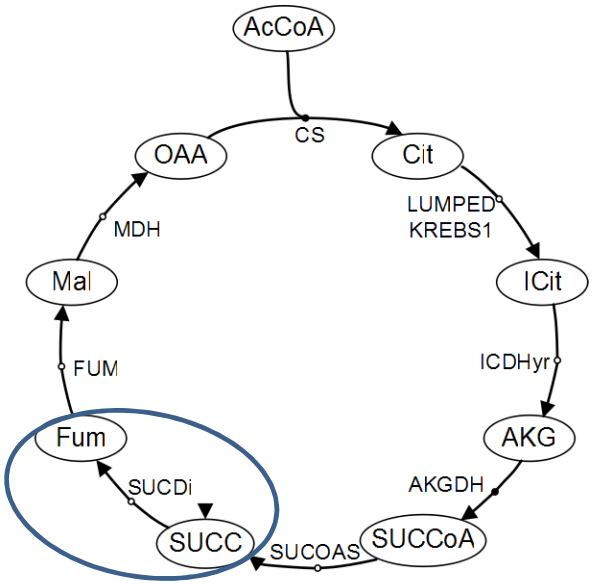
- Information included:
  - Substrates and products for an enzyme; Stoichiometric coefficients; Reversibility; Compartments, Input/output metabolites, Biomass equation

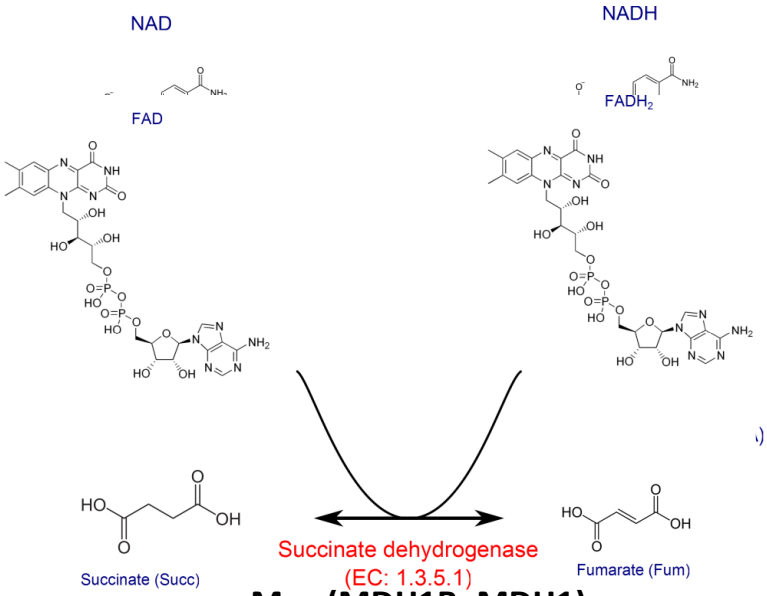


- **Gene-Protein-Reaction** (GPR) rules:





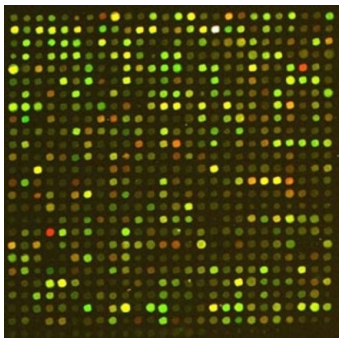




Max (MDH1B, MDH1)

SDHA and SDHB and SDHC and SDHD

Min(SDHA, SDHB, SDHC, SDHD)

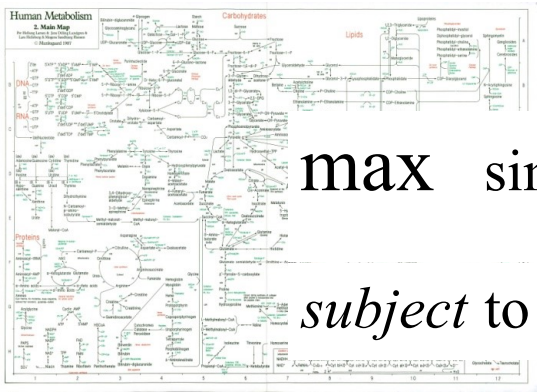


- Genomics
- Transcriptomics
- Proteomics
- Lipidomics
- Metabolomics
- Fluxomics
- ….

Cancer-specific metabolic reconstructions

Contextualize the reference metabolic network of human cells based on avaliable –omics data and, then, conduct gene knockout perturbations

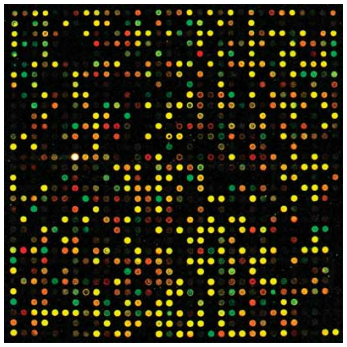
- **Cancer-specific metabolic reconstructions**:



$$\max \quad \text{similarity with expression data}$$

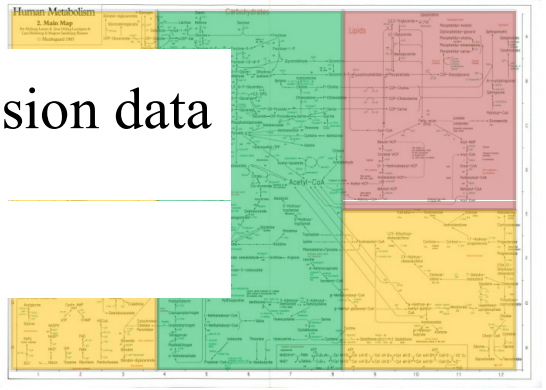
$$\textit{subject to}$$

$$Sv = 0$$

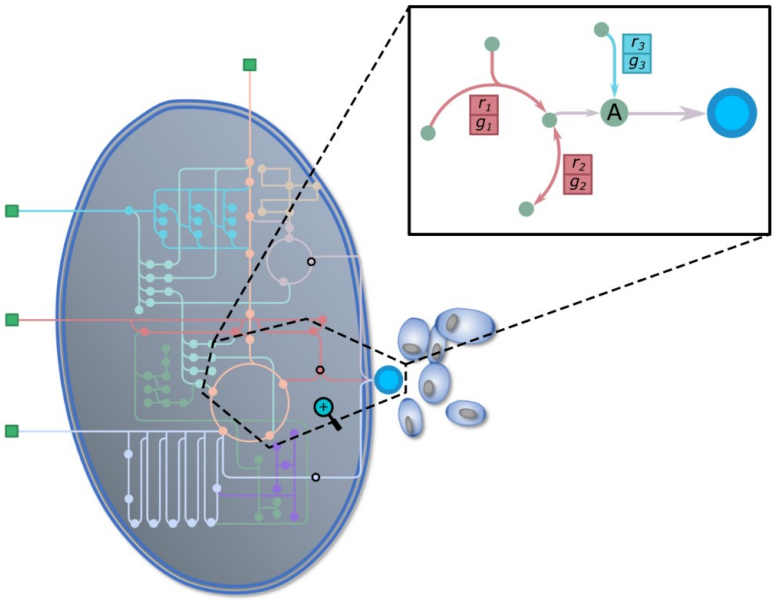


$$u_r \geq v_r \geq l_r$$

$$v_{biomass} \geq \varepsilon$$



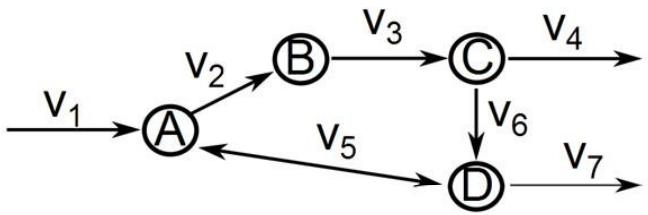
- **Cancer-specific metabolic reconstructions**:
  - **Essential metabolites for cellular growth (biomass reaction)**
    - Human biomass reaction (Folger et al, 2011)



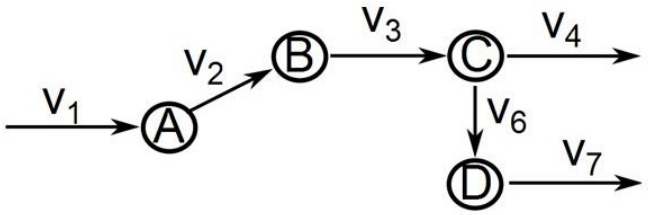
Coefficient	Name	Description
-20.6508	h2o[c]	H2O
-20.7045	atp[c]	ATP(4-)
-0.3859	glu_L[c]	L-glutamate(1-)
-0.3526	asp_L[c]	L-aspartate(1-)
-0.0361	gtp[c]	GTP
-0.2794	asn_L[c]	L-asparagine
-0.5056	ala_L[c]	L-alanine
-0.0466	cys_L[c]	L-cysteine
-0.326	gln_L[c]	L-glutamine
-0.5389	gly[c]	Glycine
-0.3925	ser_L[c]	L-serine
-0.3127	thr_L[c]	L-threonine
-0.5921	lys_L[c]	L-lysinium(1+)
-0.3593	arg_L[c]	L-argininium(1+)
-0.153	met_L[c]	L-methionine
-0.0233	pail_hs[c]	1-phosphatidyl-1D-myo-inositol(1-)
-0.039	ctp[c]	CTP
-0.1545	pchol_hs[c]	Phosphatidylcholine
-0.0554	pe_hs[c]	phosphatidylethanolamine

- **Gene essentiality and drug targets**:
  - One of these metabolites is disrupted upon gene knockout

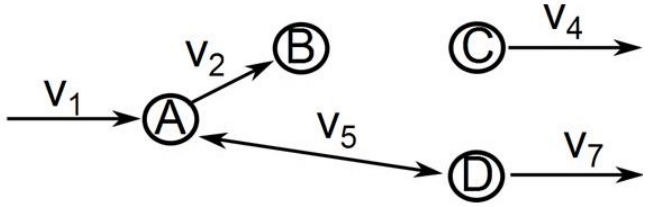
Delete Gene 1
No reaction is affected
Non-essential



Delete Gene 2
Reaction v_5 inactive
Non-Essential



Delete Gene 3
Reactions v_6 and v_3 inactive
Essential

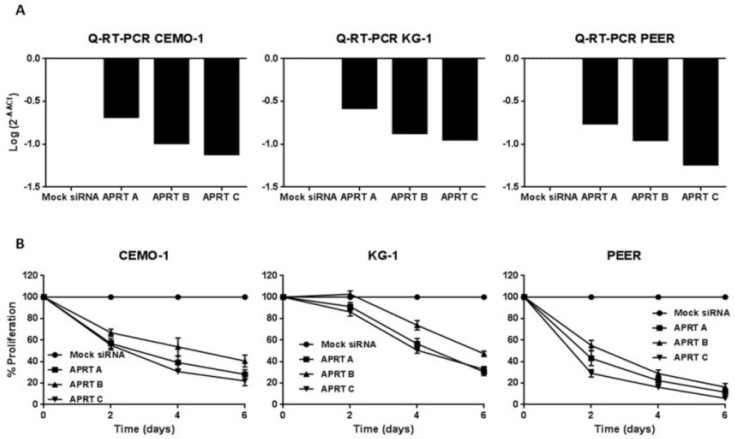


Gene essentiality and drug targets:

- Polyamines in cancer

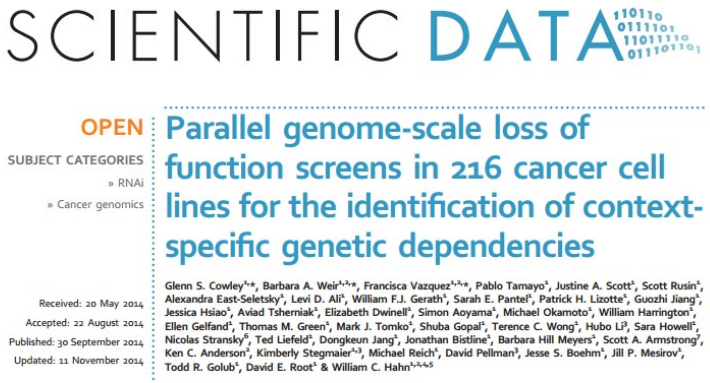
Gene(s)	Enzyme(s)	Type
262 (AMD1)	adenosylmethionine decarboxylase	Essential
4507 (MTAP)	5'-methylthioadenosine phosphorylase	Essential
4953 (ODC1)	Ornithine Decarboxylase	Essential
6723 (SRM)	spermidine synthase	Essential
4143 (MAT1A) & 27430 (MAT2B)	methionine adenosyltransferase	Synthetic
4143 (MAT1A) & 4144 (MAT2A)	methionine adenosyltransferase	Synthetic
353 (APRT) & 4860 (PNP)	**purine-nucleoside phosphorylase adenine phosphoribosyltransferase**	**Synthetic**
383(ARG1) & 4942(OAT)	ornithine transaminase reversible arginase	Synthetic

Error in the database led to APRT as an essential gene in leukemic cells



Pey et al, 2017, Scientific Reports,

- **Large-scale validation of predicted essential genes**:
  - **Project Achilles data**: large-scale gene silencing (knocking out) experiments in order to identify and catalogue genetic vulnerabilities in cancer.



SCIENTIFIC DATA

OPEN

SUBJECT CATEGORIES
» RNAi
» Cancer genomics

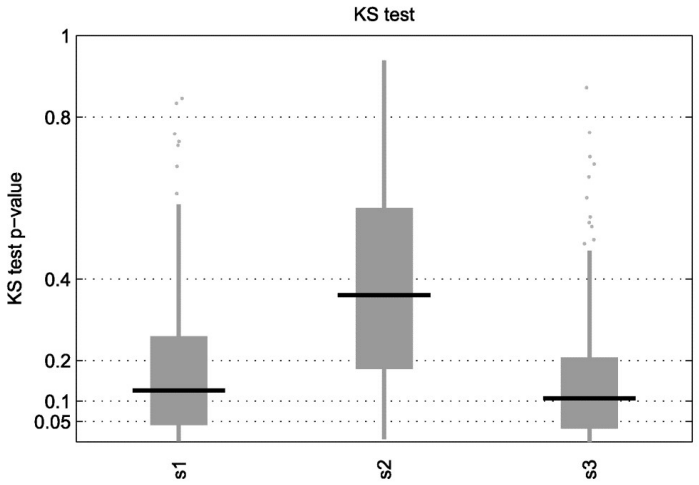
Parallel genome-scale loss of function screens in 216 cancer cell lines for the identification of context-specific genetic dependencies

Glenn S. Cowley[1,*], Barbara A. Weir[1,2,*], Francisca Vazquez[1,2,*], Pablo Tamayo[1], Justine A. Scott[1], Scott Rusin[1], Alexandra East-Seletsky[1], Levi D. Ali[1], William F.J. Gerath[1], Sarah E. Pantel[1], Patrick H. Lizotte[1], Guozhi Jiang[1], Jessica Hsiao[1], Aviad Tsherniak[1], Elizabeth Dwinell[1], Simon Aoyama[1], Michael Okamoto[1], William Harrington[1], Ellen Gelfand[1], Thomas M. Green[1], Mark J. Tomko[1], Shuba Gopal[1], Terence C. Wong[1], Hubo Li[1], Sara Howell[1], Nicolas Stransky[6], Ted Liefeld[1], Dongkeun Jang[1], Jonathan Bistline[1], Barbara Hill Meyers[1], Scott A. Armstrong[7], Ken C. Anderson[2], Kimberly Stegmaier[1,3], Michael Reich[1], David Pellman[3], Jesse S. Boehm[1], Jill P. Mesirov[1], Todd R. Golub[1], David E. Root[1] & William C. Hahn[1,2,4,5]

Received: 20 May 2014
Accepted: 22 August 2014
Published: 30 September 2014
Updated: 11 November 2014

depmap

Lack of accuracy



L. Tobalina et al, 2016, PLoS One

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QUESTION

Why and when a metabolic gene is essential for a particular molecular context using our modeling perspective?

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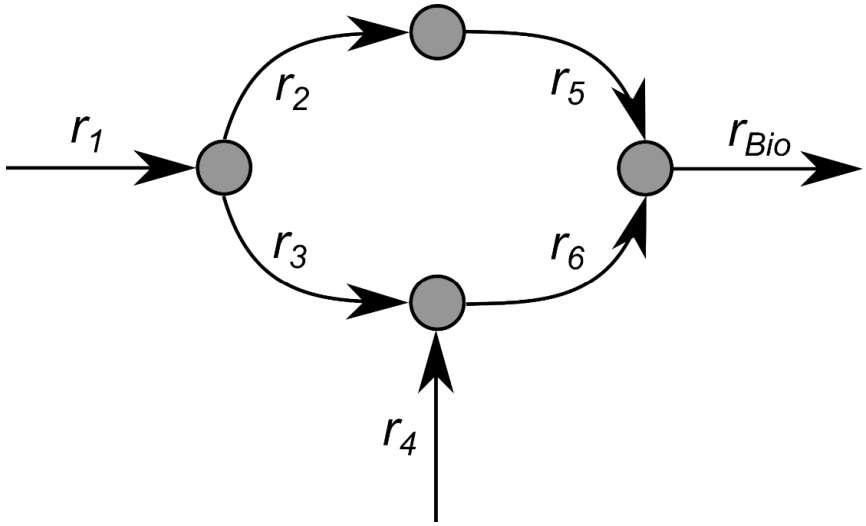
Minimal Cut Sets – MCSs (Steffen Klamt's group)

Identification of groups of metabolic **reactions**, that, when simultaneously inhibited, render celular proliferation impossible.

MCSs- Introduction



Based on:

- Optimization Theory
- Duality Theory
- Linear Algebra

Inputs:

- Template Metabolic Network
- Target metabolic task

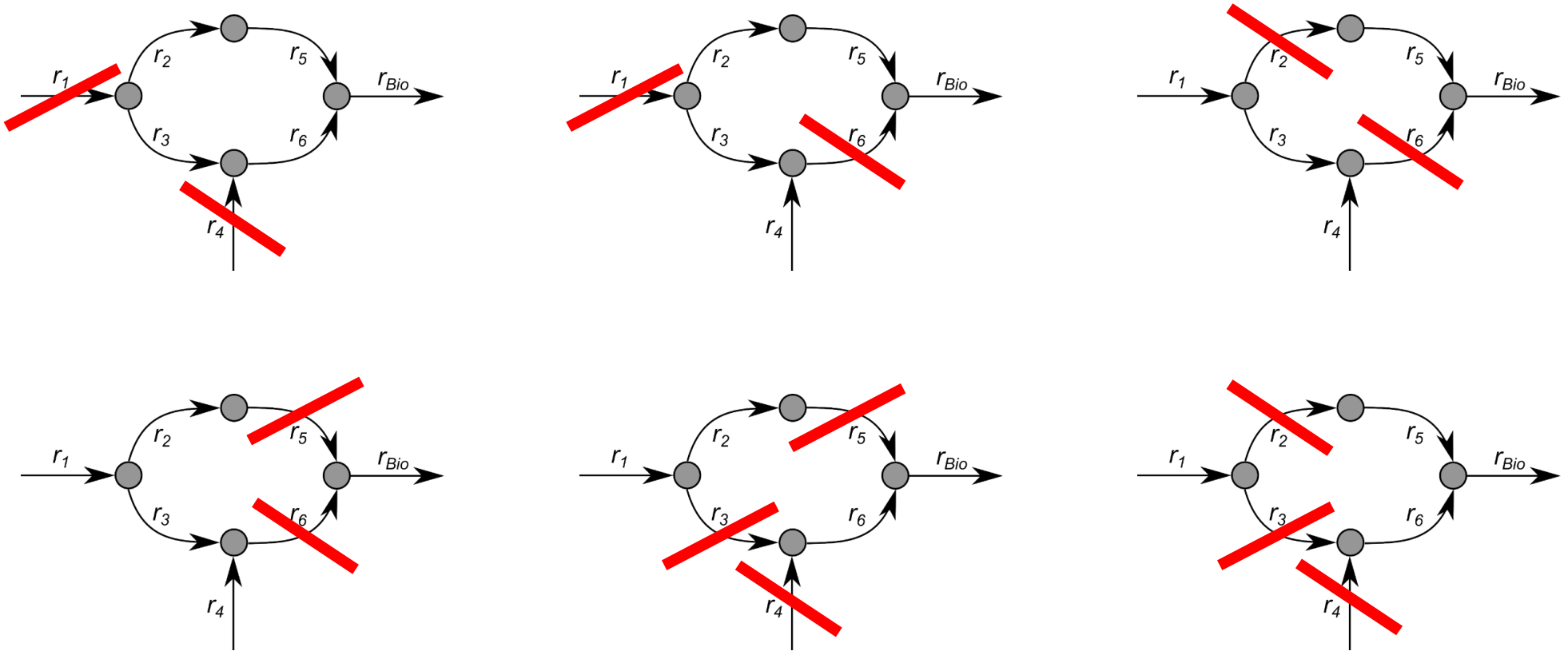
Minimal Cut Sets:

- r_1, r_4
- r_1, r_6
- r_2, r_6
- r_5, r_6
- r_3, r_4, r_5
- r_2, r_3, r_4

A. von Kamp and S. Klamt. 2014, PLoS Computational Biology

L. Tobalina et al, 2016, Bioinformatics

MCSs – Results



MCSs – Limitations

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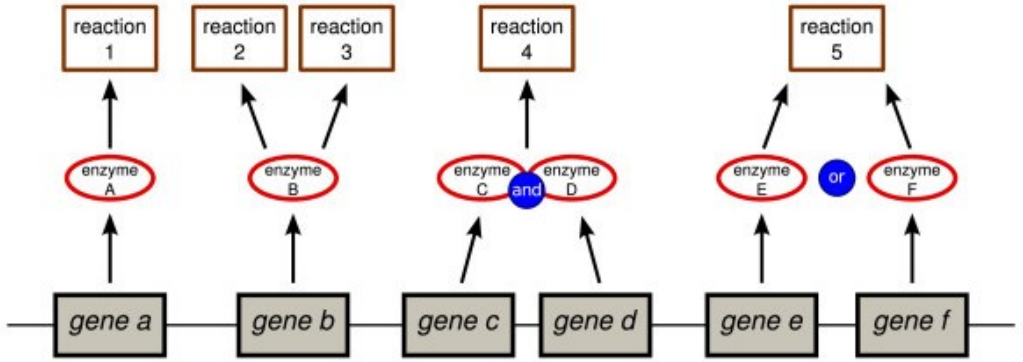
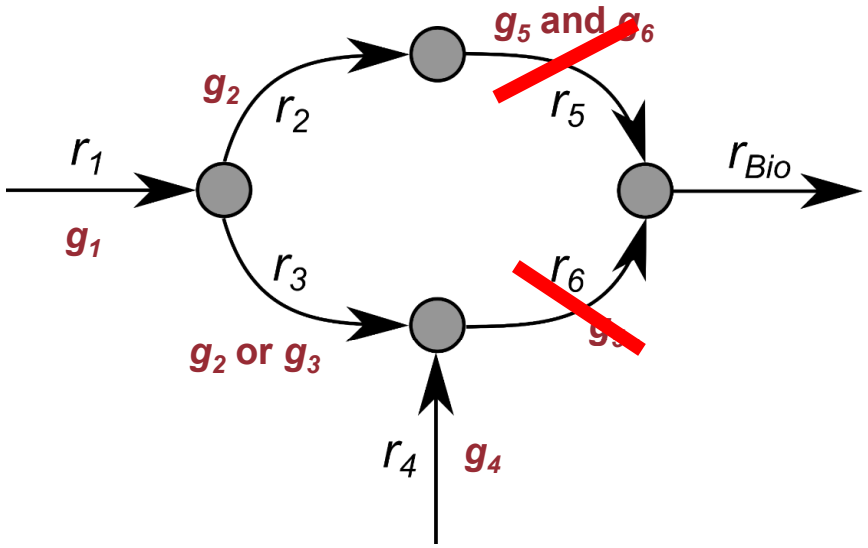
PROBLEM

Due to complex **GPR rules**, minimal reaction knockout strategies may not be minimal at the gene level.



P. Jensen et al, 2011, BMC systems biology

MCSs – Limitations



GPR rules

Minimal Cut Set	Gene knockout
r_1, r_4	g_1, g_4
r_1, r_6	~~g_1, g_5~~
r_2, r_6	~~g_2, g_5~~
r_5, r_6	g_5
	~~g_5, g_6~~
r_2, r_3, r_4	g_2, g_3, g_4
r_3, r_4, r_5	~~g_2, g_3, g_4, g_5~~
	~~g_2, g_3, g_4, g_6~~

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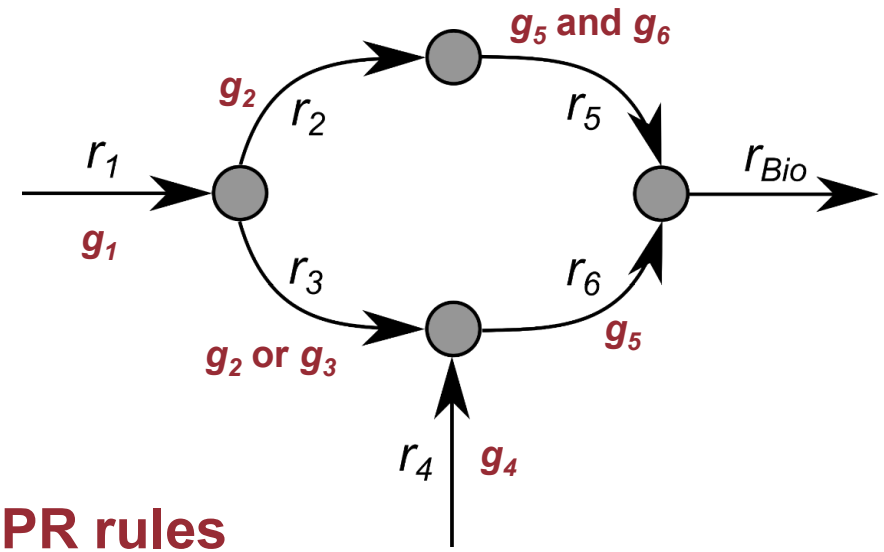
genetic Minimal Cut Sets – gMCSs

Identification of groups of metabolic **genes**, that, when simultaneously inhibited, render celular proliferation impossible.

gMCSs – Our Approach



GPR rules

Based on:

- Optimization Theory
- Duality Theory
- Linear Algebra

Inputs:

- Template Metabolic Network
- Target metabolic task
- **GPR rules**

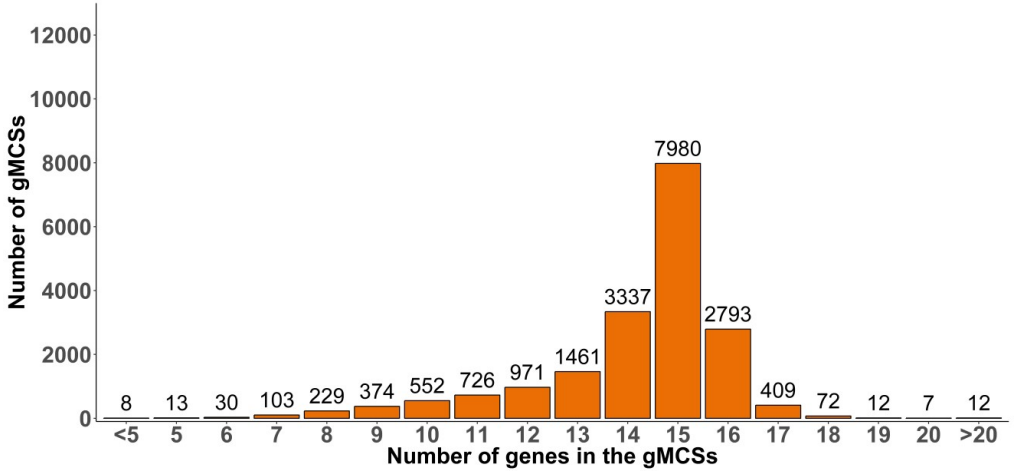
genetic Minimal Cut Sets:

- g_5
- g_1, g_4
- g_2, g_3, g_4

I. Apaolaza, 2017, Nature Communications

gMCSs – Our Approach

- A more efficient tool for the calculation of gMCSs was later implemented in the COBRA Toolbox, **gMCS function.**
- Technical details can be found in **I. Apaolaza et al, 2018, Bioinformatics**.
- Some results (see **poster 92 of Luis V. Valcarcel**):
  - 20,000 gMCSs for Recon3D in less than 48 hours (4 cores at 2.70 GHz, 16GB RAM).

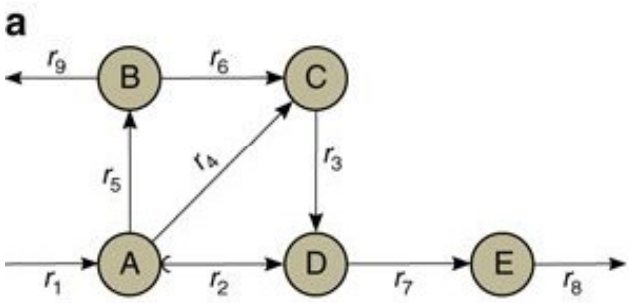


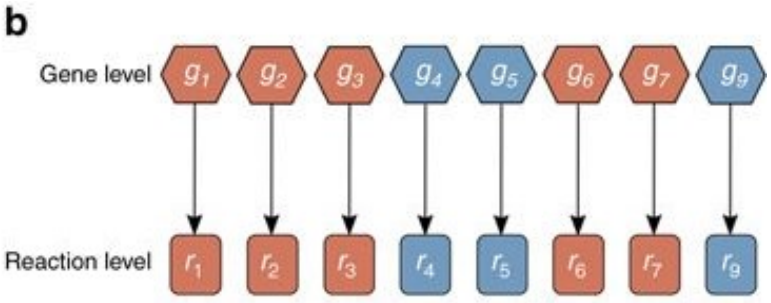
Heirendt et al, 2018,
Nature Protocols (accepted)

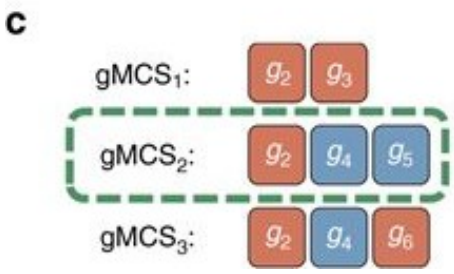
gMCSs - Cancer

Returning to our fundamental question:

A particular gene is essential if it is the only expressed gene in at least one gMCS







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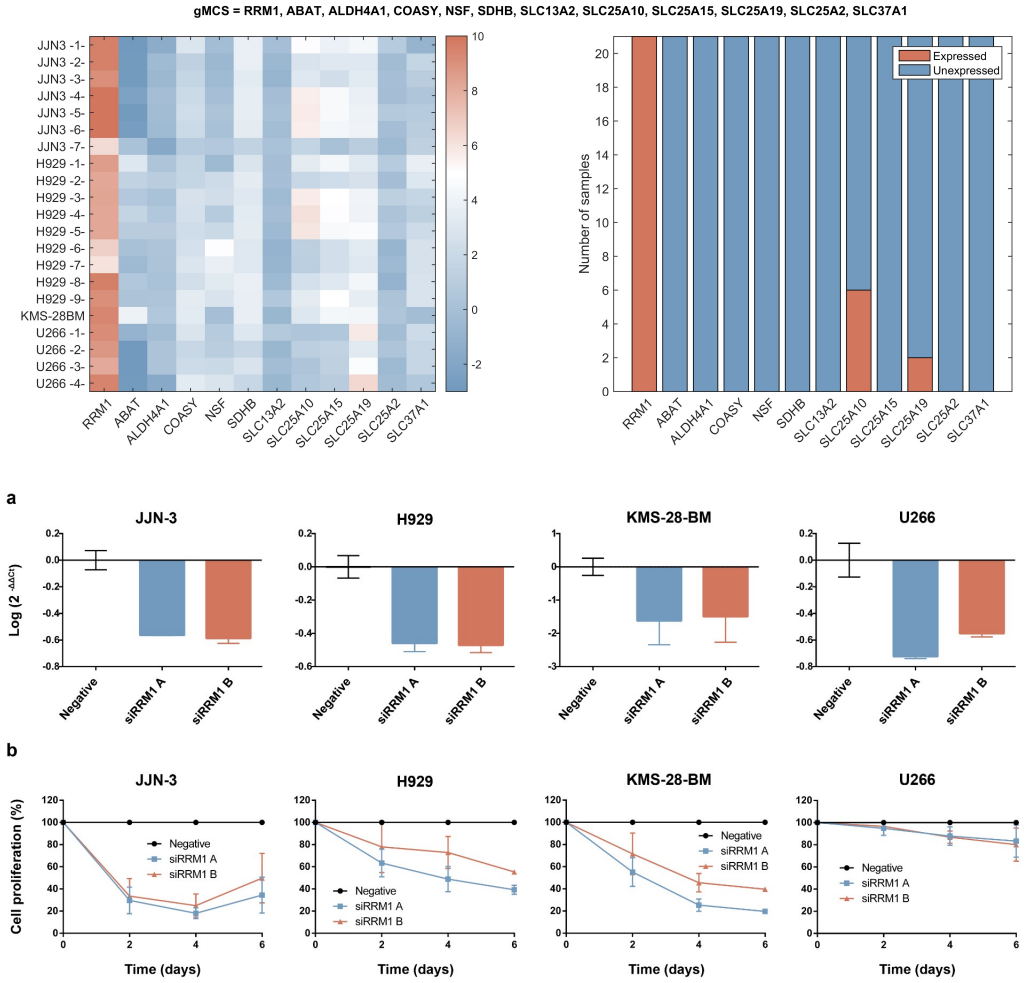
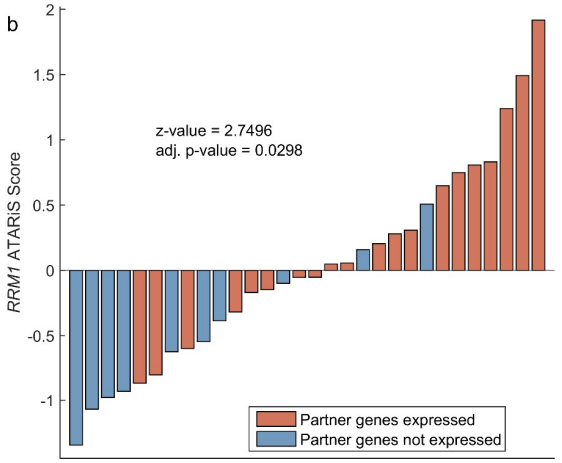
		gMCS approach		
		Essential	Non-essential	Total
Achilles	Essential	167	141	308
	Non-essential	111	340	451
	Total	278	481	759

p-value = 3.78×10−16, Odds Ratio (OR) = 3.62

I. Apaolaza, 2017, Nature Communications

gMCSs - Cancer





Essentiality of RRM1 (Ribonucleotide Reductate Catalytic Subunit M1**) in different cancer cell lines**

I. Apaolaza, 2017, Nature Communications

gMCSs – Cancer

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- **Reconstruction process is avoided** to identify cancer-specific essential genes.
- Possibility to calculate **gMCSs involving a particular gene knockout**.
- Possibility to calculate **gMCSs among a selected subset of genes** (e.g. lowly expressed genes).
- The expression of partner genes of a cancer-specific essential gene (e.g. RRM1) can be used as **response biomarkers**.

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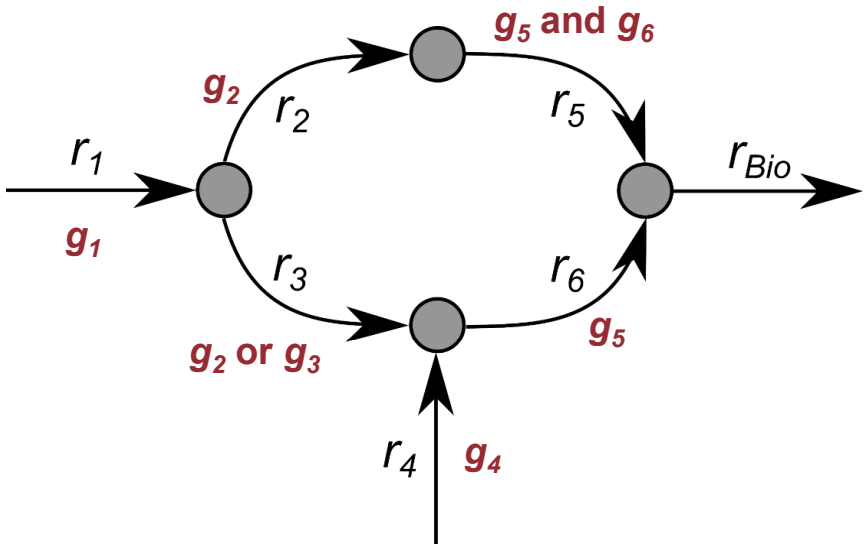
gene & drug Minimal Cut Sets – gdMCSs

Minimal subsets of **metabolic inhibitors (drugs)** and **gene knockouts**
that render celular proliferation impossible.

gdMCSs – Our Approach



Based on:

- Optimization Theory
- Duality Theory
- Linear Algebra

Inputs:

- Template Metabolic Network
- Target metabolic task
- **GPR rules**
- **Drug – Target Relationships**

Example:

Will d_1 be effective for a given patient?

Translation:

Is there a gdMCS which contains d_1 and lowly expressed genes for the patient under study?

Drug Target Relationships:

- d_1: g_1

Solution:

$\{d_1, g_4\}$ is a gdMCS. If g_4 is not expressed, the patient will benefit from a therapy with d_1.

In addition, g_4 is a biomarker for the effectiveness of therapy with d_1.

gdMCSs – Methotrexate

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- Targets Dihydrofolate Reductase, **DHFR**.
- DHFR is a **metabolic gene** which converts dihydrofolate into tetrahydrofolate.
- Methotrexate is an interesting drug for our analysis since **its mainly interacts with metabolic targets**.

gdMCSs – Methotrexate

gdMCS_1
TK1
TK2
Methotrexate

- It is a gdMCS in **Recon2.v04** (I. Thiele et al, 2013, Nature Biotechnology) and **Recon3D_301** (E. Brunk et al, 2018, Nature Biotechnology).
- TK2 commonly not expressed.

HYPOTHESIS

The expression level of TK1 will explain de effectiveness of Methotrexate.

gdMCSs – Methotrexate

Published online 23 November 2012 *Nucleic Acids Research, 2013, Vol. 41, Database issue* **D955–D961**
doi:10.1093/nar/gks1111

Genomics of Drug Sensitivity in Cancer (GDSC): a resource for therapeutic biomarker discovery in cancer cells

Wanjuan Yang[1], Jorge Soares[1], Patricia Greninger[2], Elena J. Edelman[2], Howard Lightfoot[1], Simon Forbes[1], Nidhi Bindal[1], Dave Beare[1], James A. Smith[3], I. Richard Thompson[1], Sridhar Ramaswamy[2], P. Andrew Futreal[1], Daniel A. Haber[2,4], Michael R. Stratton[1], Cyril Benes[2], Ultan McDermott[1,*] and Mathew J. Garnett[1,*]

[1]Cancer Genome Project, Wellcome Trust Sanger Institute, Hinxton CB10 1SA, UK, [2]Center for Molecular Therapeutics, Massachusetts General Hospital Cancer Center, Harvard Medical School, Charlestown, MA 02129, USA, [3]Core Software Services, Wellcome Trust Sanger Institute, Hinxton CB10 1SA, UK and [4]Howard Hughes Medical Institute, Chevy Chase, MD 20815, USA

Received August 29, 2012; Revised October 15, 2012; Accepted October 20, 2012

ABSTRACT

Alterations in cancer genomes strongly influence clinical responses to treatment and in many instances are potent biomarkers for response to drugs. The Genomics of Drug Sensitivity in Cancer (GDSC) database (www.cancerRxgene.org) is the largest public resource for information on drug sensitivity in cancer cells and molecular markers of drug response. Data are freely available without restriction. GDSC currently contains drug sensitivity data for almost 75 000 experiments, describing response to 138 anticancer drugs across almost 700 cancer cell lines. To identify molecular markers of drug response, cell line drug sensitivity data are integrated with large genomic datasets obtained from the Catalogue of Somatic Mutations in Cancer database, including information on somatic mutations in cancer genes, gene amplification and deletion, tissue type and transcriptional data. Analysis of GDSC data is through a web portal focused on identifying molecular biomarkers of drug sensitivity based on queries of specific anticancer drugs or cancer genes. Graphical representations of the data are used throughout with links to related resources and all datasets are fully downloadable. GDSC provides a unique resource incorporating large drug sensitivity and genomic datasets to facilitate the discovery of new therapeutic biomarkers for cancer therapies.

INTRODUCTION

There is compelling evidence that alterations in cancer genomes can strongly influence clinical responses to anticancer therapies. Indeed, there are now several examples where genomic changes can be used as molecular biomarkers to identify patients most likely to benefit from a treatment. For example, the use of drugs to target the protein product of the *BCR–ABL* translocation in chronic myeloid leukemia, or the *BRAF* gene in malignant melanoma, has transformed the treatment of these diseases and substantially improved survival rates (1,2). Despite these notable successes, many cancer drugs in use or development have not been linked to specific genomic markers that could direct their clinical use to maximize patient benefit. Moreover, even among appropriately selected patients, a poorly explained range of clinical responses is observed (2,3). Thus, there exists a need for the development of new and improved biomarkers to guide therapies and ultimately improve clinical responses.

Recent years have seen significant advances in our understanding of the molecular nature of cancer (4). This has been driven in part by advances in high-throughput technologies and, in particular, DNA sequencing technologies that allow us to sequence on a scale that was previously unthinkable. In the near future, sequencing efforts will provide a complete description of the genomic changes that occur in many cancer subtypes. A complete list of the repertoire of cancer genes will provide profound insights into the origins, evolution and progression of cancer and will act as an impetus for the development of new cancer therapies.

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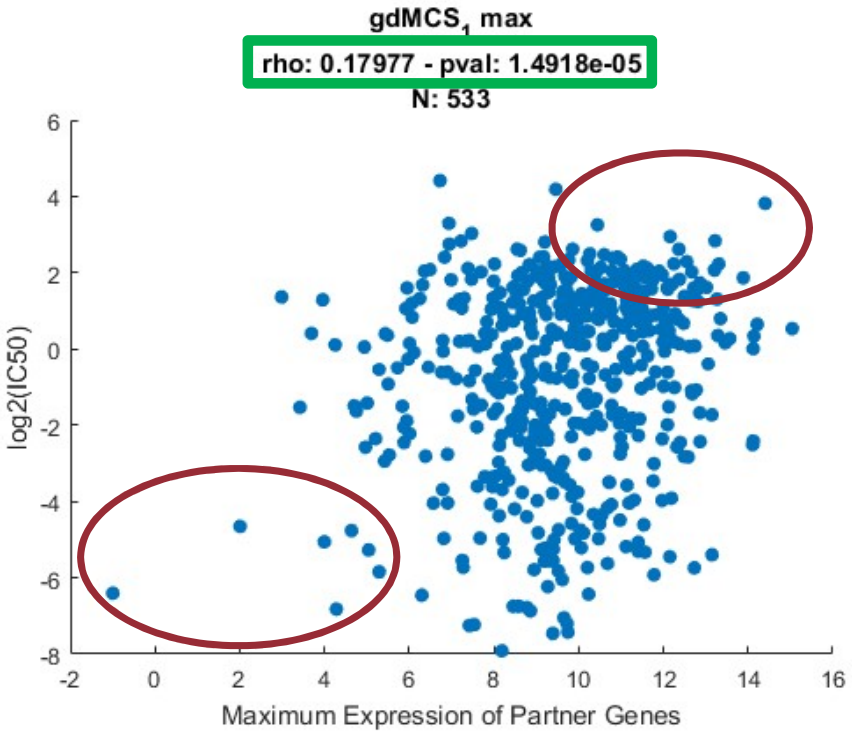
IC50 values of Methotrexate for 533 cell lines from **Genomics of Drug Sensitivity (GDSC)** and gene expression data from the **Cancer Cell Line Encyclopedia**

We expect **a higher expression of the partner genes (TK1 and TK2) in those cell lines with a higher IC50** value of Methotrexate.

gdMCSs – Genomics of Drug Sensitivity in Cancer (GDSC)

gdMCS_1
TK1
TK2
Methotrexate



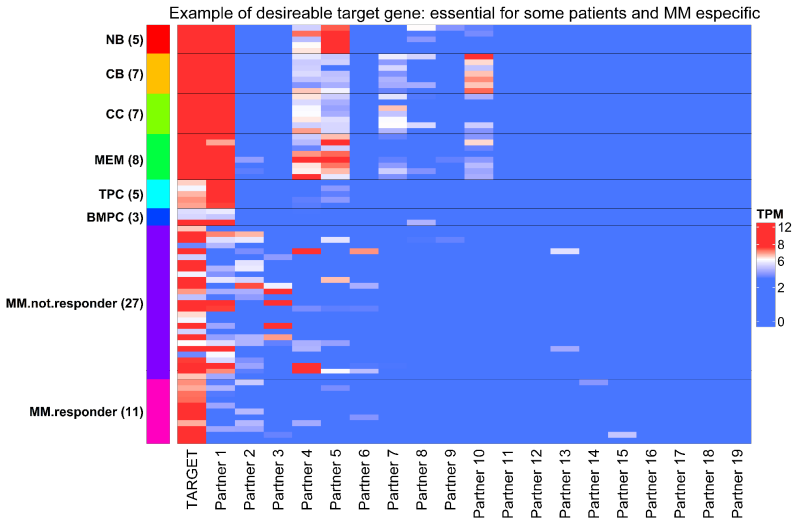
SENSITIVE

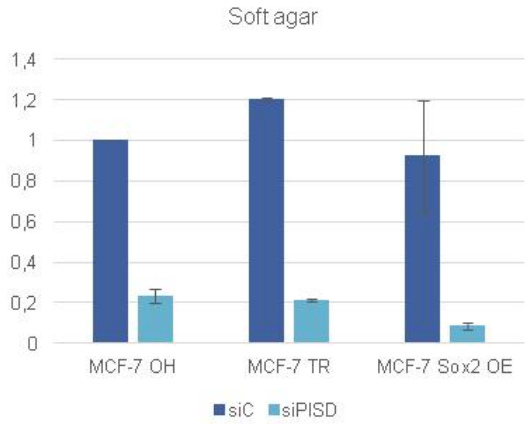
KE-37
JVM2
PF-382
P12-ICHIKAWA

RESISTANT

A498
LOUNH91
U87 MG
BT549

Future Directions





- Integration of **RNA-seq data from MM patients and healthy cells** (Poster 92).
- Application of our approach to target **tamoxifene-resistance breast cancer tumors**
- **In-vitro validation of synergy of TK1 knockout and methotrexate.**
- Minimal strategies involving **nutrient restrictions and gene knockouts**.
- Integration of **tracer-based metabolomics data**.
- Accounting for **cellular adaptation to our intervention**.
- Extend our approach to **signalling and regulatory networks.**

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Synthetic lethality in cancer research via genetic Minimal Cut Sets

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