Assessing gene essentiality using genome-wide CRISPR screens

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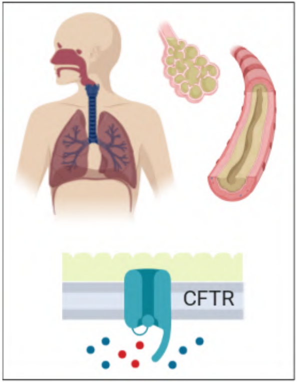
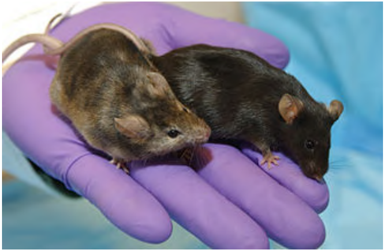
Reverse vs. forward genetics

Forward genetics:
Find the genetic basis for a specific observed phenotype.



Discovery of CFTR gene mutation causing Cystic fibrosis.

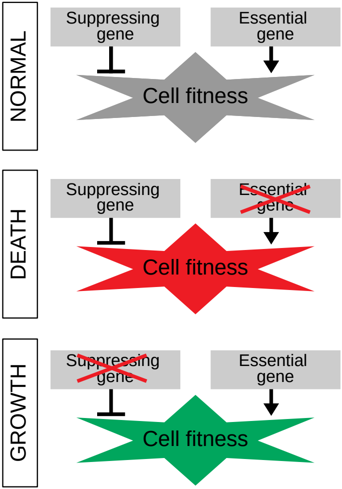
Reverse genetics:
Modify gene sequence and analyze the resulting phenotype.



Wikipedia

Knockout of gene affecting hair growth.

Biological motivation for reverse genetics screens



Core essential genes:

- *RPL13* - ribosomal component
- *POLR1B* - RNA polymerase

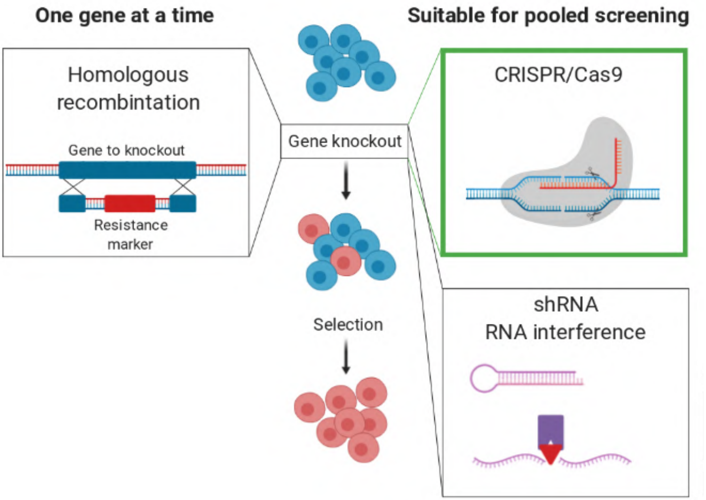
Growthsupressing genes:

- "tumor suppressor"
- *TP53*
- *BRCA1*

Synthetic lethality to target tumor cells:

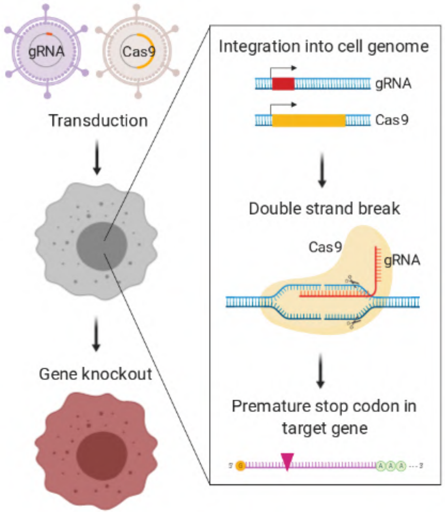
- *PARP* in *BRCA1* mutated tumors
- *BRAF* in *KRAS* mutated tumors

Advantages of using CRISPR-Cas9 for gene knockout



* shRNA based screens have problems with off-target effects and weak phenotypes.

Guide RNA (gRNA) simultaneously serves as perturbagen and barcode

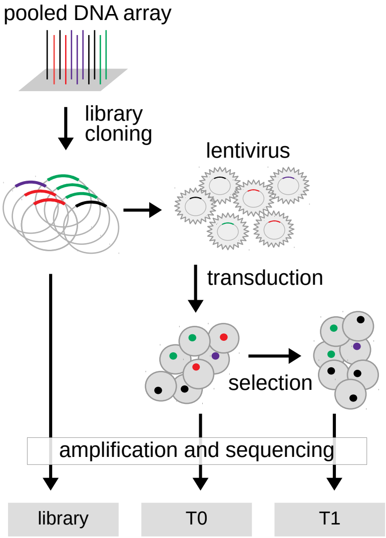


- gRNA can be PCR amplified from genome
- serves as a proxy for gene knockout

Different types of CRISPR mediated genetic perturbations

Name	**CRISPR associated enzyme**	**perturbation**
CRISPR-KO	Cas9	gene knockout
CRISPRi	dCas9 + transcription inactivator	expression inhibition
CRISPRa	dCas9 + transcription activator	expression activation
CRISPR-BE	dCas9 + base editor	base editing (C-G, A-T)

Experimental procedure of pooled CRISPR screens

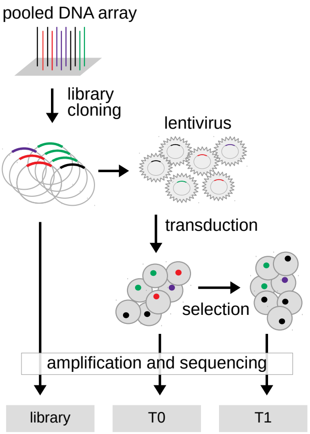


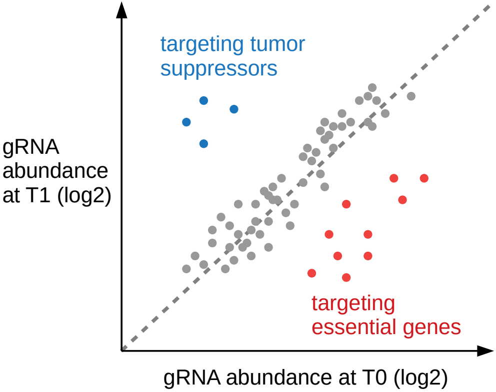
Experimental design principle

- **guide RNA/ gRNA**: perturbagen and barcode
- library size around 100K gRNAs
- perturbed cells growing in a pool
- individual growth rate depends on gene knockout
- compare abundance T0 vs. T1

For protocol see e.g. Joung et al. 2017

Phenotype detection in pooled CRISPR screens



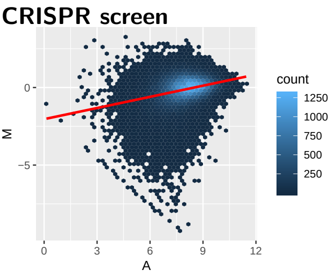


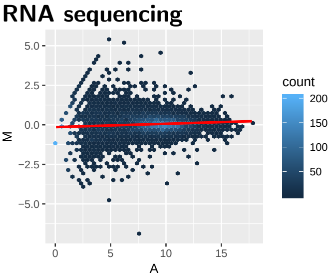
Differences between RNA-seq and CRISPR screening data

M-A plot

Logarithmic fold change: $M = log_2(\frac{S_1}{S_2})$

Mean abundance: $A = \frac{1}{2}log_2(S_1 S_2)$



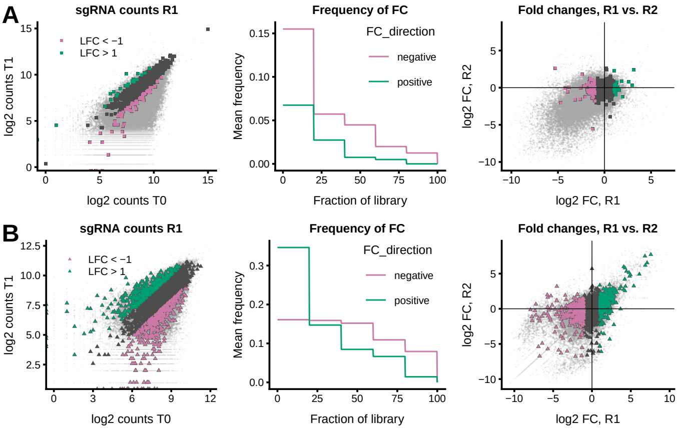


Screening data is skewed towards negative fold changes

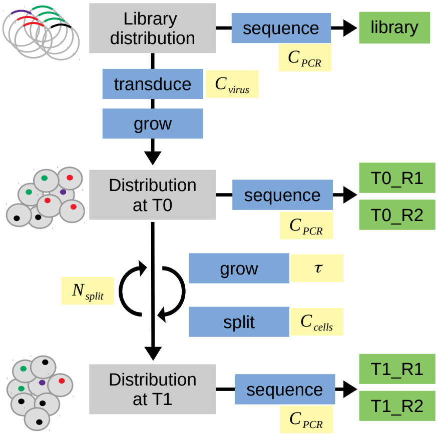
ASYMMETRY: T0 vs. T1 gRNA abundance

- negative logFC are more frequent
- especially for gRNAs that have low initial frequency

gRNAs with no expected effect on cell fitness

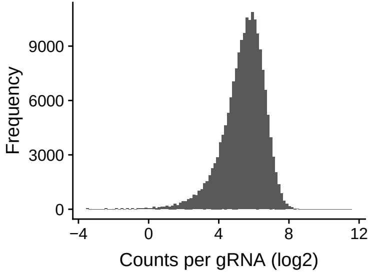


Computational simulation of screen to test influence of experiment design

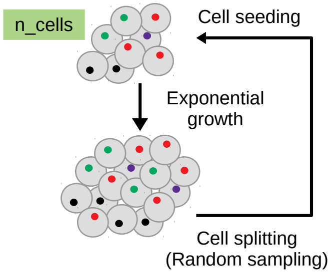


- gRNA counts modeled as a tuple of integer numbers
- result: counts after sequencing
- functions modify the counts (multiplication, random sampling)
- number of cell splittings N_{split}, cell duplication time τ
- "coverages" C_{PCR}, C_{virus}, C_{cells}

Mean gRNA coverage in pooled CRISPR screens determines cell number



Experiments assume a narrow distribution and are designed based on mean gRNA coverage.



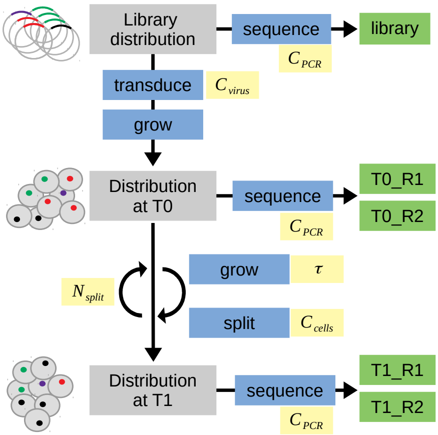
Mean coverage of gRNAs:

how many times is one gRNA on average represented in a pooled experiment?

$$\text{coverage} = \frac{n_{cells}}{n_{gRNAs}}$$

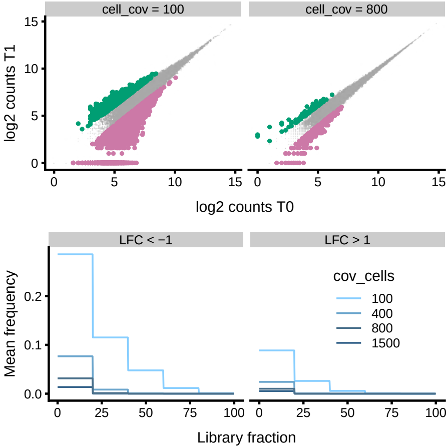
For example (coverage 500):
10^7gRNAs $\times$ 500 $= 5 \times 10^9$cells

Computational simulation of screen to test influence of experiment design



Cell splitting causes asymmetry in gRNA abundance changes

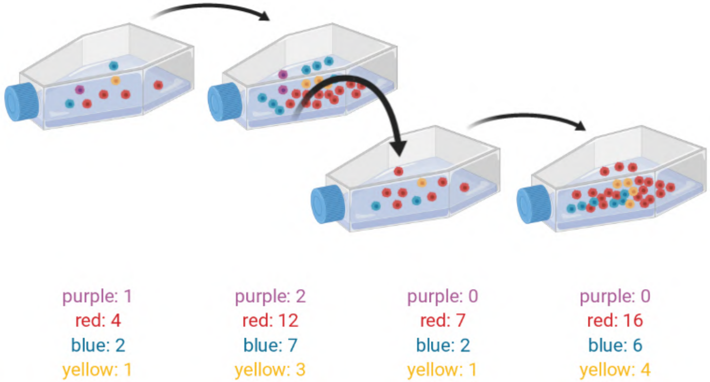
Simulation with different coverage



Lower gRNA coverage increases asymmetry of gRNA abundance changes.

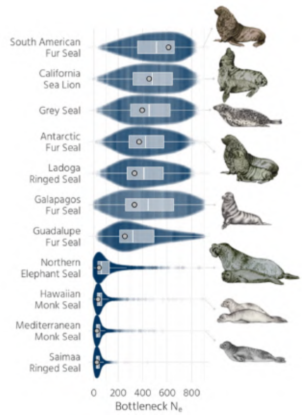
Asymmetry is caused by repetitive cell splitting

Bottle neck effect



Population bottlenecks in the Northern Elephant Seal

Bottle neck event: Hunting in 19th century, reduction of population size to 20 individuals. Today's 30,000 seals have a strongly reduced genetic diversity.

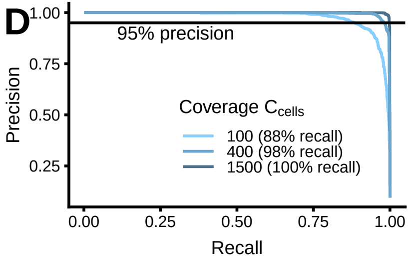


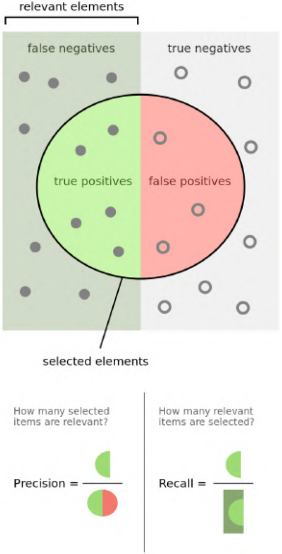
Stoffel et al.

It is not OK to assume symmetry of null-distribution!

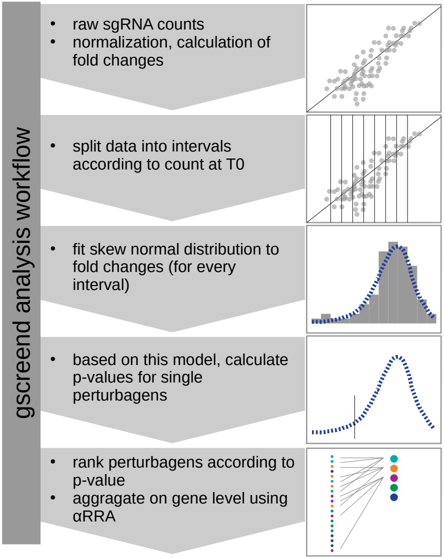
Current analysis tools loose detection power when asymmetry increases.

Detection of essential genes by MAGeCK-RRA (Li et al. 2014)





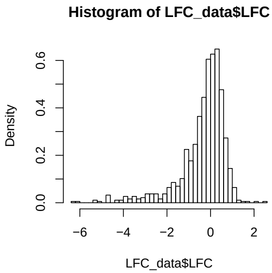
Software package gscreend with improved statistical test



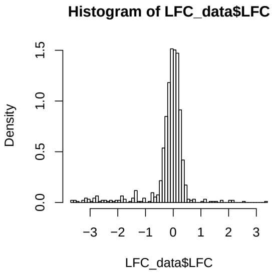
Step 1: Data preparation

- Normalization or scaling to the total counts in the reference sample.
- Calculation of logarithmic fold changes, addition of pseudo-counts: $LFC = log_2(\frac{n_{T_1} + 1}{n_{T_0} + 1})$
- Partitioning into groups according to abundance in reference sample.

Low abundant gRNAs (20-30% percentile)

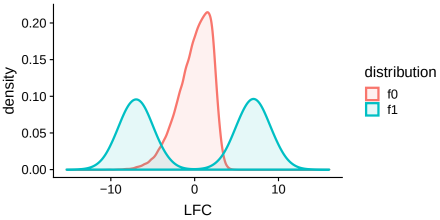


High abundant gRNAs (80-90% percentile)



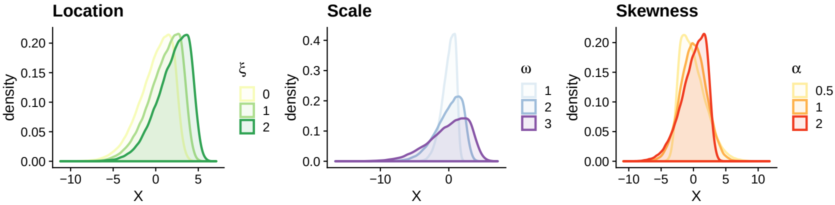
Step 2: Statistical modeling of gRNA level data

- Modeling of the data as a mixture of null-distribution f_0 and unknown distribution f_1 of the gRNAs with fitness effect:
  $f(x) = (1 - \lambda)f_0(x) + \lambda f_1(x)$



Step 2: Statistical modeling of gRNA level data

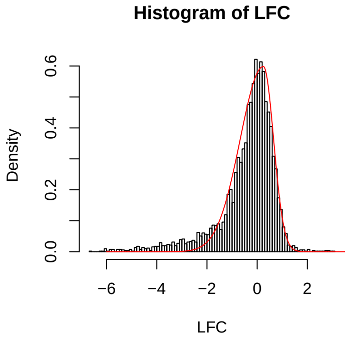
- Modeling of the data as a mixture of null-distribution f_0 and unknown distribution f_1 of the gRNAs with fitness effect:
  $f(x) = (1 - \lambda)f_0(x) + \lambda f_1(x)$
- f_0 is a skew normal distribution with 3 parameters:
  location ξ, scale parameter ω, skewness parameter α



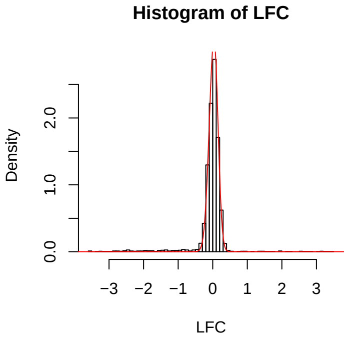
Step 3: Fitting the null-distribution

- Fit ξ, ω, and α from the actual LFC data.
- Ignore strong positive or negative LFCs, only consider the central 90% data point (using approach derived from least quantile of squares regression (*Rousseeuw et al. 1987*).

Low abundant gRNAs
(20-30% percentile)
$\xi = 0.16$, $\omega = 0.69$, $\alpha = 1.57$

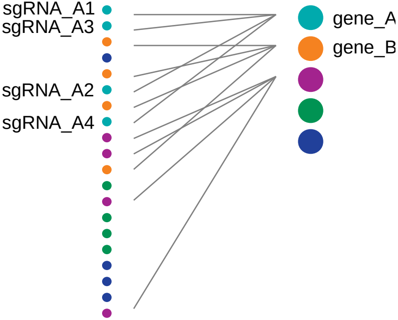


High abundant gRNAs
(80-90% percentile)
$\xi = -0.02$, $\omega = 0.13$, $\alpha = 1.09$



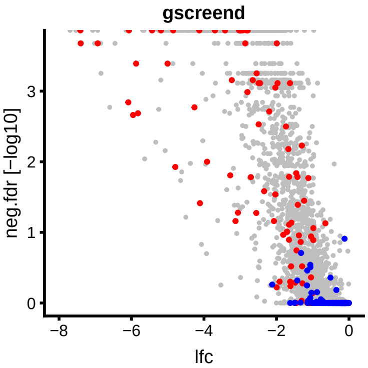
Step 4: Aggregation of gRNA level data to gene level

- Calculation of p-value for every gRNA.
- Ranking of gRNAs according to p-values.
- Robust rank aggregation (*Kolde et al. 2012*) to aggregate on gene level (typically 3-10 gRNAs per gene).
- Do the observed gRNA ranks for a given gene lie significantly outside of what you would expect by random sampling? (Permutation test)



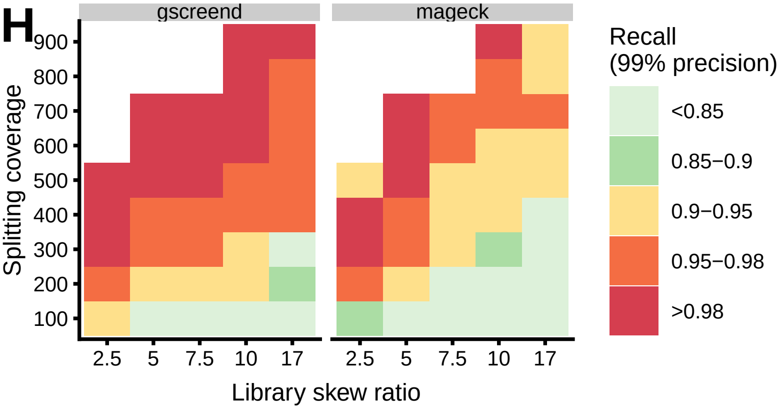
Results from a screen performed in HCT116 cells

components of the ribosome
non-essential genes



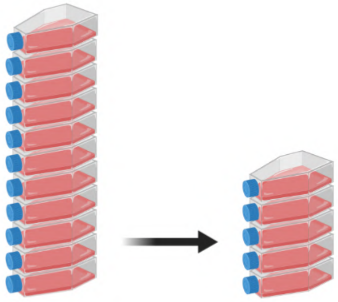
gscreend performance on simulated data

Ranking accuracy is improved using **gscreend** compared to other method.



This has major implications for experiment design

- We can predict the minimal necessary experiment size.
- gscreend allows reduction of experiment size by up to 50%.



Conclusions

- Understand the data from an experimental point of view!
- Changes in gRNA abundance are asymmetric in pooled CRISPR screens (unlike RNA-seq data).
- We provide recommendation for optimal experimental design.
- **gscreend**: more accurate phenotype detection at smaller experiment size.

gscreend (in preparation for Bioconductor submission):

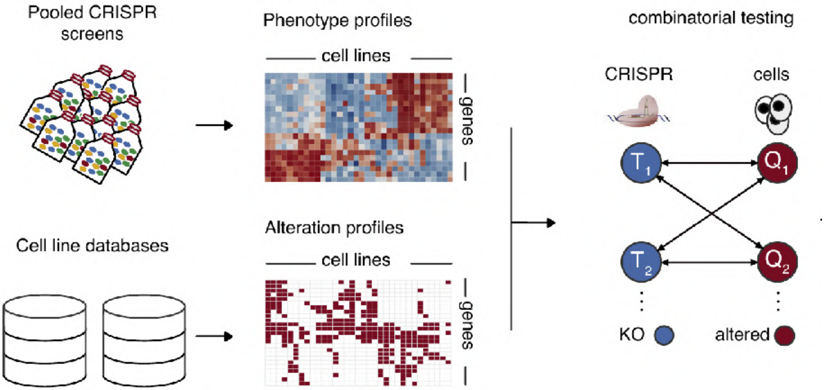
https://github.com/imkeller/gscreend

bioRxiv
Modeling asymmetric count ratios in CRISPR screens to decrease experiment size and improve phenotype detection
Katharina Imkeller, Giulia Ambrosi, Michael Boutros, Wolfgang Huber
doi: https://doi.org/10.1101/699348

Example for applications of CRISPR screens...

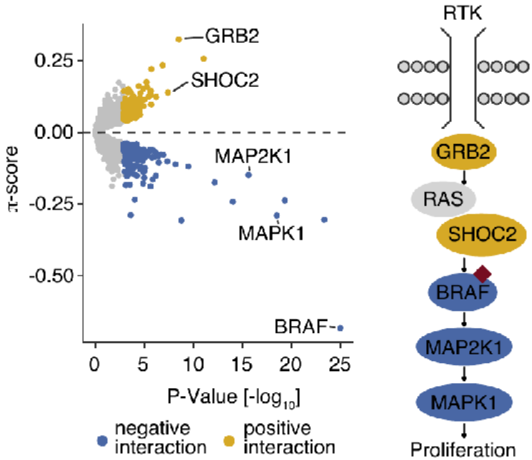
Context dependent lethality

Cancer dependency map: https://depmap.org/portal/



Rauscher et al. 2018, Henkel et al. 2019

BRAF mutation context dependency



Rauscher et al. 2018, Henkel et al. 2019

Resources

- Some of the graphics in this presentation were generated with Biorender (www.biorender.com)

- Rousseeuw, PJ, Leroy, AM. Robust regression and outlier detection. Wiley Series in Probability and Statistics 329 (1987).
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