Functional genomics screens

1. Introduction to loss-of-function approaches
2. Case studies in drug discovery

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30th of April 2025

A bit about me

Regular high school in Krakow, Poland

THE UNIVERSITY of EDINBURGH

Masters of Chemistry in Medicinal and Biological Chemistry with a Year Abroad

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Universität Basel

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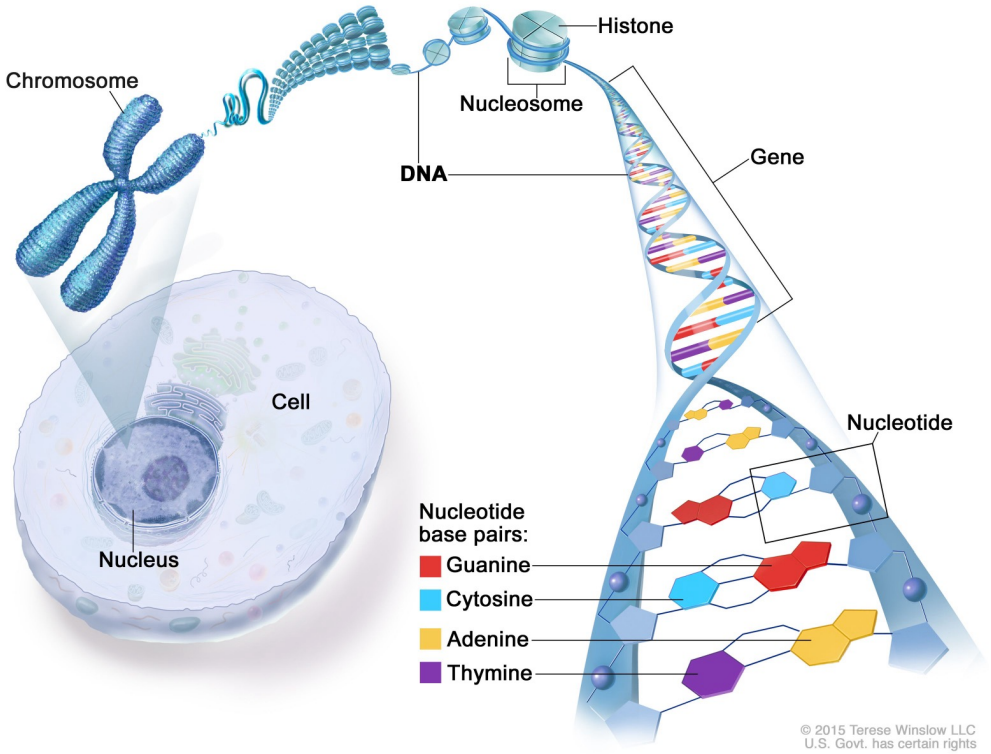
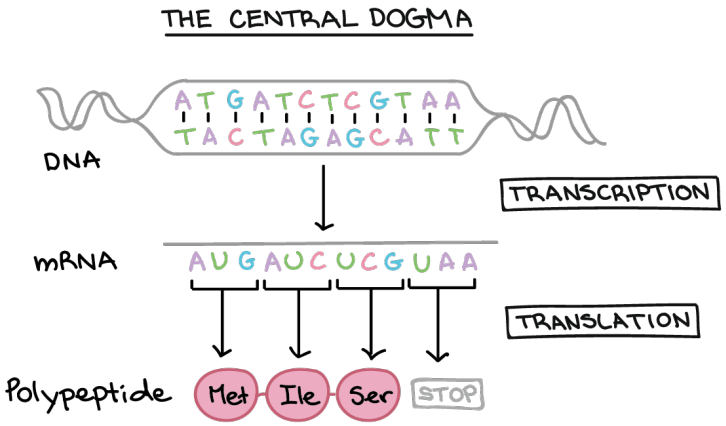
Dana-Farber Cancer Institute

connect via LinkedIn

The central dogma





Images adapted from NCI and Khan Academy

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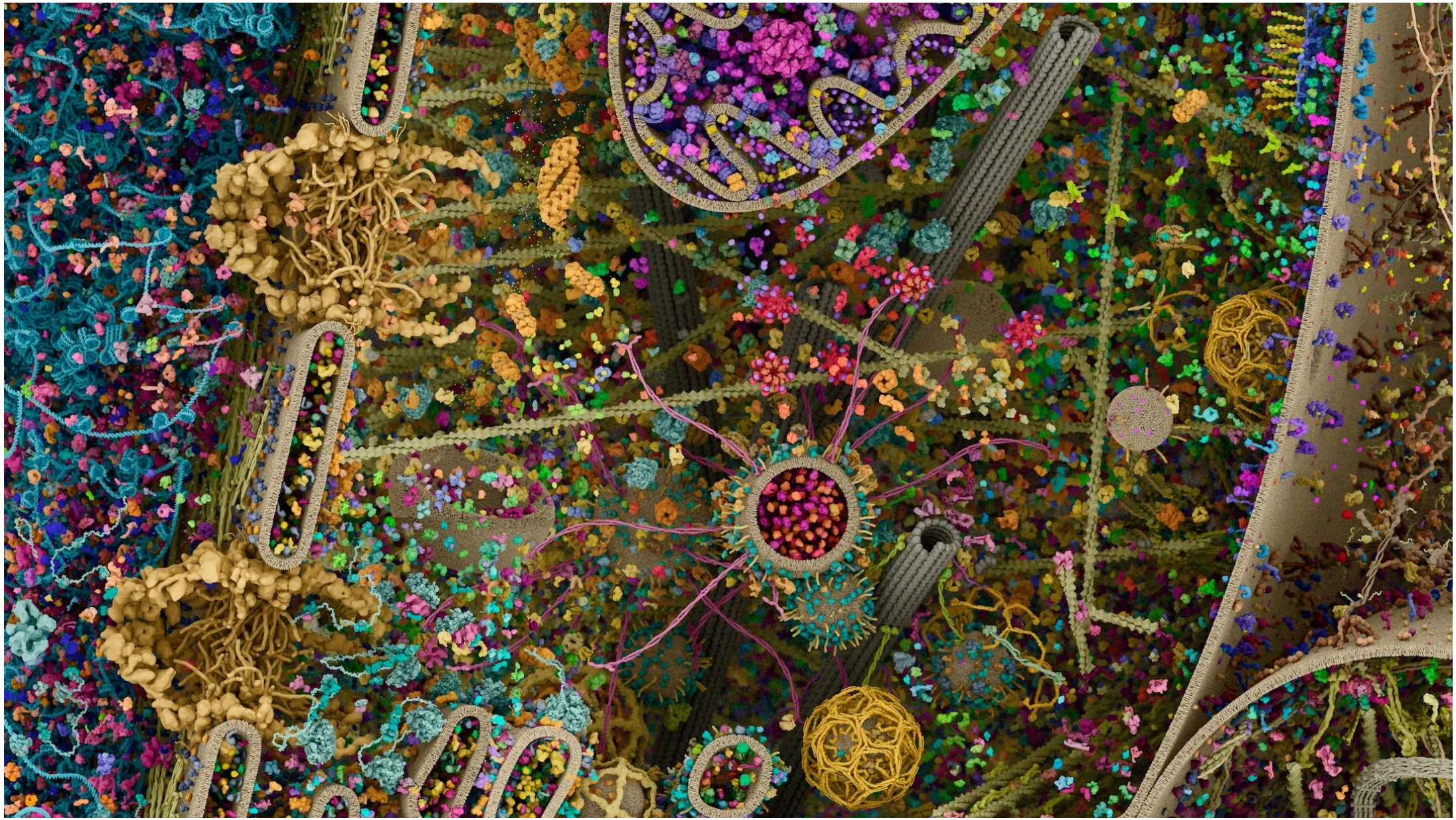

How???

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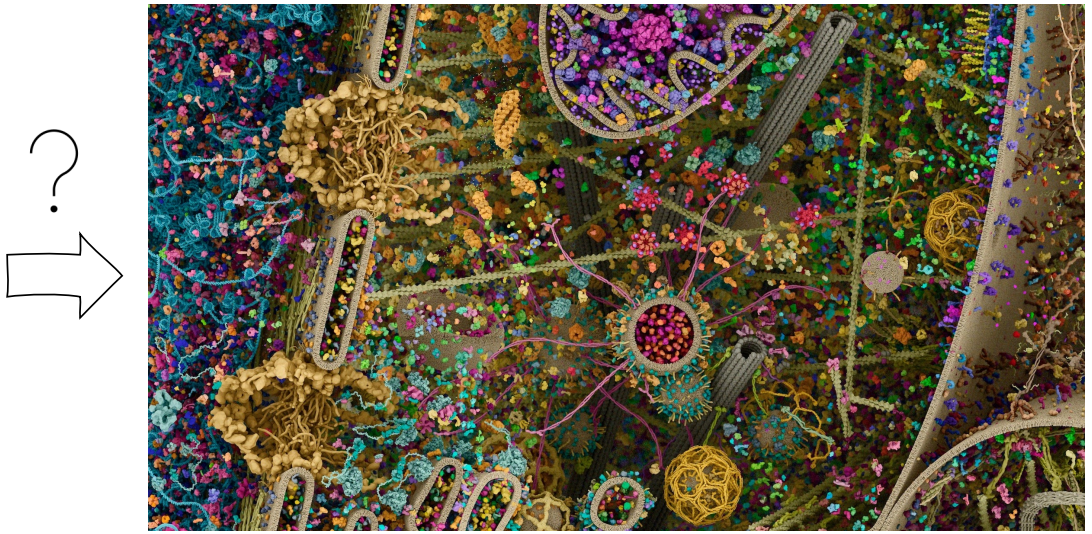
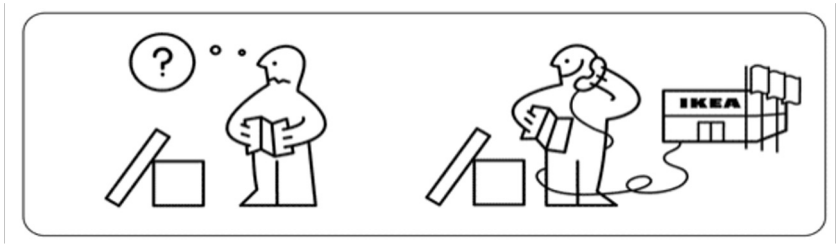
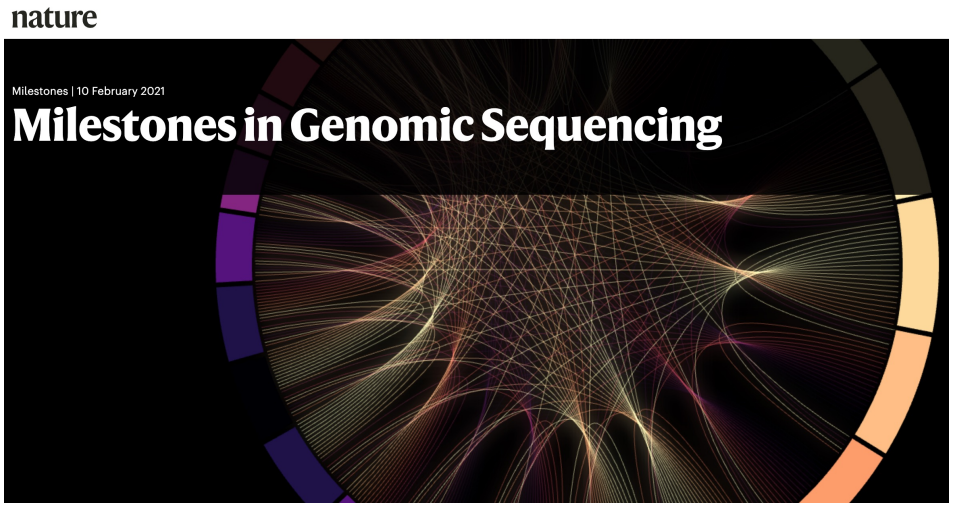




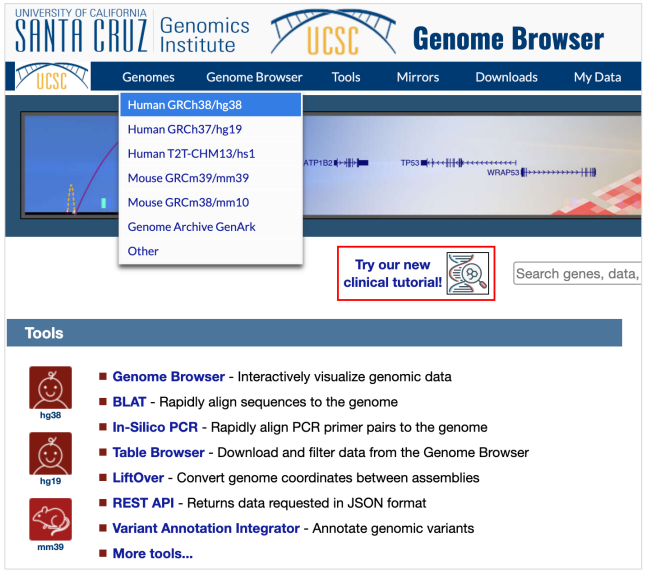
The Human Genome Project (1990-2003)

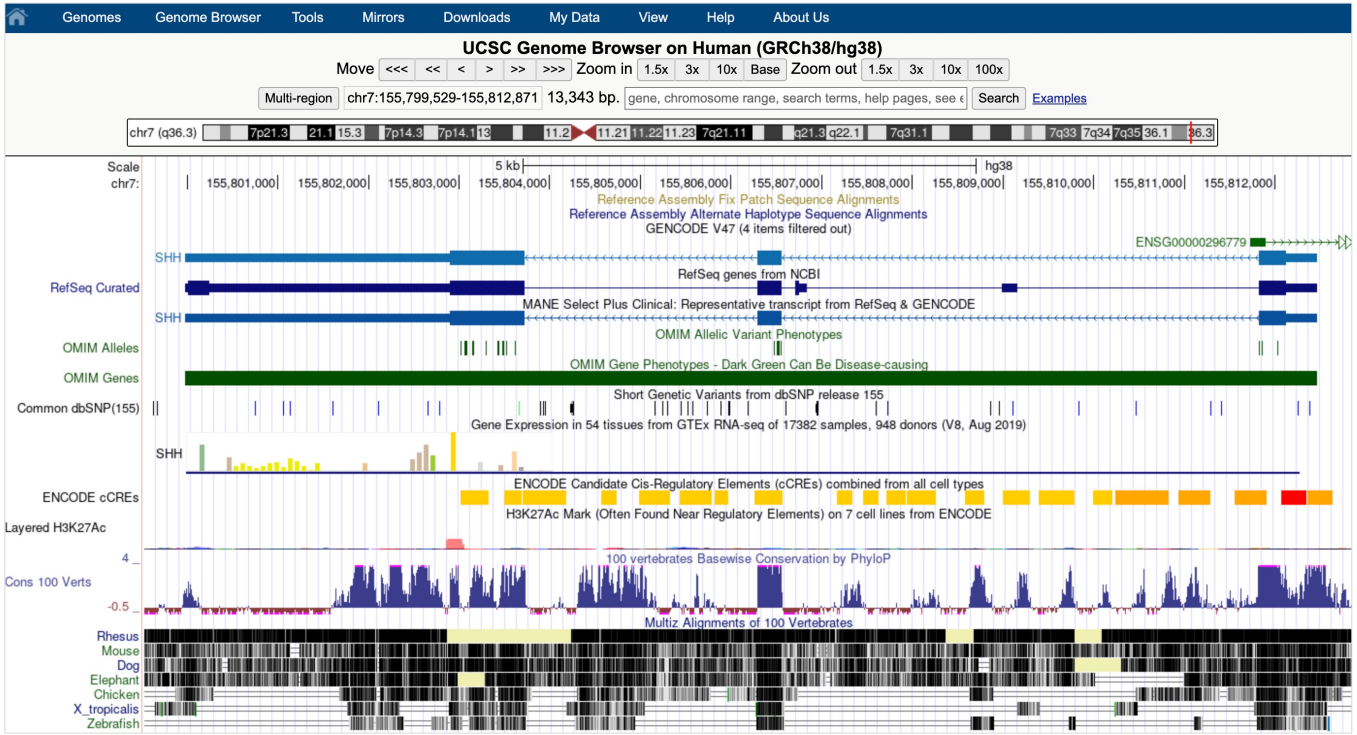
- $2’700’000’000 and 13 years to sequence ~90% of the human genome – a huge milestone
- Preceded by 30 years of technological advances as well as viral, bacterial, yeast, fly genomes
- See interactive visualization https://www.nature.com/immersive/d42859-020-00099-0/index.html

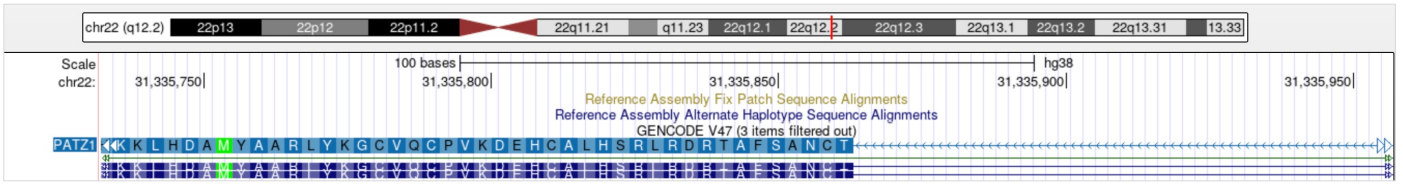


Images adapted from Nature and NCI

Today: simply (?) browse for your genes of interest

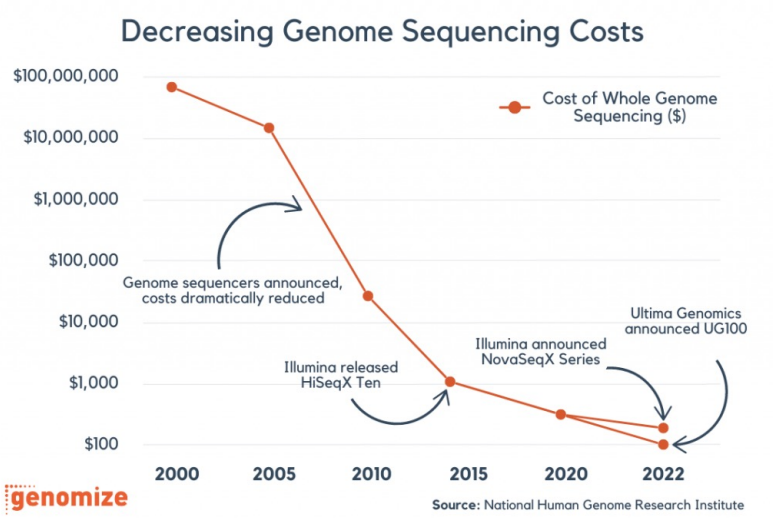




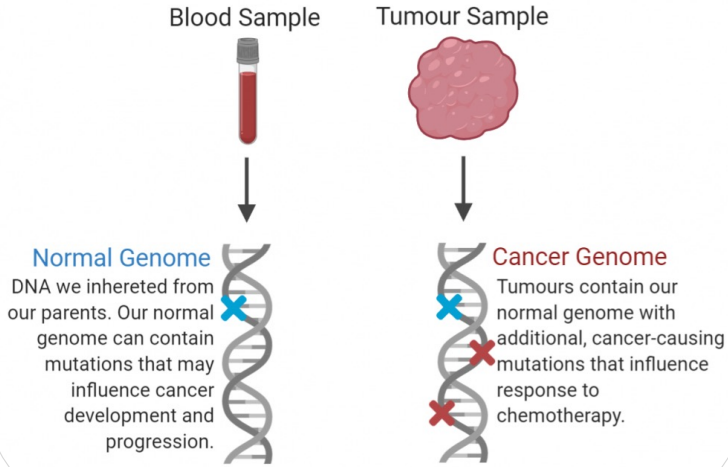


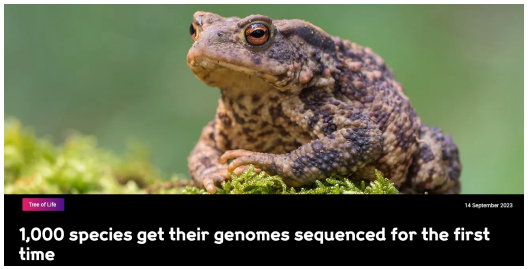
https://genome.ucsc.edu/index.html

Sequencing revolution – the promise of personalized medicine



some cancer patients get their whole genomes (or exomes) sequenced to inform treatment options:





Tree of Life Programme, Wellcome Sanger Institute

NEWS | 30 November 2023

World's biggest set of human genome sequences opens to scientists

The whole genomes of 500,000 people in the UK Biobank will help researchers to probe our genetic code for links to disease.

https://www.nature.com/articles/d41586-023-03763-3

Images adapted from Nature, genomize, and bcgsc

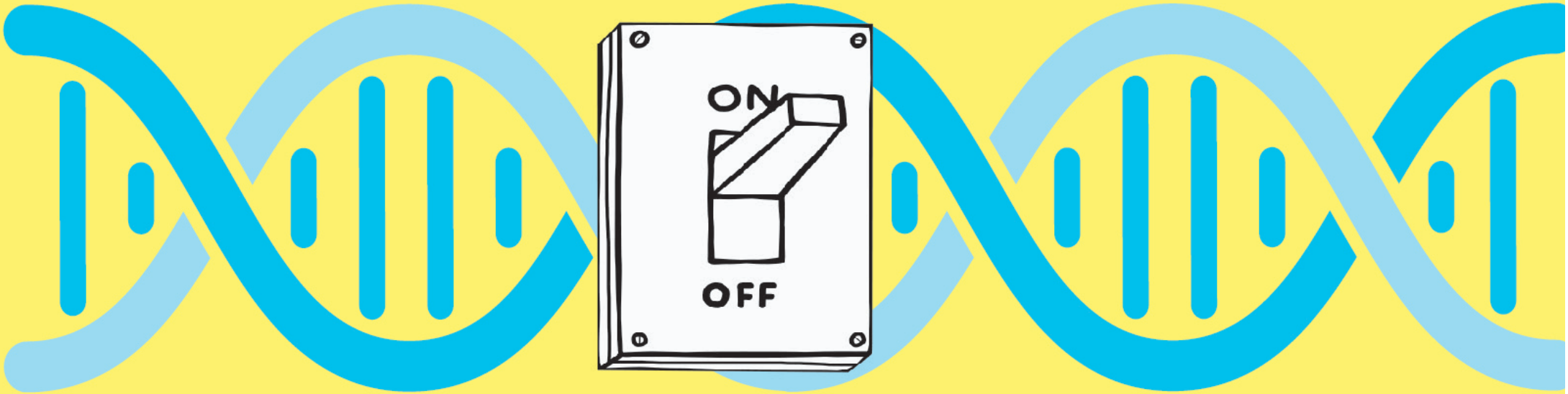
So: we (more or less) know what the parts are

- But …
- To this day 20-40% of human genes have largely unknown functions

- Armed with gene annotation we can use functional genomics to address questions like:
    - What are all the genes involved in my phenotype of interest?
    - How do genes interact with each other?
    - What genes drive disease or determine treatment sensitivity?
    - What genes are selectively essential in specific contexts (e.g. in cancer cells vs normal cells)?

genome sequencing identifies the parts —
functional genomics helps uncover their roles

How to interrogate gene function?



Adapted from www.fmi.c

Old-school genetics: from phenotype to genes responsible

Question: How does a fertilized egg develop into a complex organism with distinct body segments and organs in the right places?

Strategy: systematic population mutagenesis in flies (*Drosophila melanogaster*)

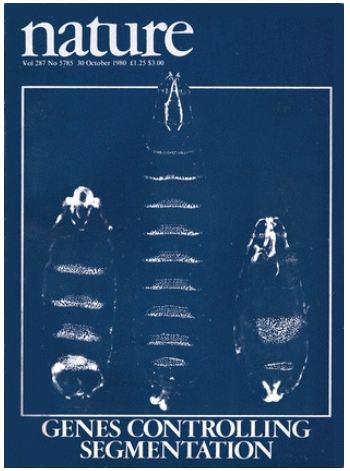
- induce random mutations using ethyl methanesulfonate (EMS)
- look at embryos from mutagenized parents: search for abnormal segmentation or patterning defects under the microscope

Findings:

- discovery of multiple classes of genes that regulate embryo development
- *e.g.* homeotic (HOX) genes that control segment identity
  - HOX mutations result in homeotic transformations, where one body part is replaced with another (e.g., antenna replaced by a leg)

Impact:

- these developmental genes are highly conserved across species
  - e.g. HOX genes in humans define regional identity along the anterior-posterior axis (e.g., limb positioning, organ placement)



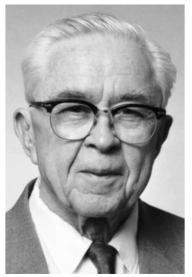


Photo from the Nobel Foundation archive.
Edward B. Lewis
Prize share: 1/3

Photo from the Nobel Foundation archive.
Christiane Nüsslein-Volhard
Prize share: 1/3

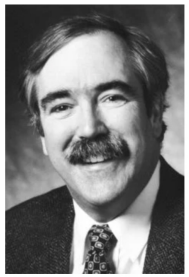


Photo from the Nobel Foundation archive.
Eric F. Wieschaus
Prize share: 1/3

Nobel Prize in Physiology or Medicine (1995)

Original paper: https://www.nature.com/articles/287795a0

How to interrogate gene function in modern genetics? Key tool: RNAi

Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*

Andrew Fire*, SiQun Xu*, Mary K. Montgomery*, Steven A. Kostas*†, Samuel E. Driver‡ & Craig C. Mello‡

Nature (1998)

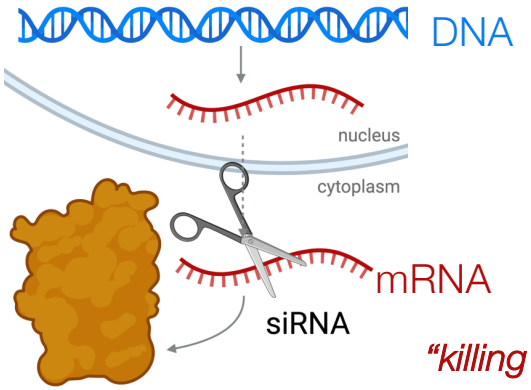
CNN
http://money.cnn.com › fortune_archive › 2003/05/26

Biotech's Billion Dollar Breakthrough

Fortune (2003)

The Nobel Prize in Physiology or Medicine 2006

Royal Swedish Academy (2006)

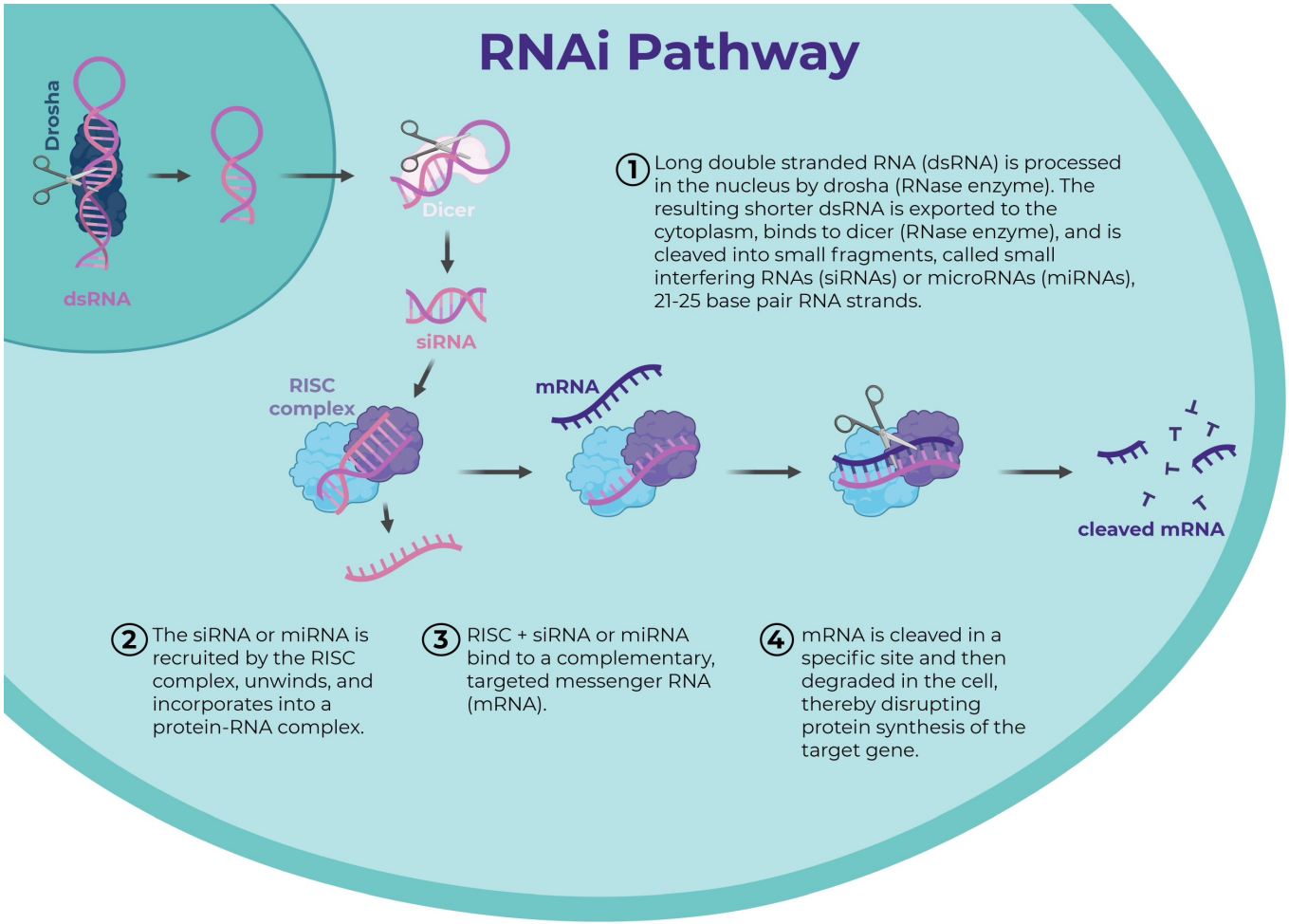


"killing the messenger"

"RNAi is a once-in-a-blue-moon breakthrough [that] within a few years should yield a rough idea of what each of our genes does"

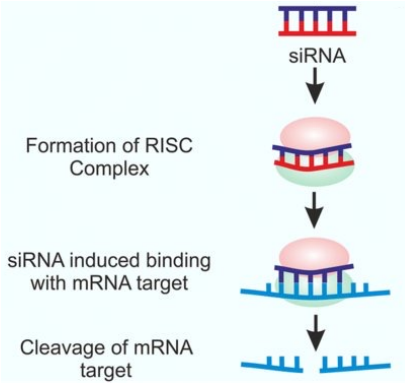
Photo: L. Cicero
Andrew Z. Fire
Prize share: 1/2

Photo: J. Mottern
Craig C. Mello
Prize share: 1/2

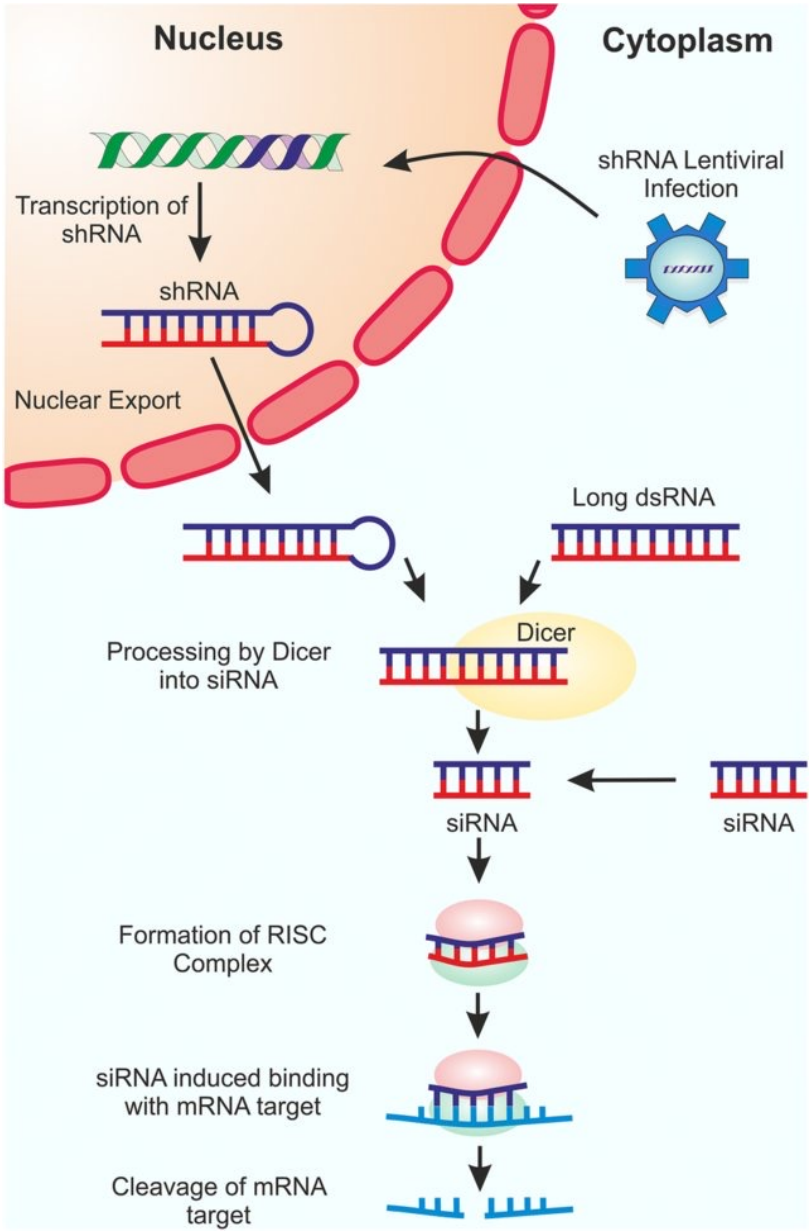


- RNAi is a conserved biological response to dsRNA
- RNAi mediates resistance to pathogenic nucleic acids and regulates the expression of protein-coding genes

pathway can be hijacked <u>by exogenous siRNAs</u> designed to target mRNA (and thus gene) of interest:



Adapted from UMass Chan Medical School



How to deliver siRNAs?
Usually don't – encode them instead:

- short hairpin RNAs (shRNAs) = short target sequence + hairpin spacer + sequence complementary to target but in reverse orientation
- shRNAs form the double-stranded short hairpin that enters the RNAi pathway and operates in the same manner as siRNAs
- can be produced in the cell when incorporated into vectors, e.g. lentivirus-based systems
- enables prolonged knock down and silencing in more complex systems (stem cells, animals)

Further reading:

RNAi review:

https://www.sciencedirect.com/science/article/pii/S1044579X03000440

example of using RNAi to discovery biology:

Identification of Host Proteins Required for HIV Infection Through a Functional Genomic Screen

ABRAHAM L. BRASS, DEREK M. DYKXHOORN, YAIR BENITA, NAN YAN, [...], AND STEPHEN J. ELLEDGE +3 authors Authors Info & Affiliations

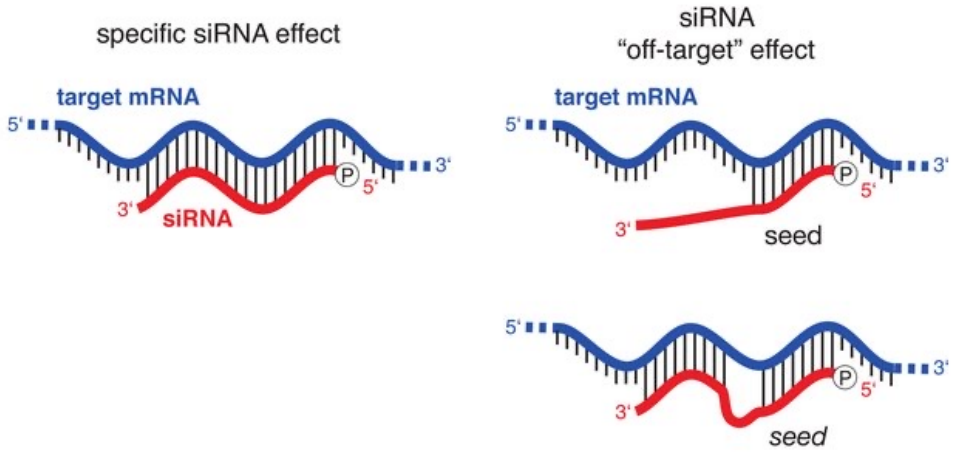
SCIENCE · 15 Feb 2008 · Vol 319, Issue 5865 · pp. 921-926 · DOI: 10.1126/science.1152725

https://www.science.org/doi/abs/10.1126/science.1152725

Adapted from UMass Chan Medical School

Important drawback: shRNA off-target effects are prevalent

- Gene-independent miRNA-like effects can dominate the signal
- shRNAs can bind partially complementary sequences (particularly in the 3'UTR of unintended transcripts)
  - "seed region" matches



→ So even if your shRNA was designed for gene A – it can downregulate genes B, C, D!

→ Leads to confounding phenotypes…

How to interrogate gene function in modern genetics? Key tool: ~~RNA~~i CRISPR

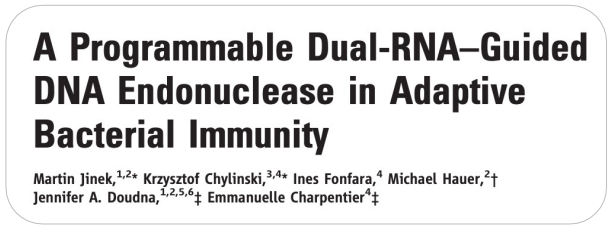
Fortune (2003)

"RNAi is a once-in-a-blue-moon breakthrough [that] within a few years should yield a rough idea of what each of our genes does"

Science (2012)

"The honeymoon is now over, and […], the yield has fallen far short of expectations. Many drug targets identified by means of si/shRNA technology in academic laboratories are not robust when tested in industrial laboratories … perhaps the most damaging of these [pitfalls] is the potential for any given si/shRNA to affect genes other than its intended target"

three weeks later CRISPR enters the scene…



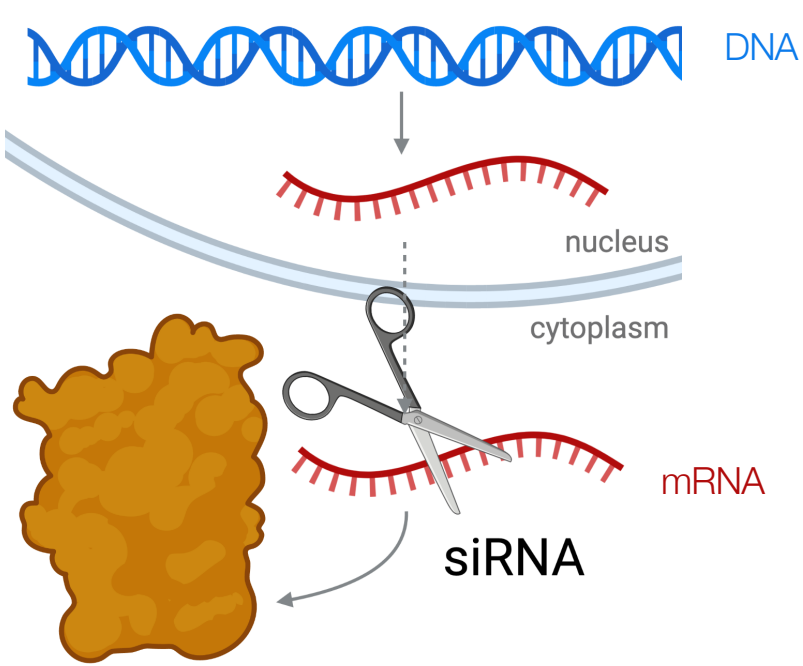
Science (2012)

"We show that the Cas9 endonuclease can be programmed with guide RNA engineered as a single transcript to target and cleave any dsDNA sequence of interest. The system is efficient, versatile, and programmable [and] could offer considerable potential for gene-targeting and genome-editing applications."

New kid on the block: CRISPR-Cas9

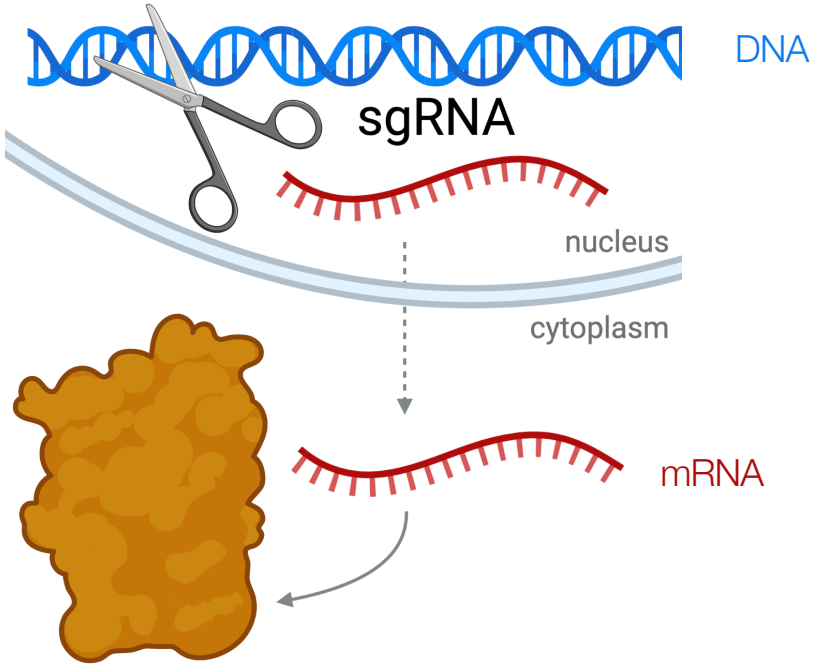
RNAi technology

"killing the messenger"



CRISPR technology

"editing the source code"



Cheat sheet – glossary:

RNAi (RNA interference): A biological process in which small RNA molecules inhibit gene expression by targeting and degrading specific mRNA molecules

siRNA (small interfering RNA): A class of double-stranded RNA molecules that guide the RNAi machinery to degrade specific mRNA, resulting in gene silencing

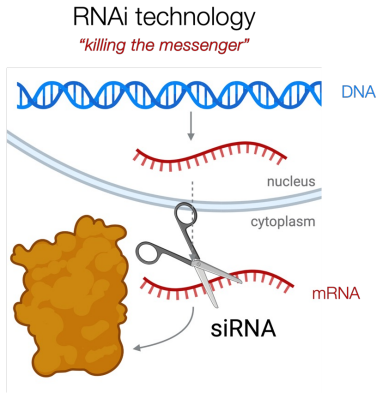
shRNA (short hairpin RNA): A DNA-encoded small RNA that forms a hairpin structure, which is processed by Dicer into siRNA to silence gene expression

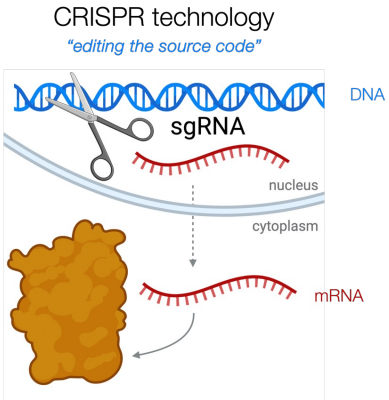
Knock-down (KD): Temporarily silences gene expression using methods like RNAi

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats): A genome-editing tool that uses a specific RNA sequence (sgRNA) to guide the Cas9 protein to target DNA for cutting, enabling precise modifications

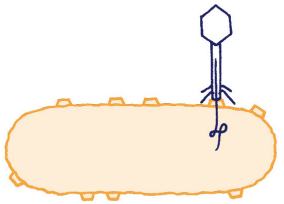
sgRNA (single guide RNA): A short RNA molecule used in CRISPR systems to direct the Cas9 enzyme to a specific target DNA sequence for editing

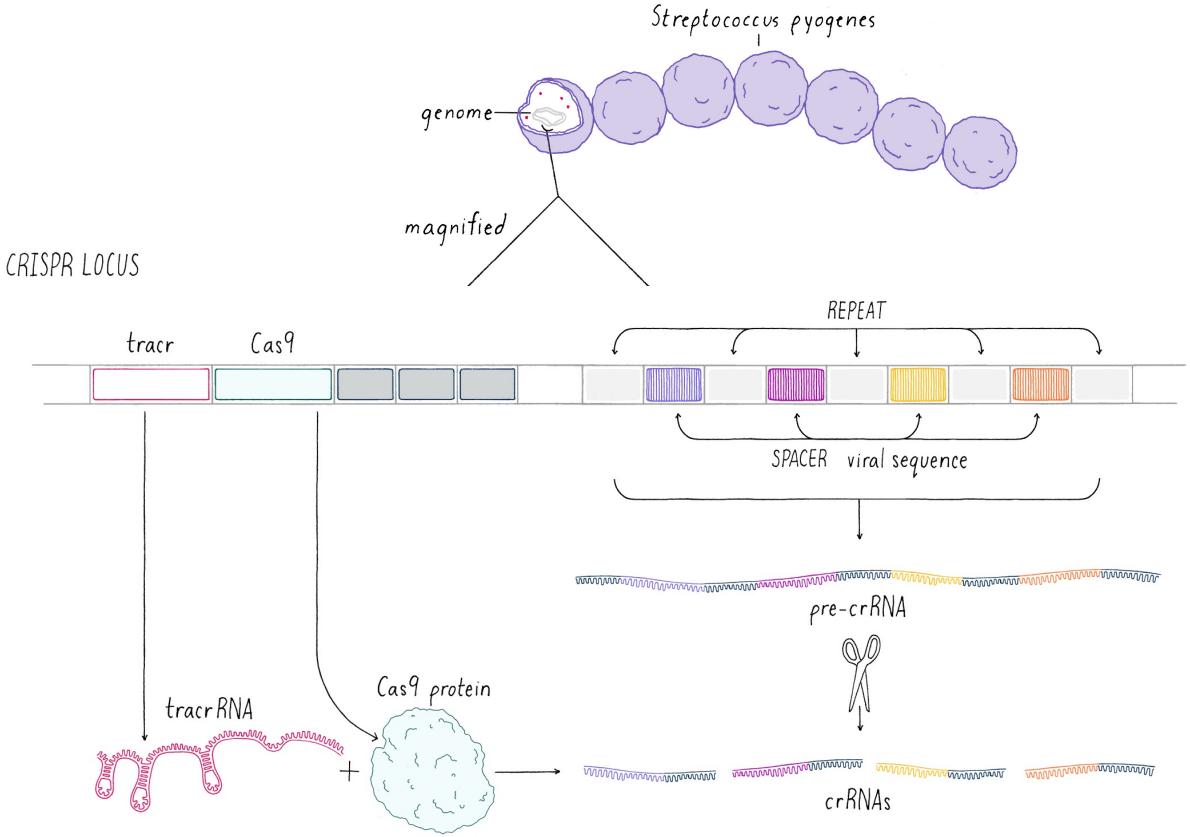
Knock-out (KO): Permanently disables a gene by introducing a loss-of-function mutation, truncation, or similar modification using methods like CRISPR





Discovery of CRISPR



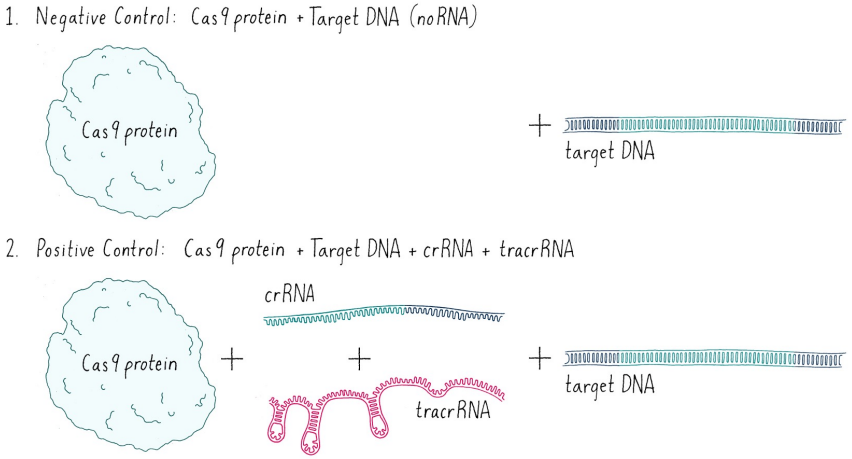


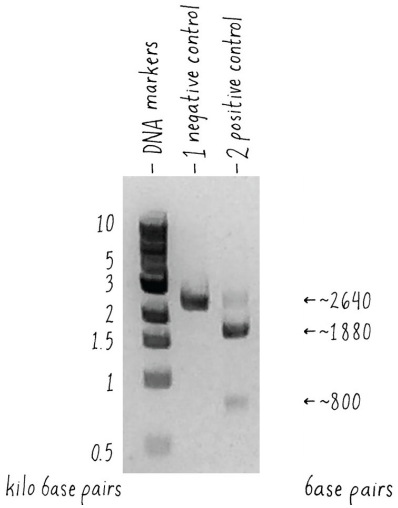
- CRISPR is a bacterial immune system that recognizes and cuts the DNA of an invading virus (bacteriophage)
- first recognised due to the presence of “spacers” (unique sequences acquired from bacteriophages) in bacterial genome
- CRISPR locus expresses a **tracrRNA,** a DNA cutting enzyme called **Cas** (from *Streptococcus pyrogenes*: Cas9), and various **crRNAs**
- work by Charpentier, Doudna, and others showed **Cas9** forms a complex with a **crRNA** and **tracrRNA** and then cuts across the double helix of the viral genome

Adapted from www.explorebiology.com

Proof of concept: CRISPR-Cas9 DNA cutting in a test tube

- perhaps one could “edit” genome sequences?
- components needed:
  - Cas9 protein
  - crRNA
  - tracrRNA
- Jinek et al. (2012) paper demonstrates programmable, sequence-specific DNA cutting



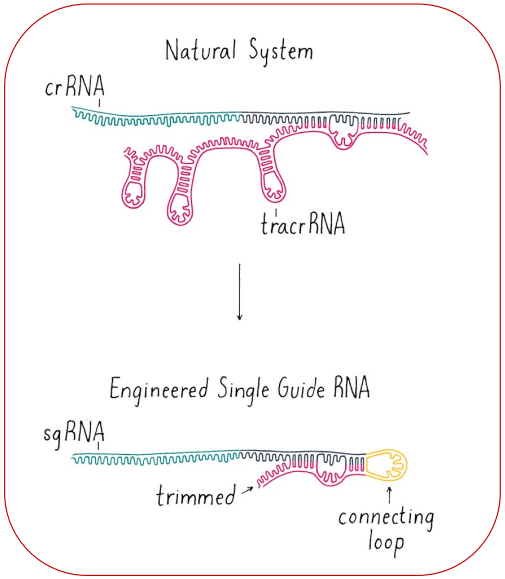


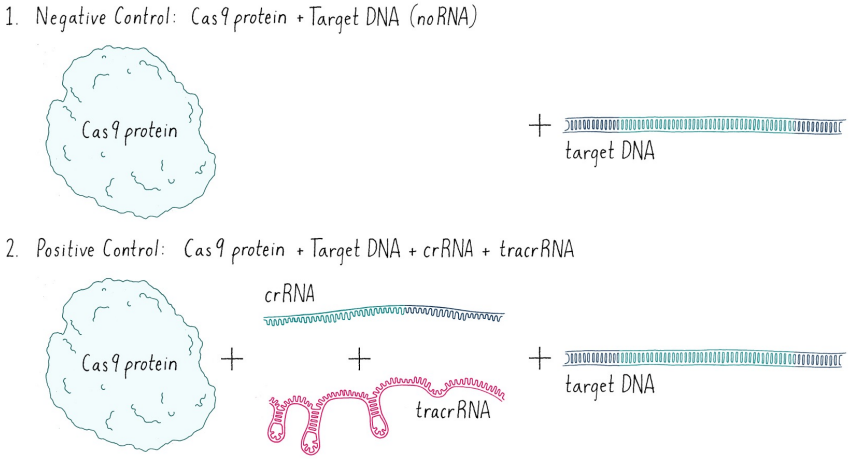
Adapted from www.explorebiology.com

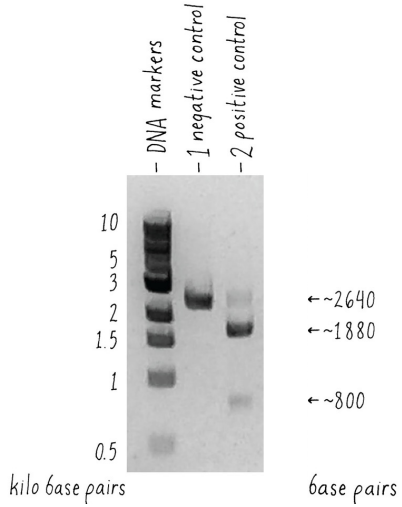
Proof of concept: CRISPR-Cas9 DNA cutting in a test tube

- perhaps one could “edit” genome sequences?
- components needed:
  - Cas9 protein
  - crRNA
  - tracrRNA

  } sgRNA → key simplification: from dual to single guide (sg) RNA
- Jinek et al. (2012) paper demonstrates programmable, sequence-specific DNA cutting

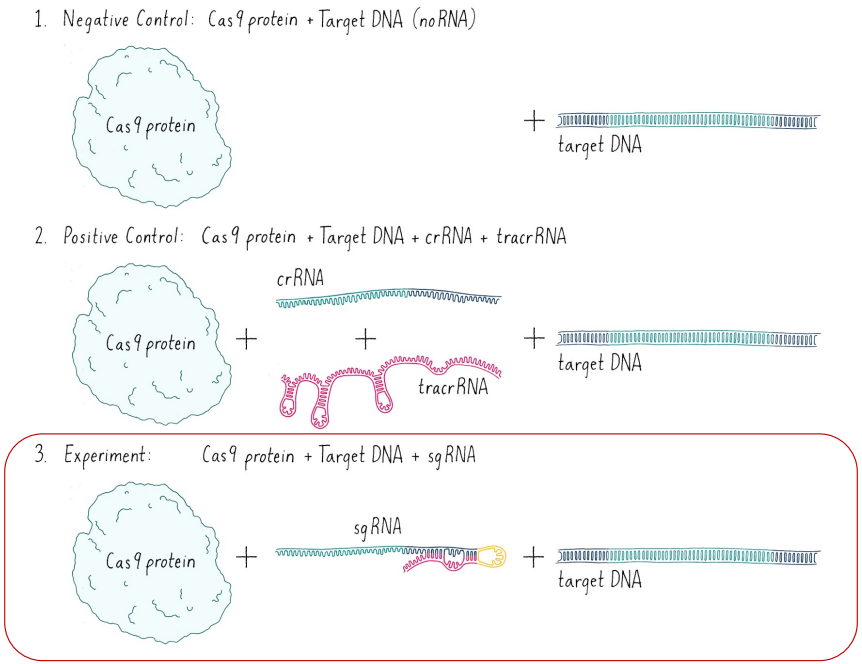


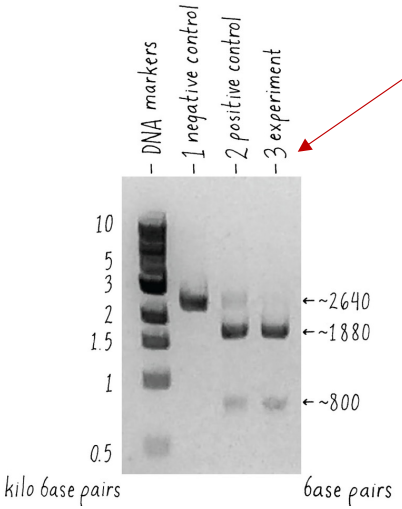




Adapted from www.explorebiology.com

CRISPR-Cas9 DNA cutting with sgRNA – a Nobel-prize winning discovery





works very well (better than the positive control!)

- programmable, sequence-specific DNA cutting: only Cas9 and an sgRNA
- Nobel Prize in Chemistry (2020) for Emmanuelle Charpentier and Jennifer Doudna
- dubbed the biggest revolution in biology since DNA cloning

Adapted from www.explorebiology.com

Interactive guide: https://www.biointeractive.org/classroom-resources/crispr-cas9-mechanism-applications

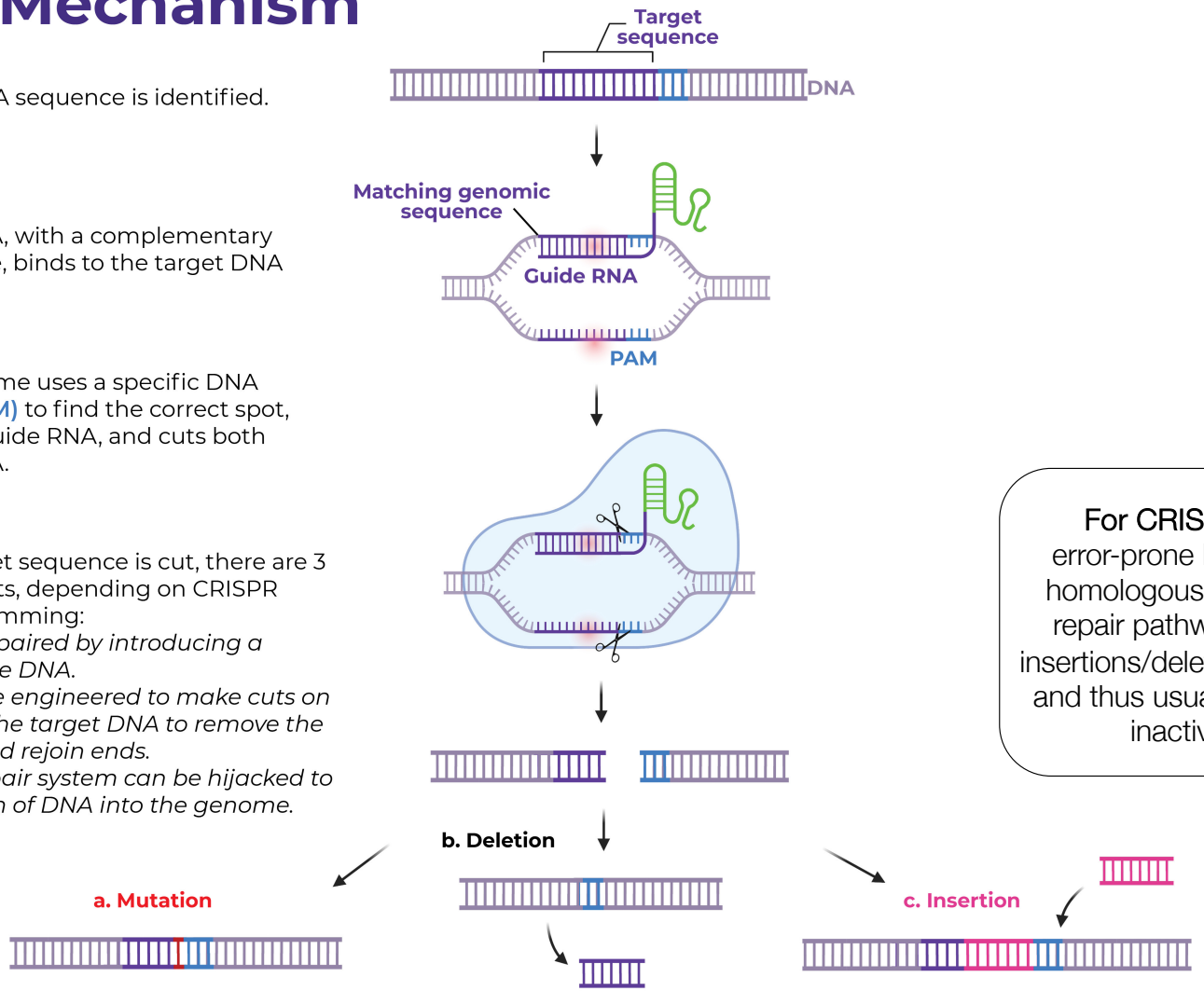
CRISPR Mechanism

1. The target DNA sequence is identified.

2. The guide RNA, with a complementary DNA sequence, binds to the target DNA sequence.

3. The Cas9 enzyme uses a specific DNA sequence **(PAM)** to find the correct spot, binds to the guide RNA, and cuts both strands of DNA.

4. Once the target sequence is cut, there are 3 potential results, depending on CRISPR system programming:
   a. The cut is repaired by introducing a mutation in the DNA.
   b. Enzymes are engineered to make cuts on either side of the target DNA to remove the target DNA and rejoin ends.
   c. The DNA repair system can be hijacked to insert a section of DNA into the genome.

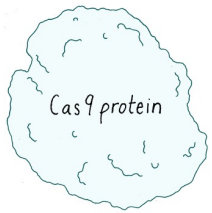


For CRISPR KOs:
error-prone NHEJ (non-homologous end joining) repair pathway leads to insertions/deletions (“indels”) and thus usually functional inactivation

Adapted from UMass Chan Medical School

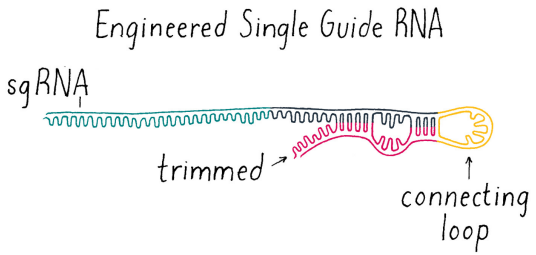
So I want to knock out a gene (in a human cell line)… What do I need?

1. Cas9



- Need the active bacterial nuclease - how to deliver it to cells?
- Could deliver protein (e.g. electroporation)
- More common to express it transiently from a plasmid
- In practice usually engineer cells to express Cas9 stably (e.g. using lentiviral transduction)

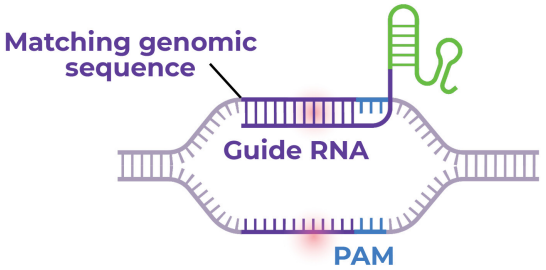
2. sgRNA



- Need a ~20 nt sequence complementary to target (remember the PAM - for Cas9: “NGG”)
- In practice use sophisticated tools: e.g. CHOPCHOP (https://chopchop.cbu.uib.no/)
- Delivery:
  - synthetic sgRNA via electroporation or lipid-based transfection
  - plasmid-encoded sgRNA delivered through lentiviral transduction or lipid-based transfection

- Possible to also deliver Cas9 + sgRNA together: both plasmid-encoded or electroporated as a complex

Basics of sgRNA design



Step 1: indentify a PAM (NGG) sequence in the DNA region you want to target

```
GTT GGG GGG AGG GGT CGG CAA TTG AAC CGG TGC CTA GAG AAG GTG GCG CGG
CAA CCC CCC TCC CCA GCC GTT AAC TTG GCC ACG GAT CTC TTC CAC CGC GCC
                                                    PAM
```

Step 2: determine the 5' start of sgRNA sequence by counting 20 nts upstream of the PAM

```
GTT GGG GGG AGG GGT CGG CAA TTG AAC CGG TGC CTA GAG AAG GTG GCG CGG
CAA CCC CCC TCC CCA GCC GTT AAC TTG GCC ACG GAT CTC TTC CAC CGC GCC
                                                    PAM
                          ↑
                5' G AAC CGG TGC CTA GAG AAG G
```

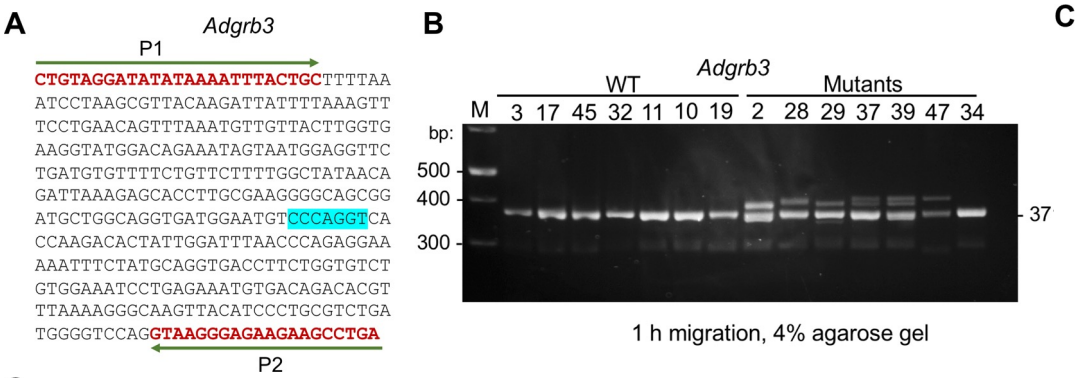
Important considerations:

- which region of the gene to target? Key domains? Splice isoforms? Fusions?
  - for knock-outs focus on critical exons early in the coding sequence
- off-target effects – check using prediction tools (e.g. http://www.rgenome.net/cas-offinder/)
- best practice to compare multiple (2-8) sgRNAs

So I want to knock out a gene (in a human cell line)… How do I know if I succeeded?

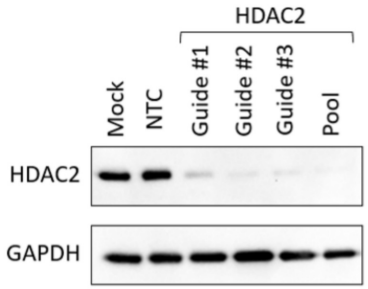
Example assays to confirm knockout:

- Genotyping PCR: detect indels at the target site



Source (see for a detailed explanation):
https://www.nature.com/articles/s41598-019-39950-4

- Western blot: confirm loss of protein expression



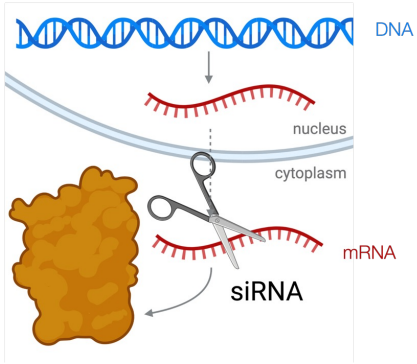
Source:
https://resources.revvity.com/pdfs/app-western-blot-validation-crispr-cas9-induced-functional-knockout.pdf

- Functional assays: look for biological impact

Comparison of RNAi and CRISPR-Cas9

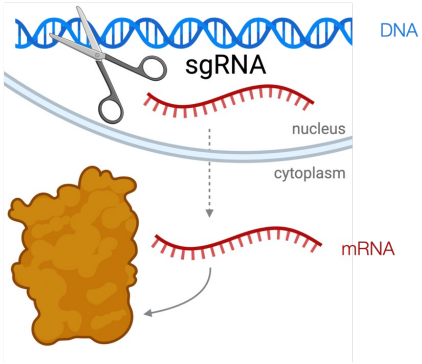
RNAi technology

"killing the messenger"



CRISPR technology

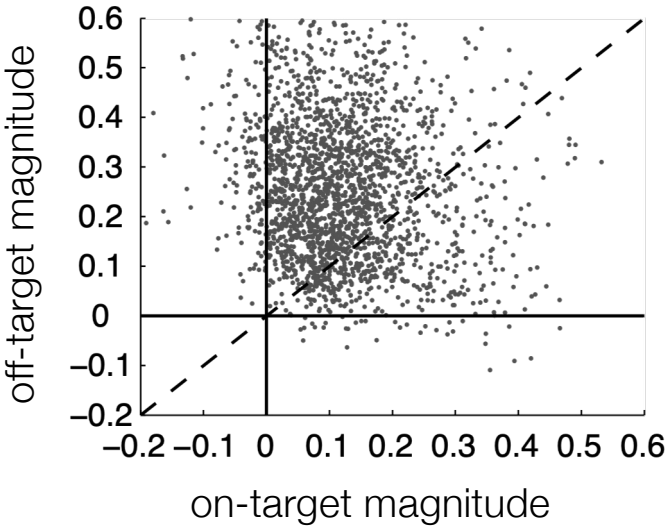
"editing the source code"



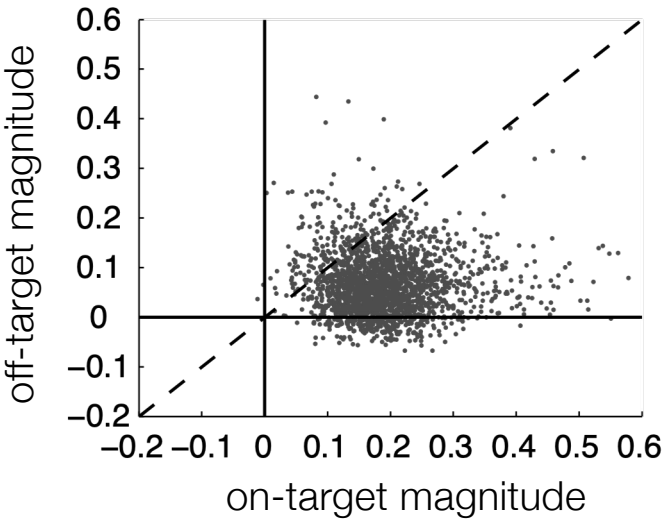
delivery	siRNA or shRNA molecules (often via plasmids or viral vectors)	sgRNA and Cas9 protein (delivered as plasmids, viral vectors, or ribonucleoprotein complexes)
mode of action	RISC complex binds target mRNA and induces degradation or translation inhibition	Cas9 introduces double-strand DNA breaks at target site, repaired by non-homologous end joining (NHEJ) or homology-directed repair (HDR)
specificity	~19–22 bp sequence complementarity; *tolerates multiple mismatches* (up to ~3–5, depending on position)	~20 bp sequence matching plus PAM sequence; highly sensitive to mismatches, especially near PAM-proximal end (tolerates ~0–3 mismatches)
end result	knockdown of gene expression (partial, not complete loss)	knockout (frameshift mutations lead to loss of function) or precise editing with HDR
use cases	preferred if studying essential genes (where full KO is lethal) or when partial suppression is desired	preferred for creating full gene knockouts, precise edits, or when permanent changes are needed

Key reason why CRISPR prevails: far fewer off-target effects than with RNAi

RNAi technology



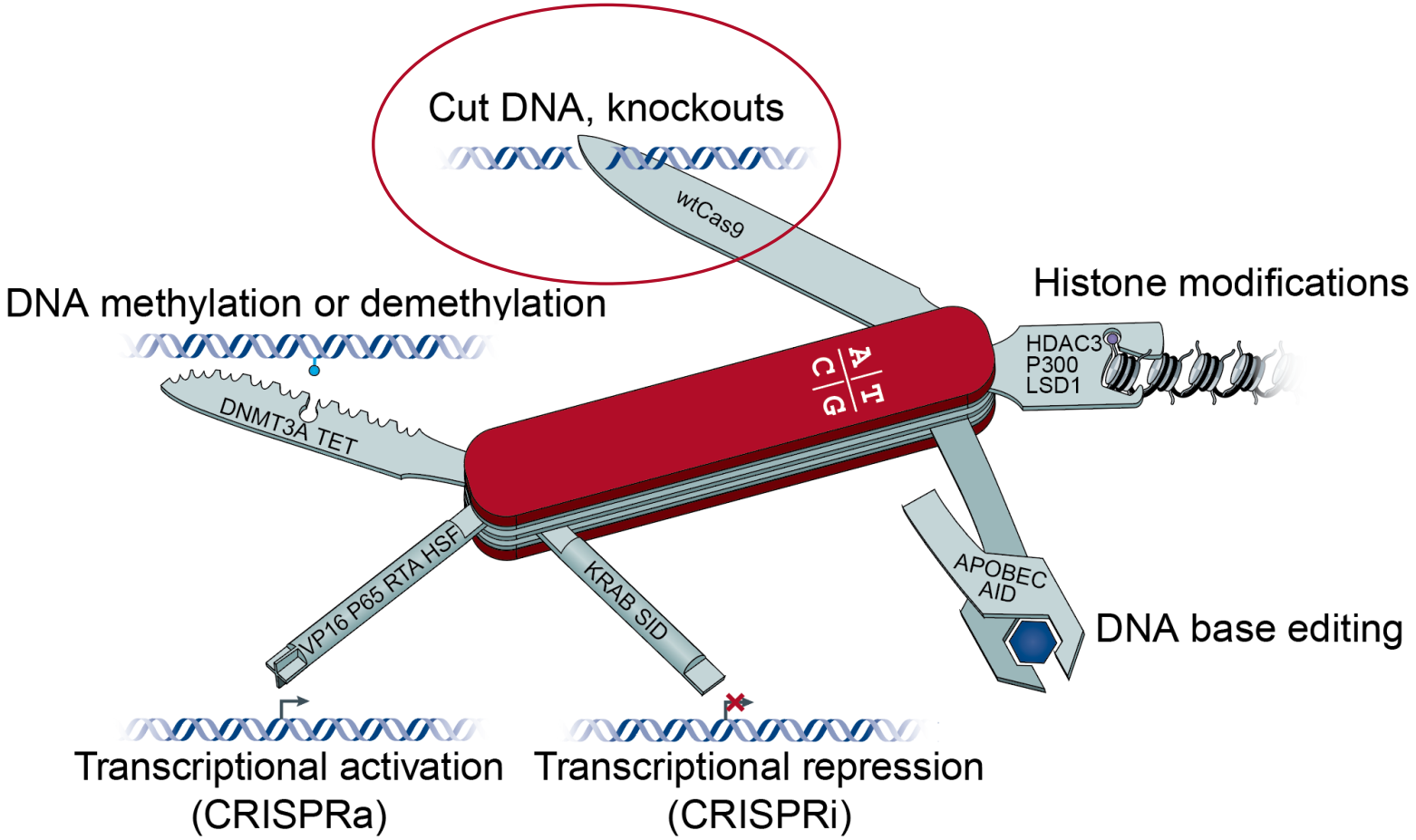
CRISPR technology



specificity	~19–22 bp sequence complementarity *tolerates multiple mismatches* (up to ~3–5, depending on position)	~20 bp sequence matching plus PAM sequence highly sensitive to mismatches, especially near PAM-proximal end (tolerates ~0–3 mismatches)

Evaluation of RNAi and CRISPR technologies by large-scale gene expression profiling in the Connectivity Map
Courtesy of John Doench (GPP, Broad Institute)

CRISPR enables diverse genomic manipulations beyond knockouts



Designing CRISPR vectors – modular systems with many flavours

Guide | Promoter | N-t. extra | Cas protein | C-t. extra | Sel. marker

Promoter
e.g. U6

Guide
Targeting
Non-targeting
Controls

tracrRNA
For SpCas9
For others, e.g.: Sa, Cas12a, Cas13d…

Localisation signals (e.g. NLS, NES)

Extras for other modalities:
KRAB (CRISPRi)
p65 (CRISPRa)
VP64 (CRISPRa)
TadA (A>G editor)
Apobec (C>T editor)
Apobec-UNG (C>G editor)

SpCas9

Or also:
SpCas9-NG
SpCas9-SpG
SpCas9-SpRY
SaCas9
AsCas12a
EnAsCas12a
RfxCas13d
Cas7-11
Cas13b

size, PAMs, etc

Nuclease
Nickase I
Nickase II
Deactivated

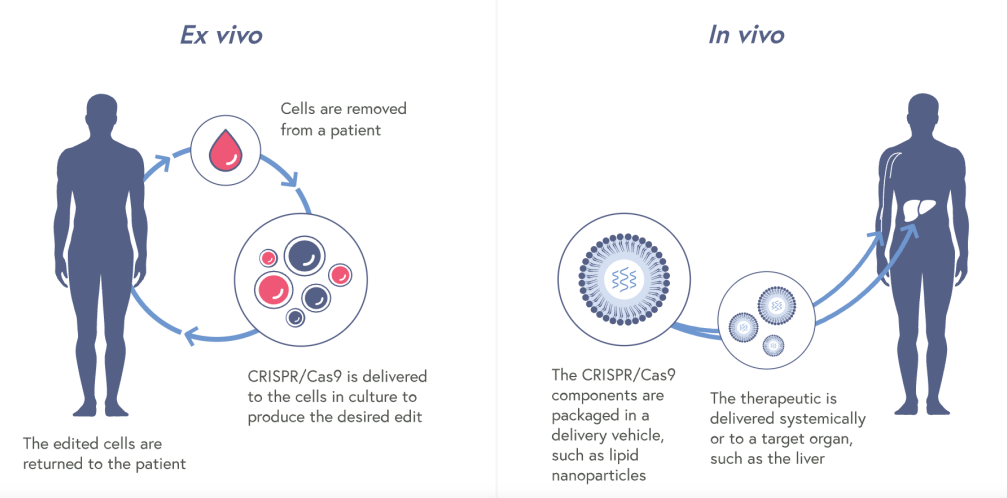
Localisation signals (e.g. NLS, NES)

Extras for other modalities:
KRAB (CRISPRi)
VP64 (CRISPRa)
VPR (CRISPRa)
MMLV RT (Prime Edit)
UGI (C>T editor)

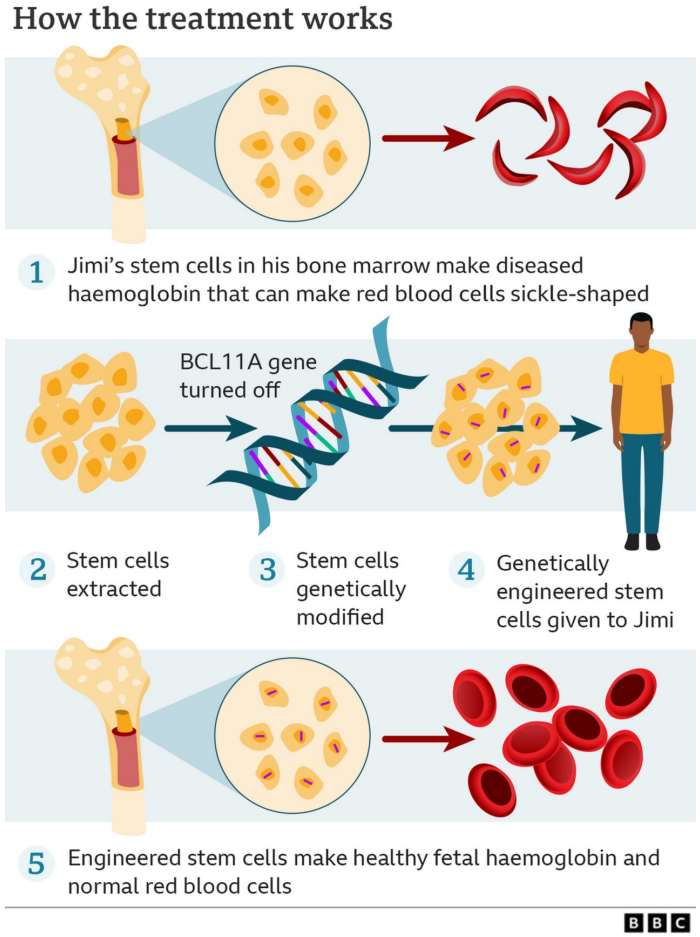
Antibiotic resistance:
Puromycin
Zeocin
Blasticidin

CRISPR in medicine: gene therapy

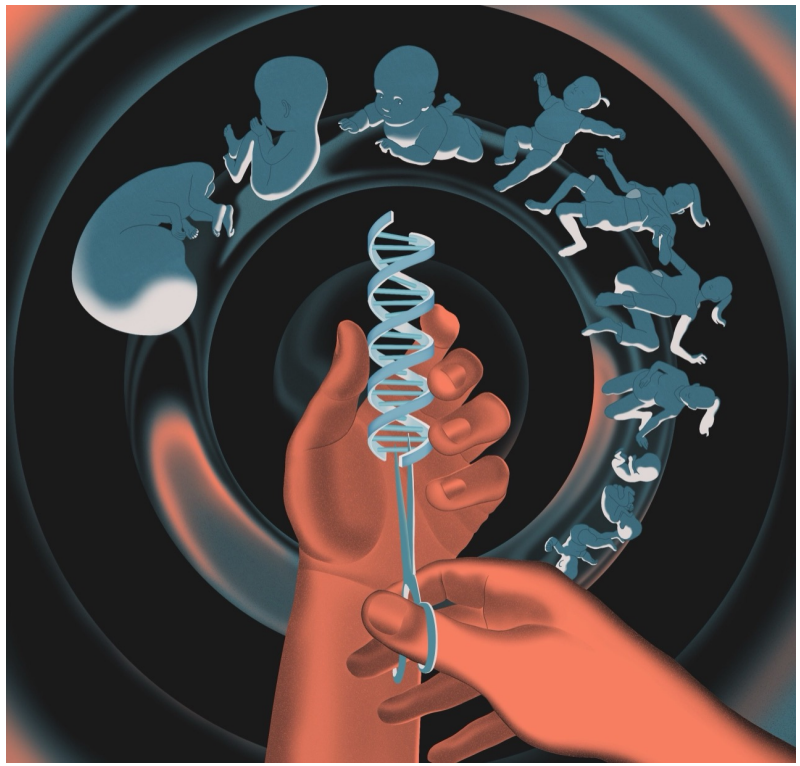
- Promising especially for treatment of monogenic diseases: repair a genetic defect
- FDA approval of CASGEVY for sickle cell disease and beta thalassemia (2024)
  - inactivates BCL11A: more fetal hemoglobin made
  - *ex vivo* treatment, one-off infusion eliminates the need for regular blood transfusions



Adapted from Vertex/CRISPR Therapeutics



Ethical considerations of somatic vs germline editing



China's CRISPR twins might have had their brains inadvertently enhanced

CRISPR Babies Scientist Sentenced to 3 Years in Prison

A Chinese Court jailed He Jiankui for an "illegal medical practice" in editing embryos' DNA

- He Jiankui edited the CCR5 gene in embryos
- Aimed to replicate HIV resistance observed in $CCR5_{\Delta 32}$ patients
- Later evidence of CCR5 involvement in brain function

Adapted from The New Yorker, The Economist, MIT Technology Review, The Spectator

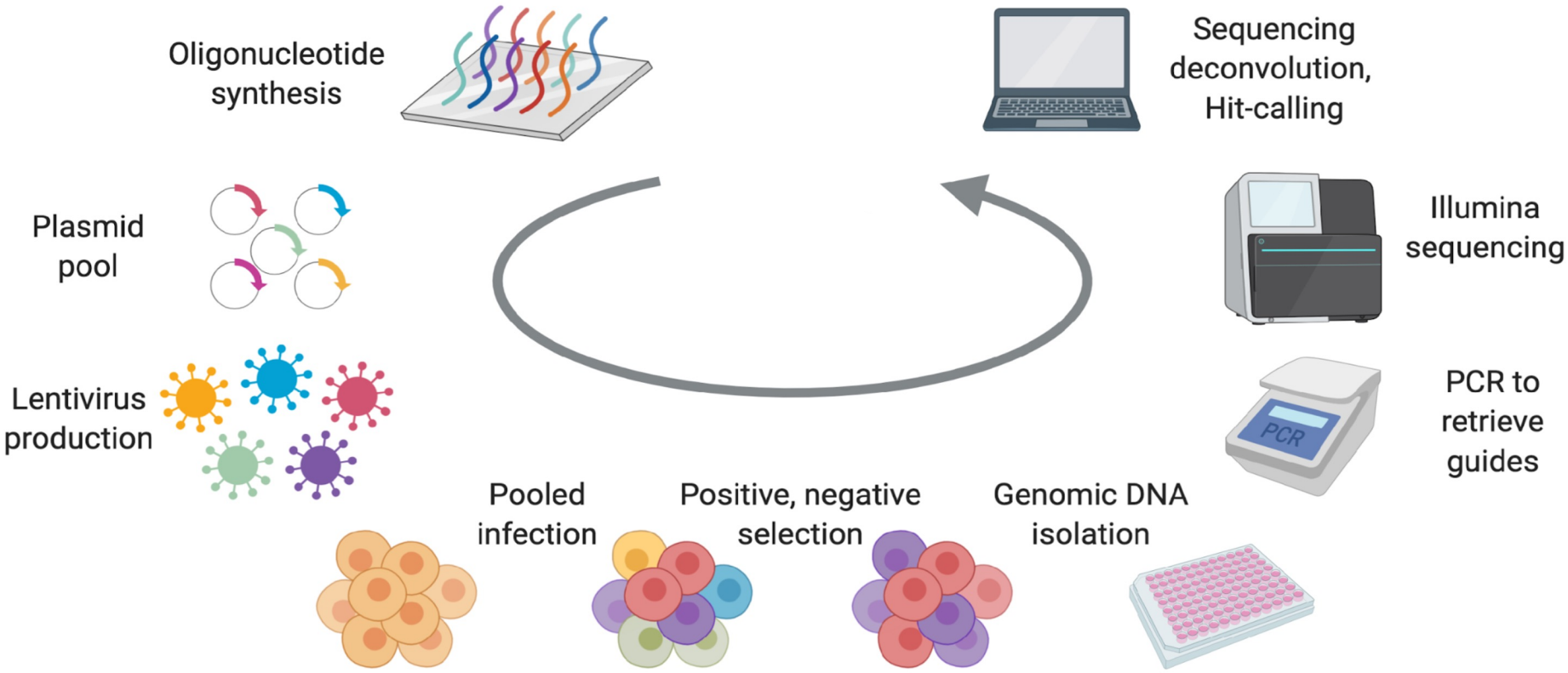
CRISPR screening

CRISPR screens: let's scale up

We can use **simultaneous CRISPR-Cas9 KO of many genes** to ask questions like:

- What genes are essential for cell survival in specific conditions?
- Which genes control my phenotype of interest (e.g., drug resistance, metastasis, differentiation)?
- What pathways are activated or suppressed after treatment with a drug?
- How do genes interact within a biological network?
- What is the cellular target of an unknown or poorly understood drug?
- How do cells develop resistance to a drug?
- Which genes are specifically essential in e.g. a given type of cancer cells but not in normal cells?

CRISPR to study gene function: genome-wide pooled screening

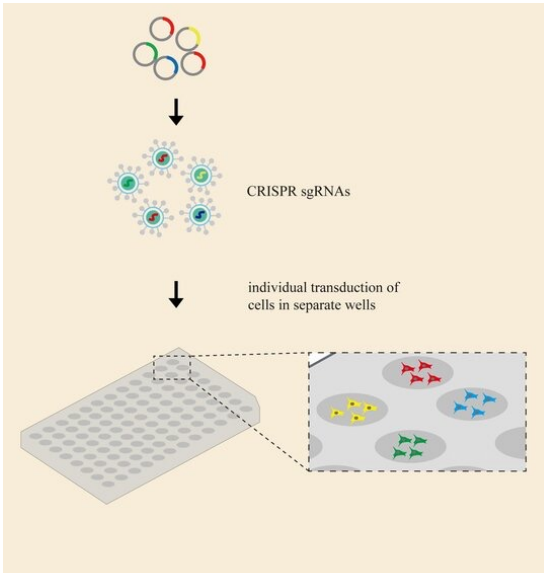


CRISPR-Cas9 screen formats

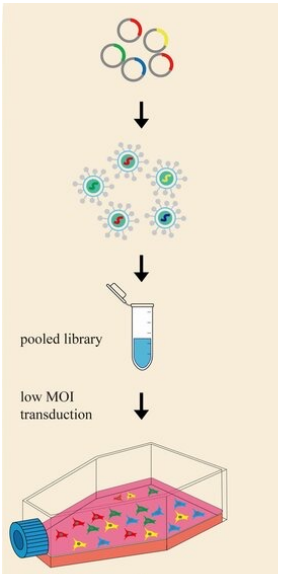
Arrayed Screens

- one perturbation per well - physical separation allows direct linking of phenotype to a specific perturbation
- if large-scale: special infrastructure required
- suitable for complex high-content phenotypic readouts (e.g., imaging)

arrayed



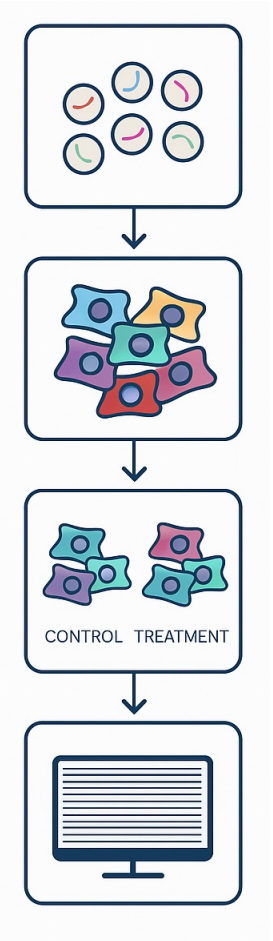
pooled



Pooled Screens

- all perturbations are delivered together into a single bulk cell population - perturbation identities are recovered later by NGS
- selection is based on a yes/no question: survival, growth, enrichment/depletion, fluorescence, etc under specific conditions

How do CRISPR-Cas9 screens work?



1

LIBRARY DESIGN
Selection of genes to target and creation of a suitable sgRNA library

2

sgRNA DELIVERY
Delivery of the sgRNA library into cells (e.g. via lentiviral transduction)

3

SCREEN ASSAY AND SELECTION
Cells are treated with the assay conditions (e.g. drug treatment, perturbation)

4

READOUT BY NGS
Deep sequencing is used to determine the abundance of each sgRNA and hence the relevance of gene

1

LIBRARY DESIGN

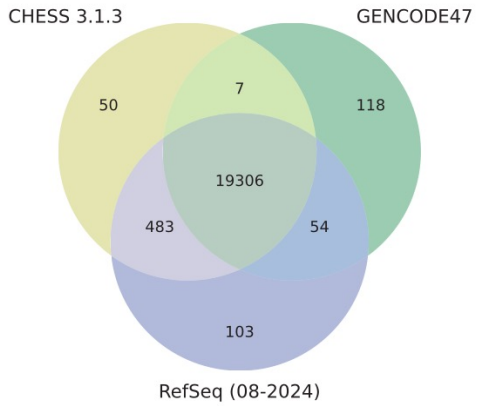
Selection of genes to target and creation of a suitable sgRNA library

Example libraries from Addgene:

Name	Cas	Activity	Number of genes	sgRNAs per gene	Controls	total sgRNAs	species
Genome-wide (*Brunello*)	SpCas9	Cutting (KO)	19,114	4	1,000	76,441	human
Ubiquitin system (*Bison*)	SpCas9	Cutting (KO)	713	4	20	2,852	human
Kinase domains (*Vakoc*)	SpCas9	Cutting (KO)	482	6	87	3,051	human

But need to be constantly updated:

- gene annotations change over time
- e.g. *Brunello* (2016) leaves out 3% of GENCODE47
- sgRNA design scoring algorithms improve regularly

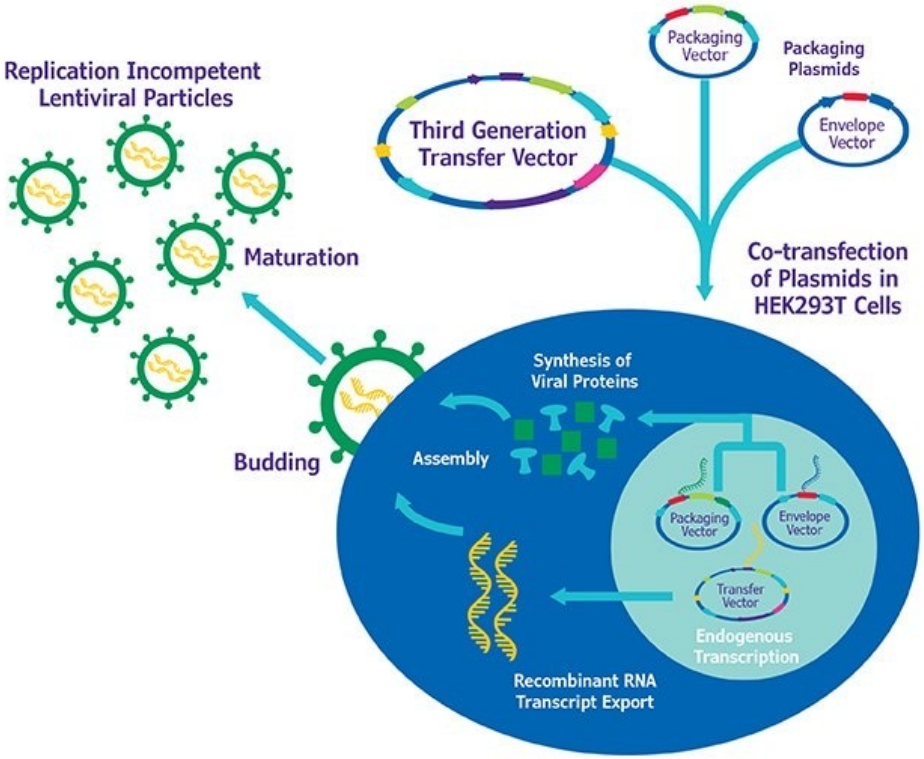


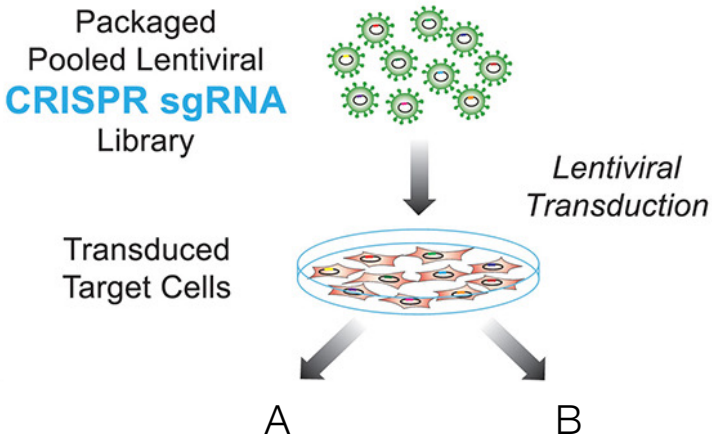
2

sgRNA DELIVERY

Delivery of the library into cells (e.g. via lentiviral transduction)

first make lentivirus → then transduce cells of interest





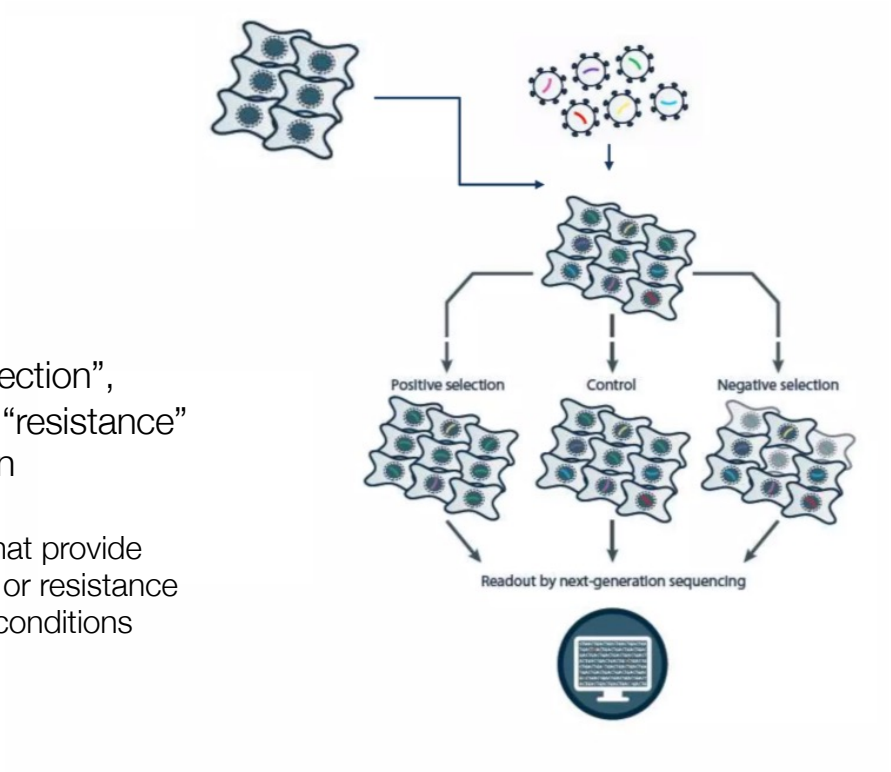
Adapted from Merck

3

SCREEN ASSAY AND SELECTION

Cells are treated with the assay conditions (e.g. drug treatment, perturbation)

- compare populations: e.g. treated versus control
- most common readout: **cell viability/growth** – but also e.g. fluorescence (see case studies)



“positive selection”, “enrichment”, or “resistance” screen

identify genes that provide growth advantage or resistance under specific conditions

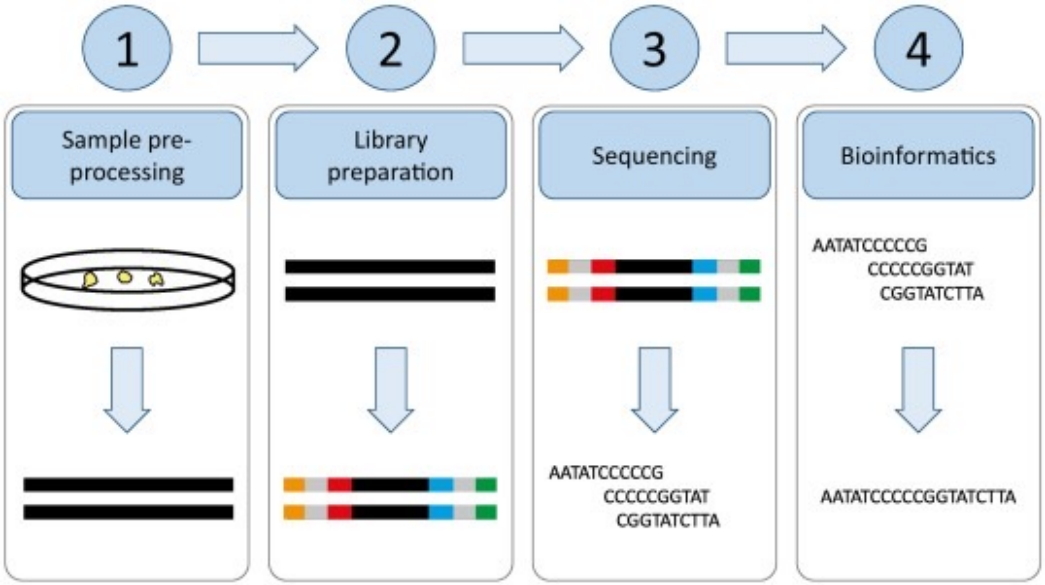
“negative selection”, “dropout”, “sensitivity”, or “viability” screen

identify genes whose knockout leads to cell death or growth inhibition under specific conditions

4

READOUT BY NGS

Deep sequencing is used to determine the abundance of each sgRNA and hence the relevance of gene



Main steps of the workflow:

- lyse cells
- extract genomic DNA
- PCR to amplify regions of integrated lentiviral cassettes and add barcodes and sequencing adapters
- check – e.g. by agarose gels
- purify amplified PCR products
- perform NGS sequencing
- bioinformatics to align reads and quantify sgRNA abundance

further reading (watching):
great explanation of NGS technology: https://youtu.be/jFCD8Q6qSTM?si=TocagwrEm2zGBa-i

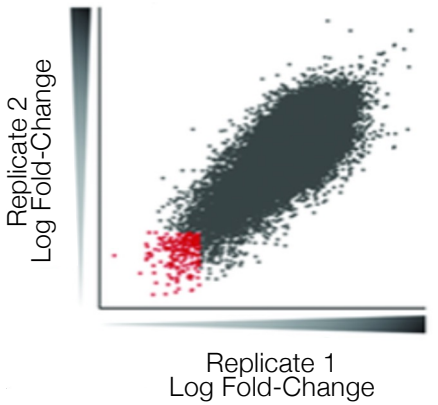
First-pass analysis: calculating LFC (your "hit" score)

Log Fold-Change (LFC) = log_2RPM of your experimental sample - log_2RPM of your reference sample

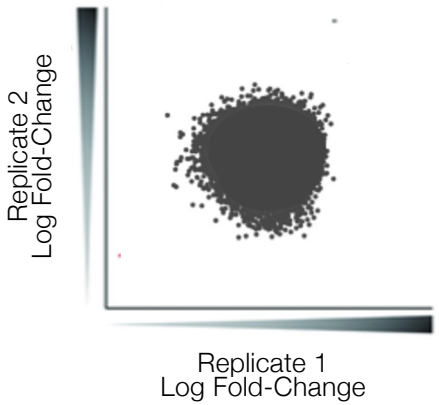
*log_2RPM =~ log_2 of normalised reads for an sgRNA

- important question: what is a good reference sample? early time point? late time point of a sample not treated with drug?
- in practice more sophisticated calculations performed but same principle
- compare replicates and assess correlation and signal-to-noise
- look at where your controls (hitting essential genes versus non-targeting) are

Exemplary screen



Screen with issues



lack of correlation or outliers indicates lack of signal

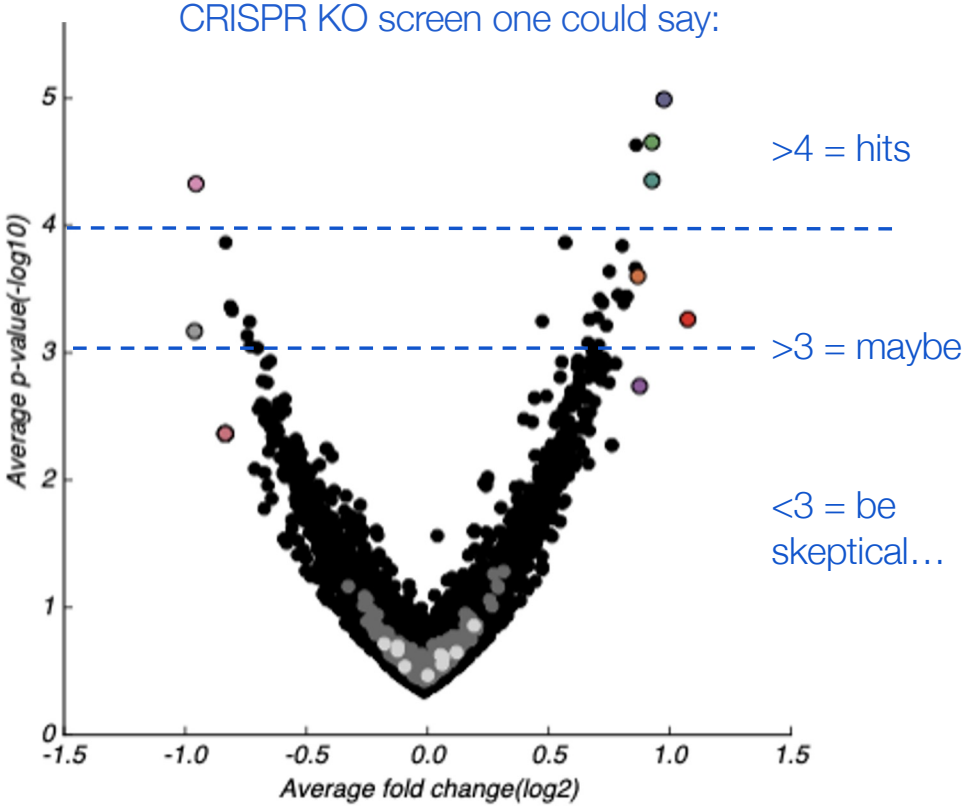
Adapted from Piccioni F, Younger ST, Root DE, Curr Protoc Mol Biol, 2018 and Doench JG, Hanna RE, Nat Biotechnol, 2020

Hit calling: what is a *true* hit?

An important and difficult question…

- Basic guidelines for hit calling:
  - generate gene-level scores (NB. 4-6 sgRNAs per gene)
  - generate a **volcano plot**
  - p values calculated based on the consistency of ranks of guides targeting the same gene
  - determine a hit cut-off based on Log Fold-Change (LFC) and/or p value
  - but also: judge based on what hits *make sense* (literature, pathways, context,…)
  - still need to VALIDATE!!!

for a standard genome-wide (Brunello) CRISPR KO screen one could say:



see: Doench JG, Hanna RE, Nat Biotechnol, 2020 Jul;38(7):813-823 for discussion of analysis tools

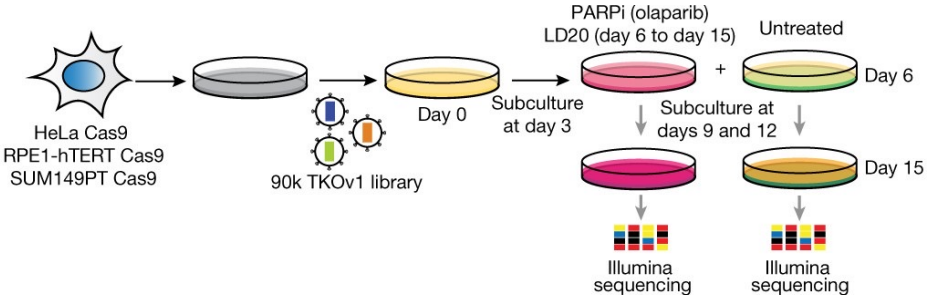
Got some hits – how to check if they *make sense*?
One idea: look at biological pathways implicated using GSEA

Example study that used GSEA:

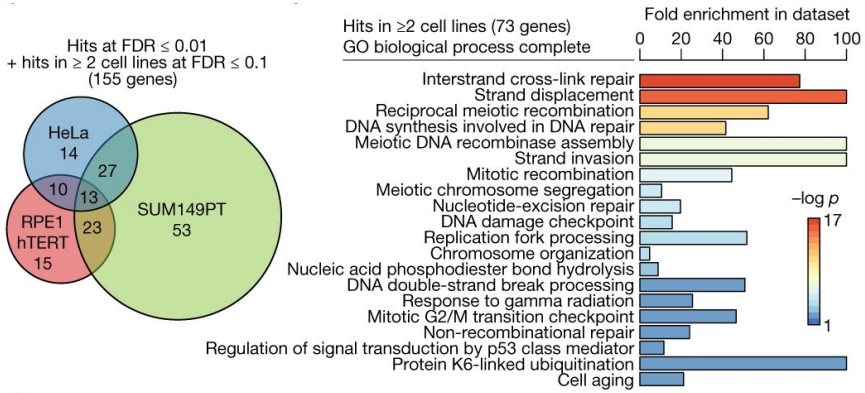
Their questions:

- What genes mediate cellular resistance to olaparib (clinically approved PARP inhibitor)?
- Can we learn something about mechanism of PARP trapping (key for PARP inhibitor toxicity)

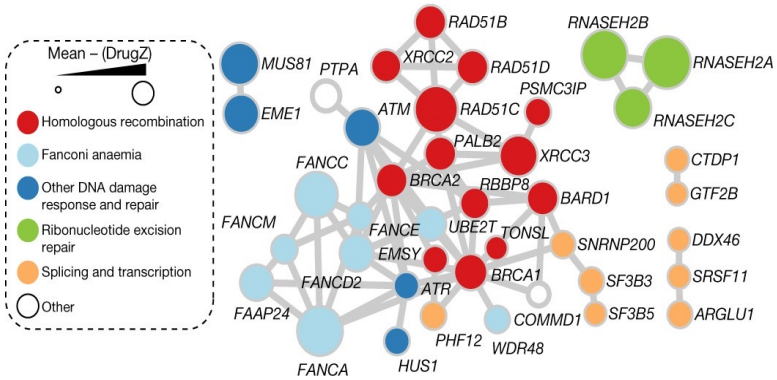
Approach:



Gene Set Enrichment Analysis (GSEA)

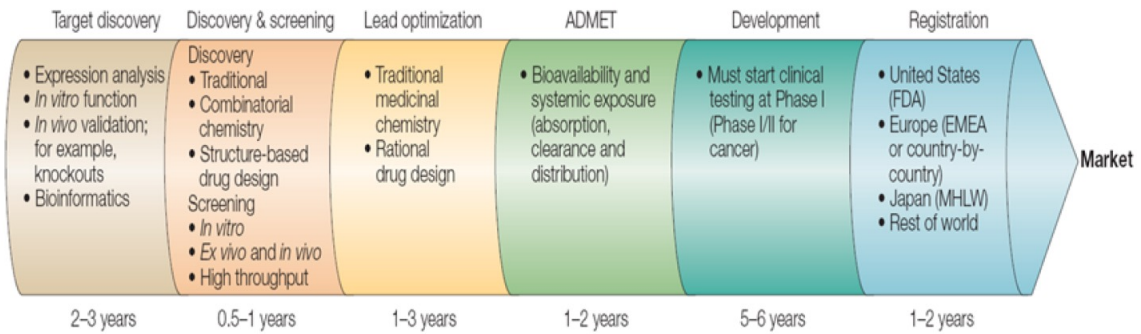


Network Analysis



Adapted from Zimmermann et al. https://www.nature.com/articles/s41586-018-0291-z

Functional genomics in drug discovery



- drug discovery and development takes 10-17 years and has <10% overall probability of success
- functional genomics can empower various phases of the process:
  - target discovery
  - target ID and de-orphanising phenotypic screens
  - MoA studies
  - drug sensitivity (patient stratification)
  - understanding resistance

Adapted from Nature Reviews in Drug Discovery

Functional genomics in drug discovery

Functional genomics in drug discovery

Some concepts and we will look at:

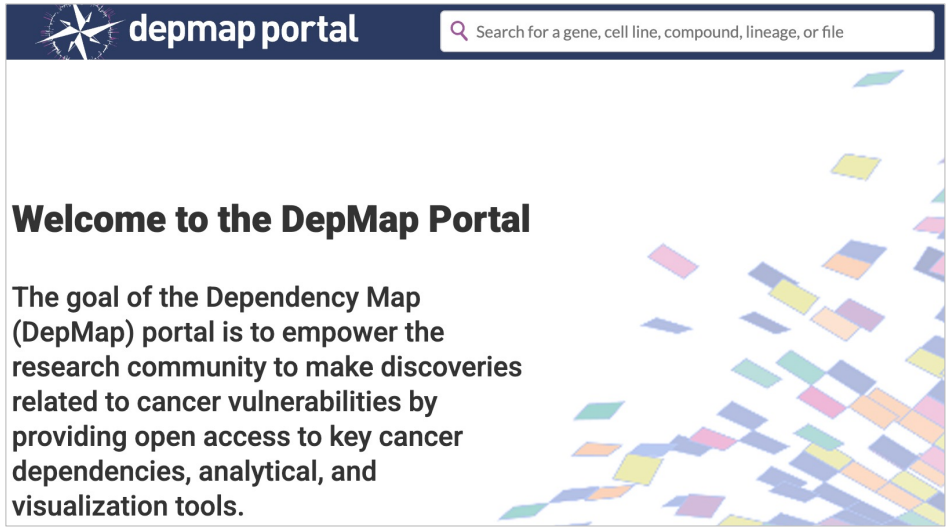
- Cancer dependencies (and depmap portal)
- Synthetic lethality
- Target deconvolution

Case studies:

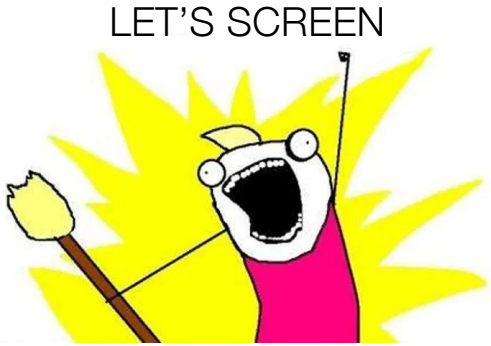
- Mode of action deconvolution: cyclin K degraders
- Mode of action deconvolution: Brd4 degraders

*Bonus: base editing and case studies in understanding drug resistance

Dependency map (**depmap**): genome-wide CRISPR screens supercharged



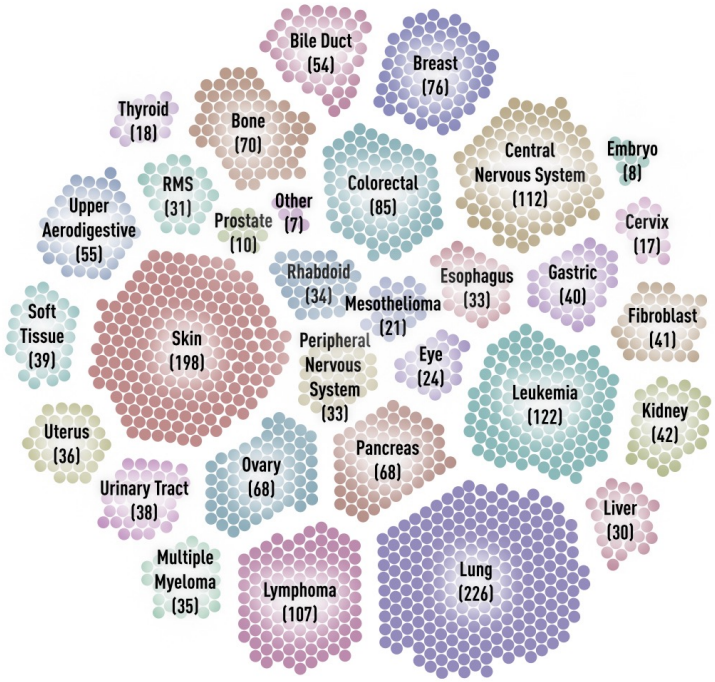
https://depmap.org/portal /



goal:
find cancer drug targets

how?
genome-wide CRISPR–Cas9 screens to disrupt every gene in many cancer models

depmap: models and data types

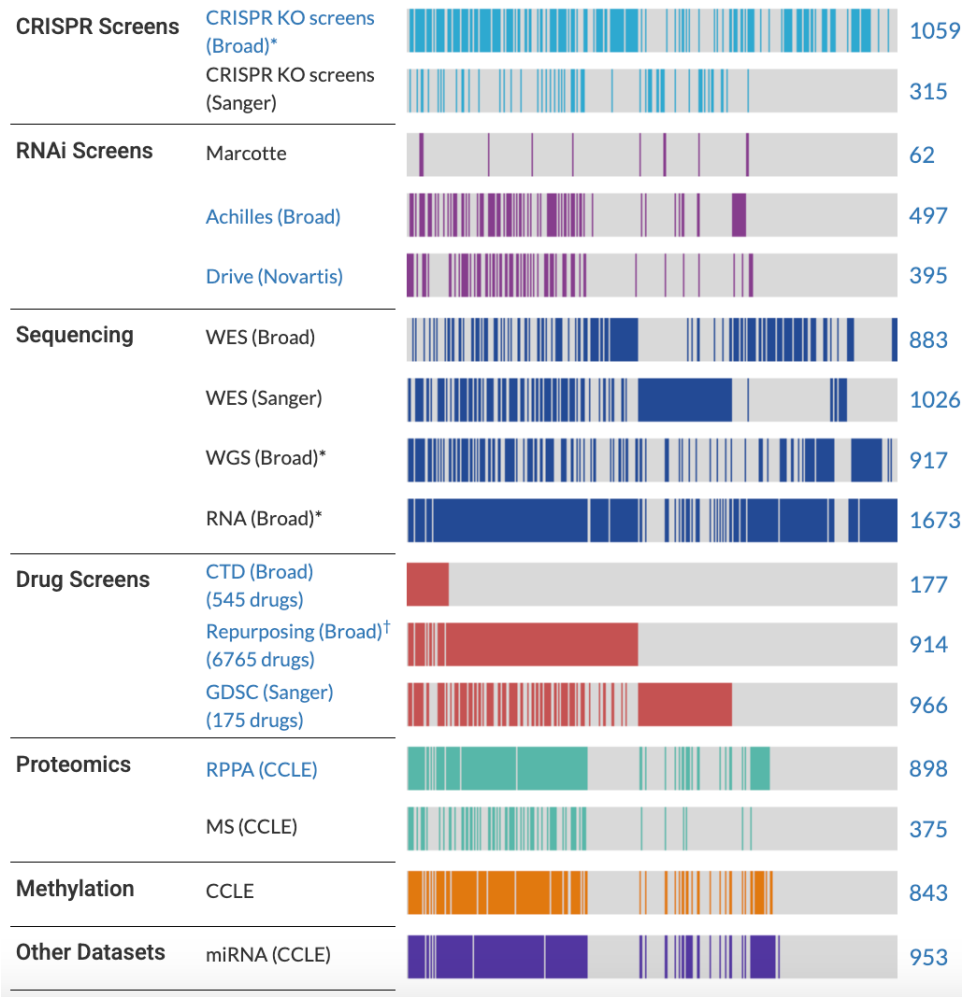


CCLE Cancer Cell Line Encyclopedia

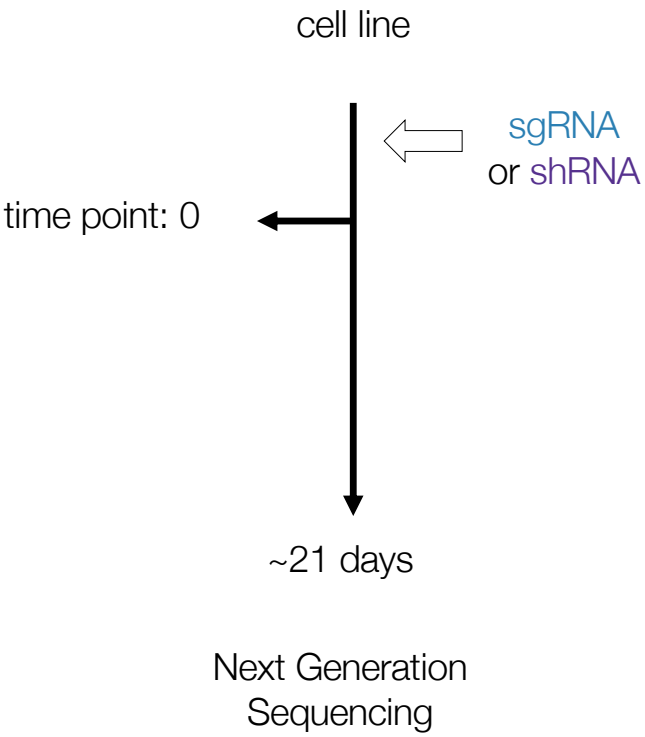
https://depmap.org/portal/ccle/

Data sources used in the DepMap portal

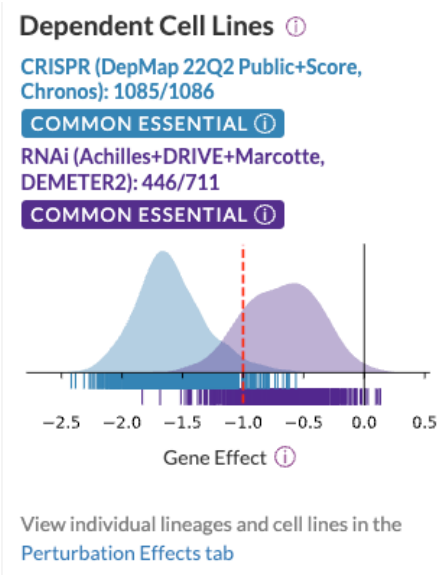
Data in the DepMap portal is aggregated from many sources, including both DepMap Release data and collaborator datasets. This figure provides an overview of how many cell lines can be found in each data source.



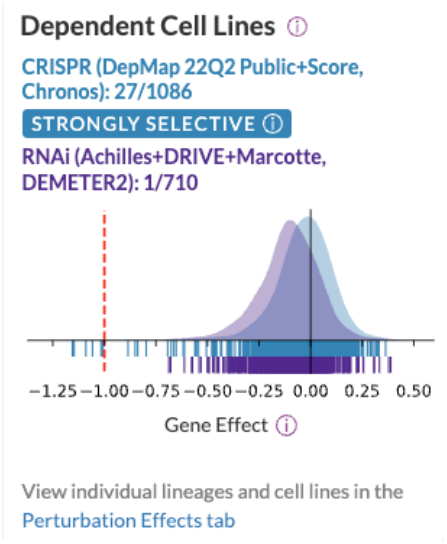
depmap: parallel genetic assessment of cancer cell dependencies



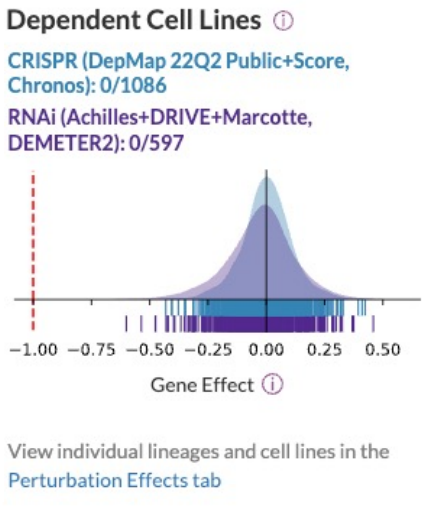
CCNK
common essential



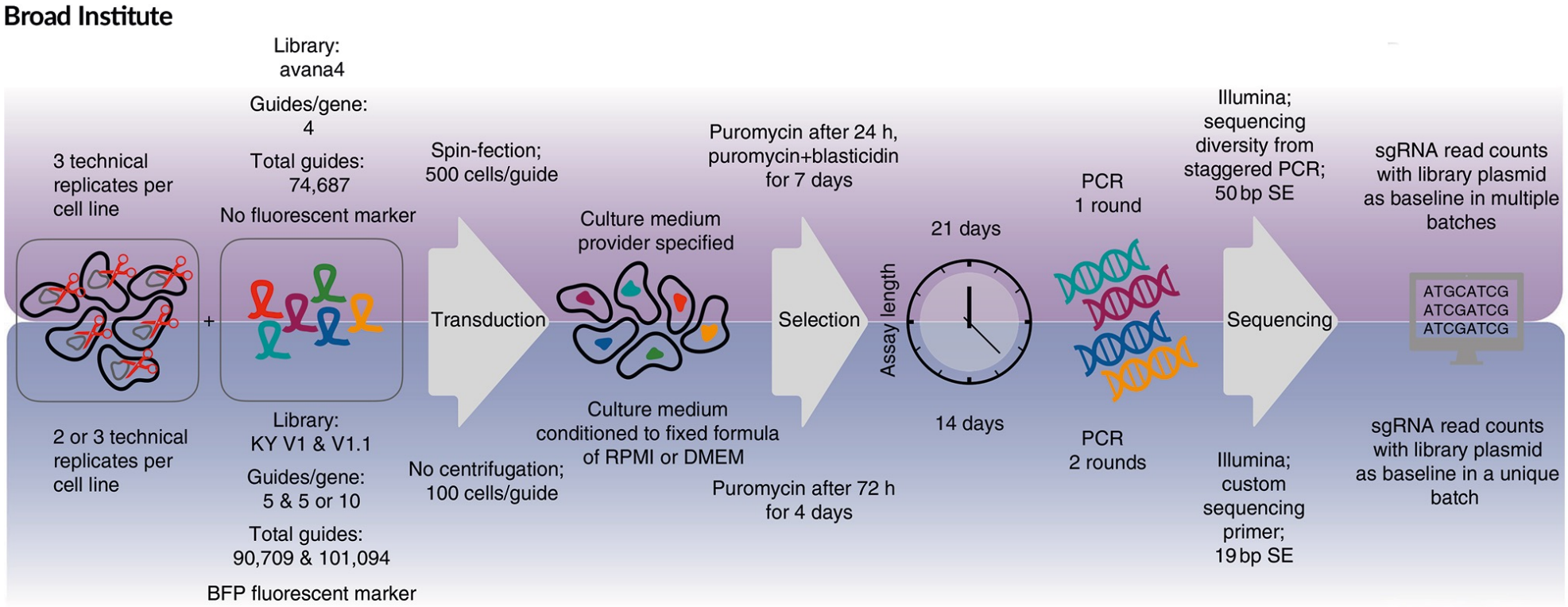
BCL6
selective essential



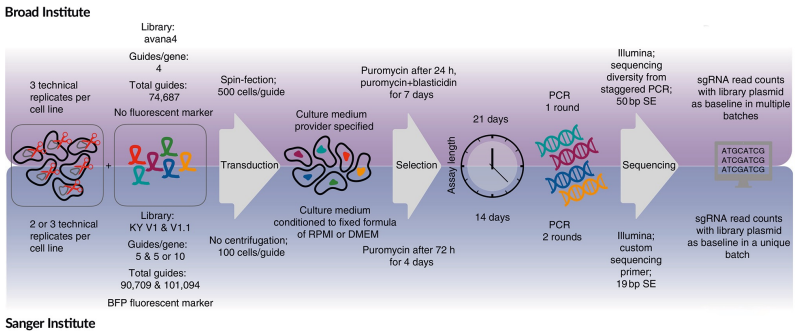
CRBN
non-essential

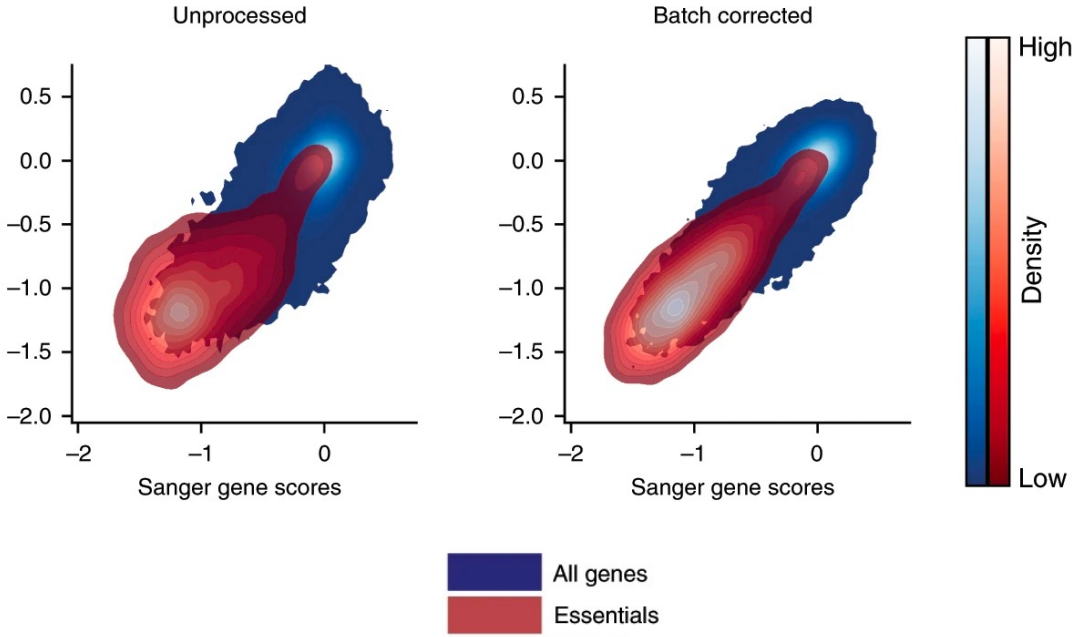


Large datasets with different CRISPR screen workflows – still in very good agreement



Large datasets with different CRISPR screen workflows – still in very good agreement





Dempster *et al*. Nat Commun (2019) https://www.nature.com/articles/s41467-019-13805-y

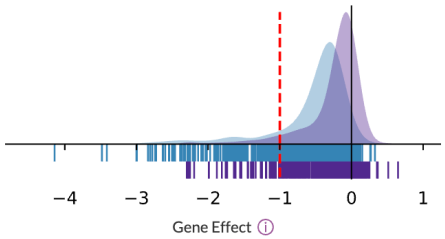
KRAS KRAS proto-oncogene, GTPase **Location:** 12p12.1 **Also known as:** KRAS2, KRAS1, K-Ras4B **Ensembl:** ENSG00000133703 **NCBI Entrez Gene:** 3845

Overview | Perturbation Effects | Characterization | Predictability

Dependent Cell Lines

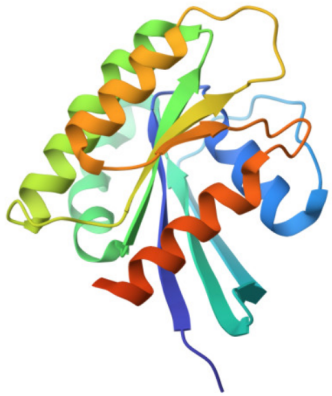
CRISPR (DepMap Public 24Q4+Score, Chronos): 449/1178
STRONGLY SELECTIVE

RNAi (Achilles+DRIVE+Marcotte, DEMETER2): 97/712
STRONGLY SELECTIVE



View individual lineages and cell lines in the Perturbation Effects tab

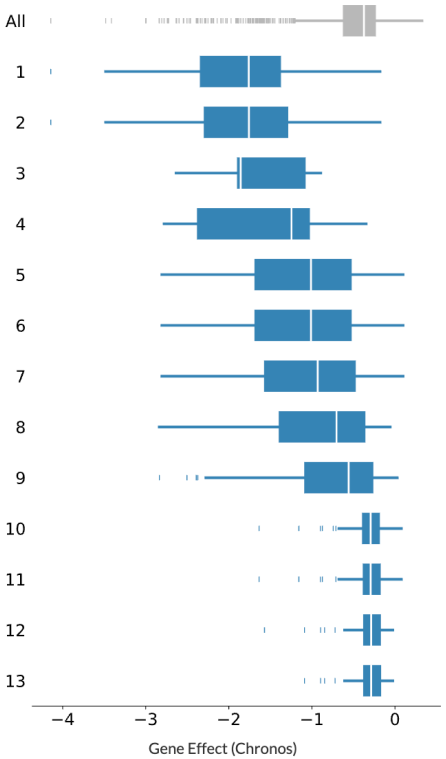
Target Tractability



Bioactive Compounds	Yes
Druggable Structure	Yes

Enriched Lineages

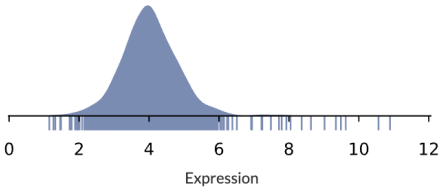
CRISPR (DepMap Public 24Q4+Score, Chronos)



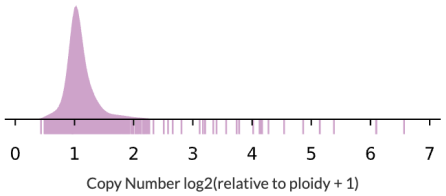
1. Pancreatic Adenocarcinoma (3.05e-59) n=45
2. Pancreas (1.26e-57) n=47
3. Immortalized Epithelial Cells, Retinal (1.02e-05) n=5
4. Rectal Adenocarcinoma (1.34e-09) n=12
5. Colorectal Adenocarcinoma (1.28e-19) n=63
6. Bowel (1.28e-19) n=63
7. Colon Adenocarcinoma (9.46e-12) n=48
8. Lung Adenocarcinoma (5.68e-07) n=53

Expression & CN

Expression Public 24Q4



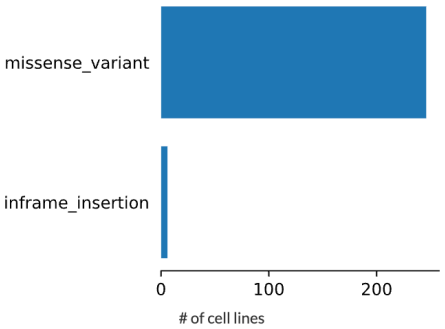
Copy Number Public 24Q4 (Log2 transformed)



View details in the Characterization tab

Mutations

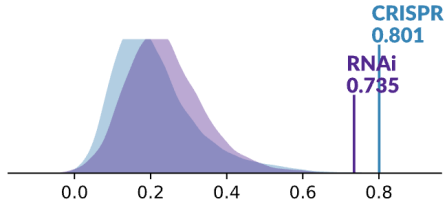
Mutation



Predictability

CRISPR (DepMap Public 24Q4+Score, Chronos): 99.9th percentile

RNAi (Achilles+DRIVE+Marcotte, DEMETER2): 100th percentile



Features of most accurate predictive model

CRISPR (DepMap Public 24Q4+Score, Chronos)

Feature	Importance	Corr.	Type
KRAS	66.3%	−	Hot. Mut.
ScreenMedianEssentialDepletion	2.3%	−	Confound...
PRDX4	1.1%	+	Expression
TXNL4A	1.0%	+	Copy num.
ScreenMADEssentials	0.7%	+	Confound...

View model details and predictive features in the Predictability tab

Description

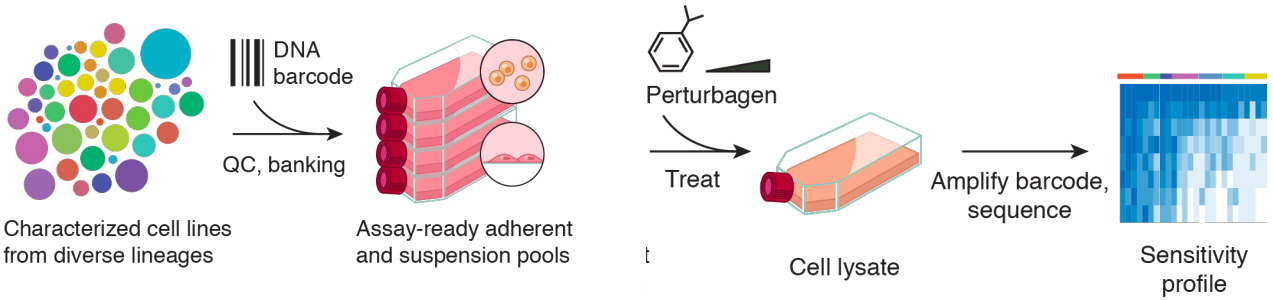
This gene, a Kirsten ras oncogene homolog from the mammalian ras gene family, encodes a protein that is a member of the small GTPase superfamily. A single amino acid substitution is responsible for an View more

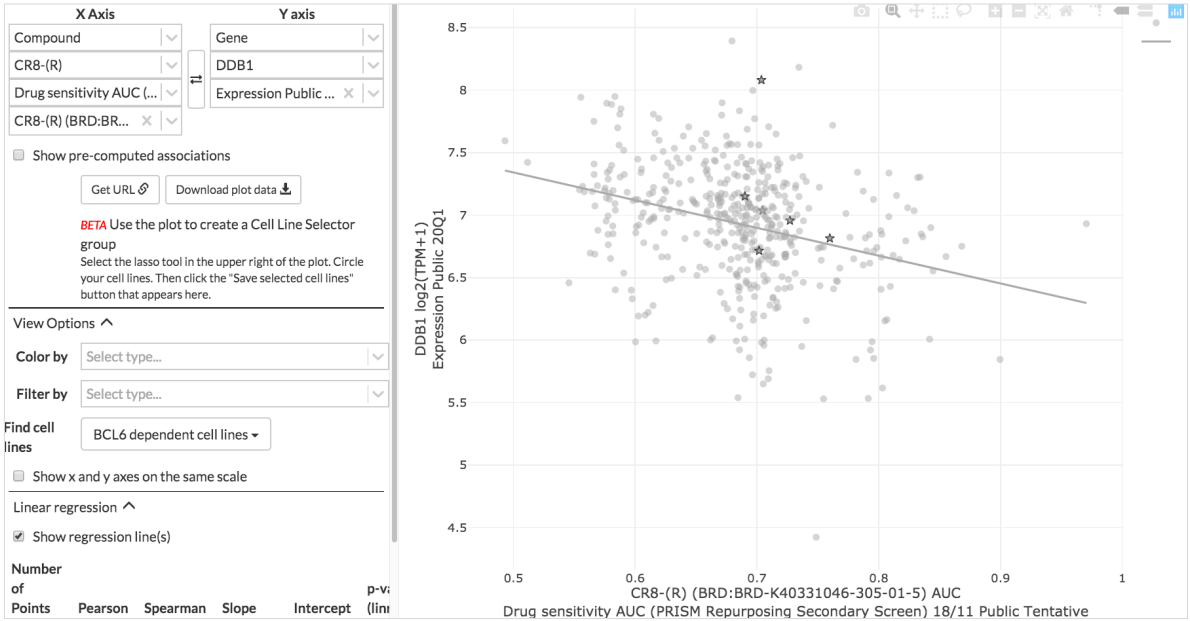
Search external sites for KRAS

- PubMed
- HUGO
- GeneCards
- Wikipedia
- Firebrowse
- Tumor Portal
- GTEx
- COSMIC
- NCBI

https://depmap.org/portal/gene/KRAS?tab=overview

depmap: PRISM – adding drug effects on all these cancer cell lines into the mix



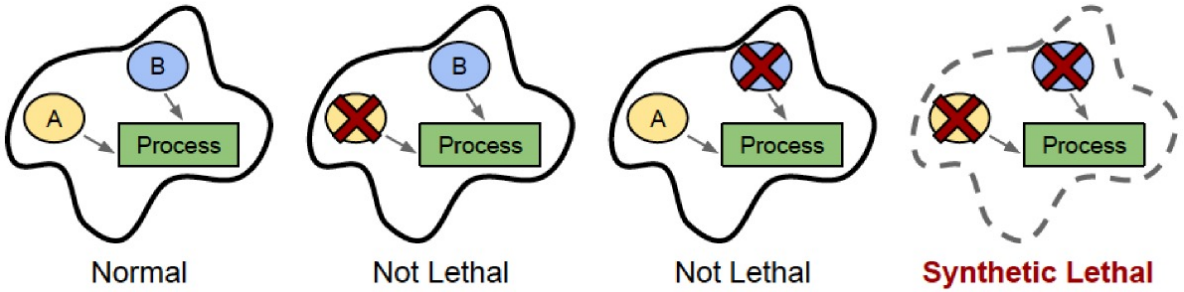


"Data Explorer" tool in depmap allows plotting of various dataset correlations:

- expression of a protein (DDB1) is correlated with sensitivity to drug (CR8) across many cell lines
- R^2 = -0.3 - is this meaningful?
  - here: yes!
  - we will revisit this example later (case study 1)…

Genetic interactions: synthetic lethality

- synthetic lethality occurs when loss of either gene A or gene B alone is tolerated, but simultaneous loss of both genes leads to cell death
- first formulated by Hartwell et al (*Integrating genetic approaches into the discovery of anticancer drugs,* 1997)

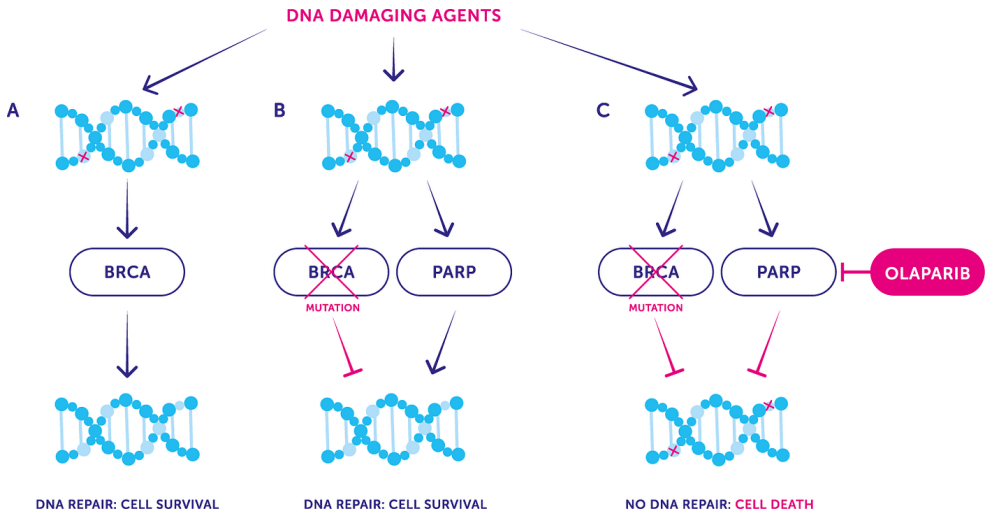


- how to look for it? *e.g.* screening in isogenic cell lines – look at perturbations in normal versus mut_A cells
- more recently: combinatorial (multiplexed) screening with engineered Cas12a: two or more guides per sgRNA construct – which combinations of genes when disrupted simoultaneously result in cell death?

further reading on SL and combinatorial screens: Thomson et al. (2021) https://www.nature.com/articles/s41467-021-21478-9

Synthetic lethality example: BRCA1^{mut} and PARP inhibitors

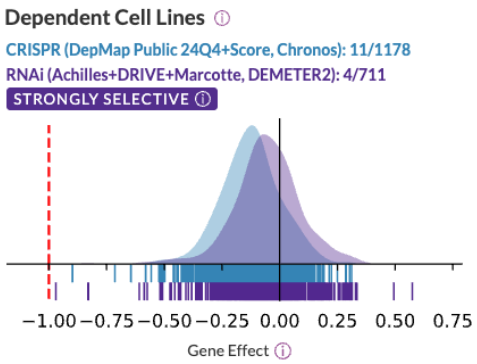
- mutations in BRCA1/2: hereditary breast-ovarian cancer syndrome due to a defect in DNA repair machinery ”BCRA-ness phenotype”
- targeting another node of DNA repair in BRCA mutant cancer leads to selective cancer cell killing (first formulated by Farmer et al, 2005)
- olaparib (PARP inhibitor) approved for ovarian cancer (2014)



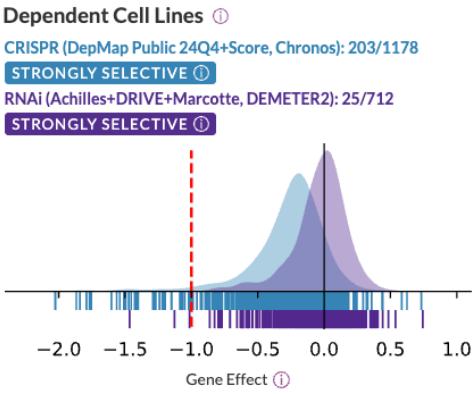
A special case of synthetic lethality: paralog redundancy

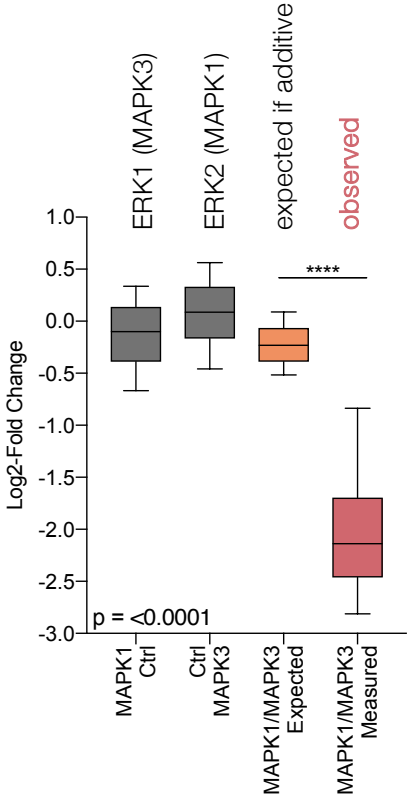
DepMap example
effect of a single sgRNA across 1078 cancer cell lines:

ERK1 (MAPK3)



ERK2 (MAPK1)

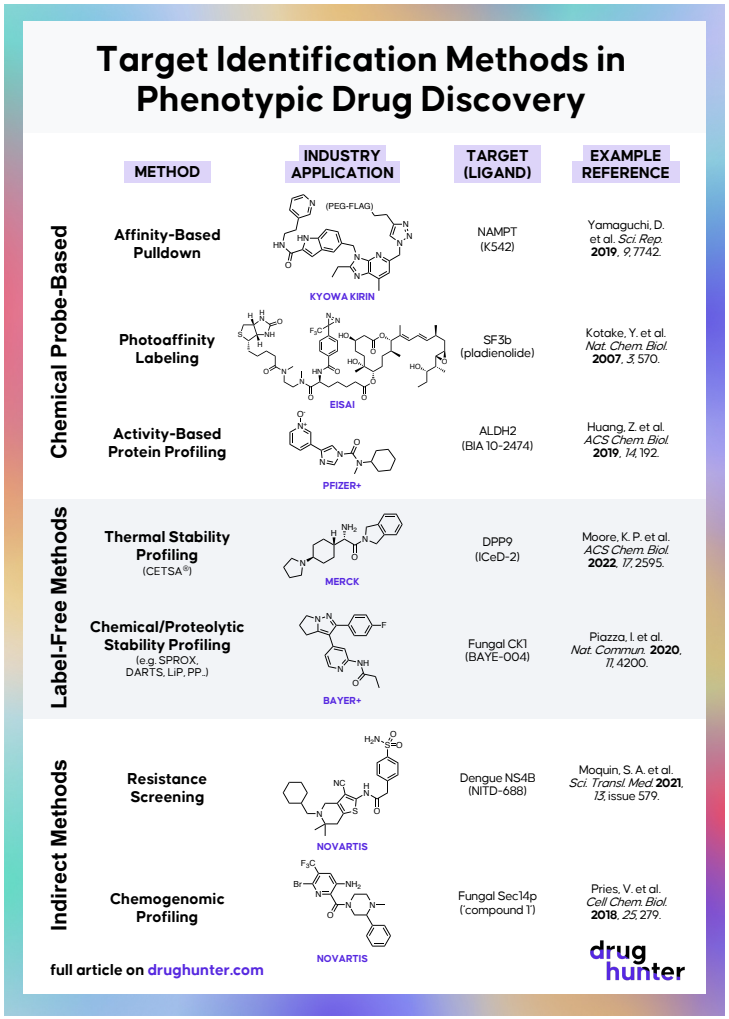




Important to bear in mind for genetic screens:

→ **redundancy** can mask dependencies and screen hits

Target identification/deorphanising of drugs: functional genomics can help!

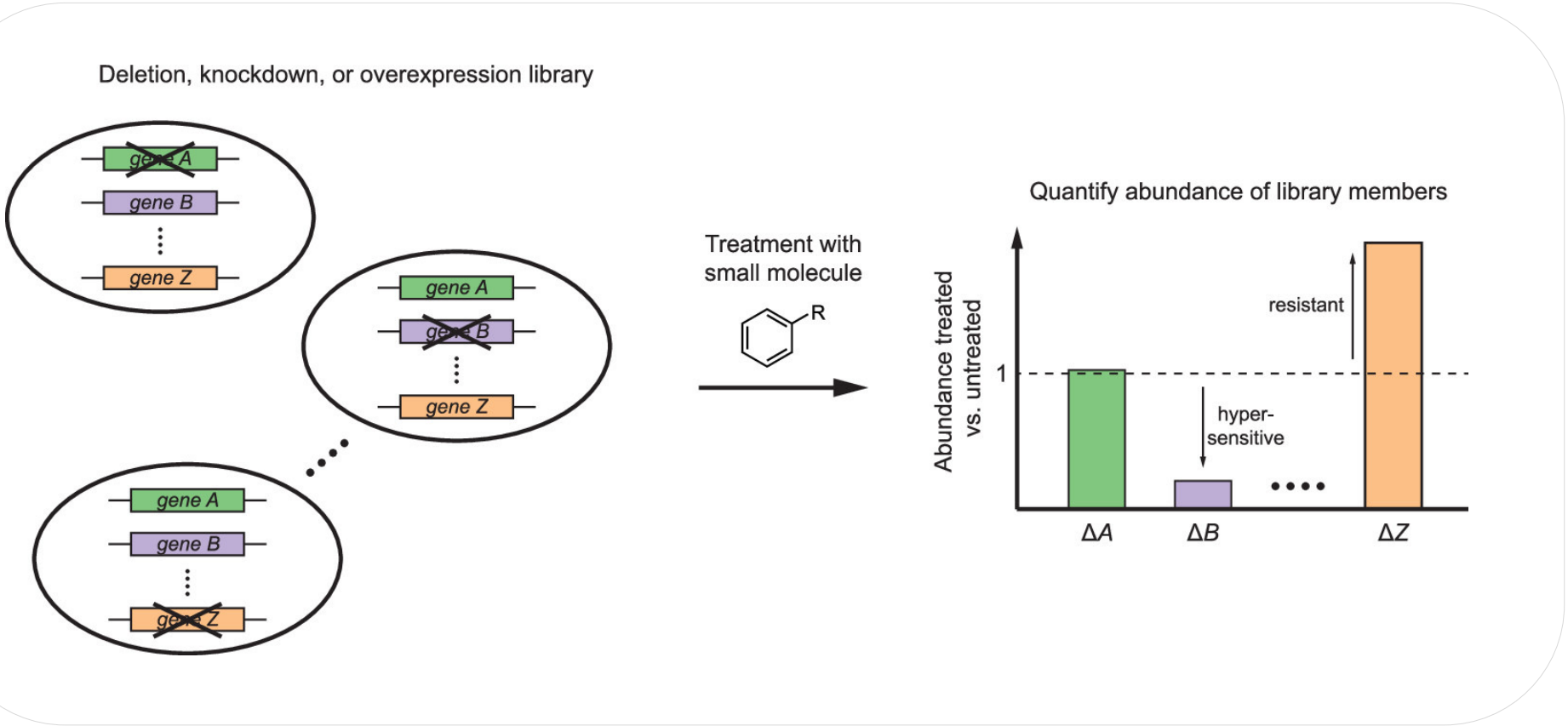


- phenotypic screens often yield active compounds with an unknown target and mode of action
- this poses challenges in clinical development
- CRISPR screens are a way (albeit indirect and effort-intensive) to understand a drug's mode of action
  - still need to confirm direct target through other means

further reading and case studies:

https://drughunter.com/resource/methods-for-drug-target-identification-after-a-phenotypic-screen

Target deconvolution for small molecules: chemogenomics



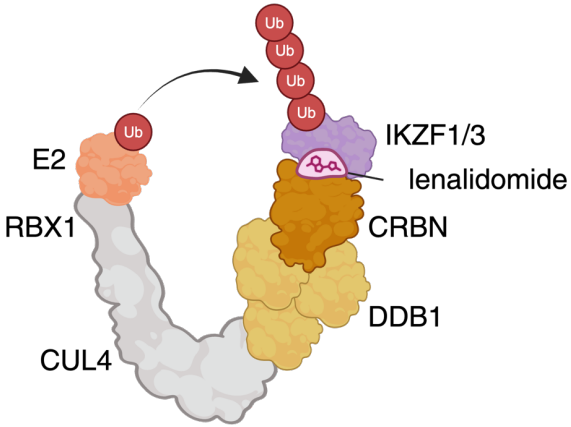
adapted from Jost and Weissman https://pubs.acs.org/doi/10.1021/acschembio.7b00965

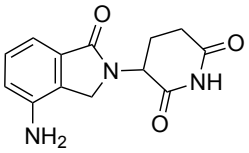
Case studies

Case study: CRISPR screen to understand drug MoA – how does the compound degrade my target? (1)

cyclin K degraders

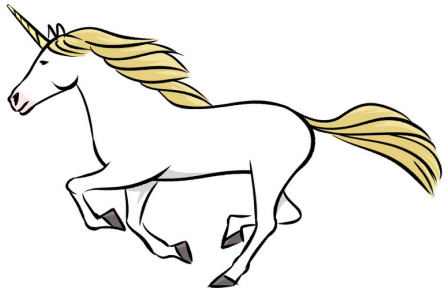
Preface: molecular glue degraders – an exciting therapeutic modality



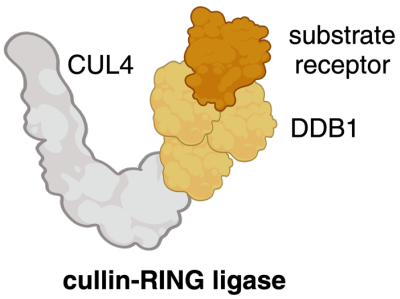


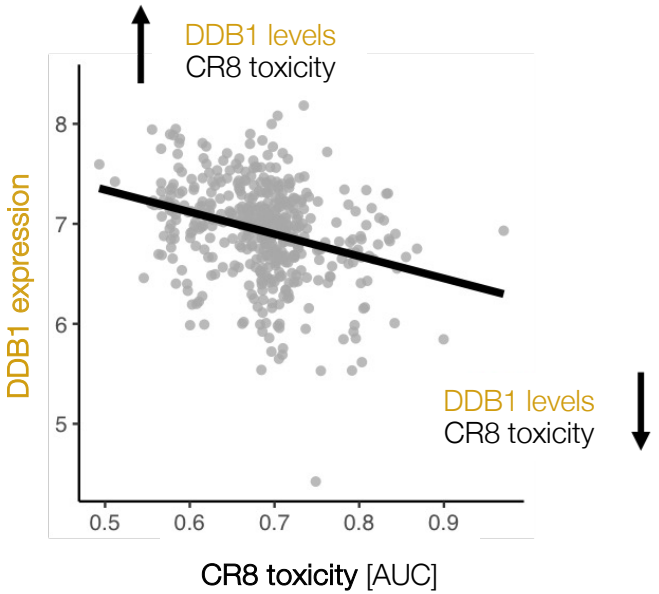
Revlimid®
(lenalidomide) capsules
2.5 · 5 · 10 · 15 · 20 · 25 mg

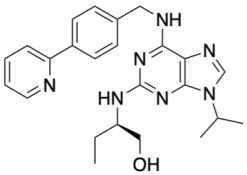
among top 3 drugs
by revenue worldwide
2017-2020



Chapter 1: searching for molecular degraders (using DepMap)





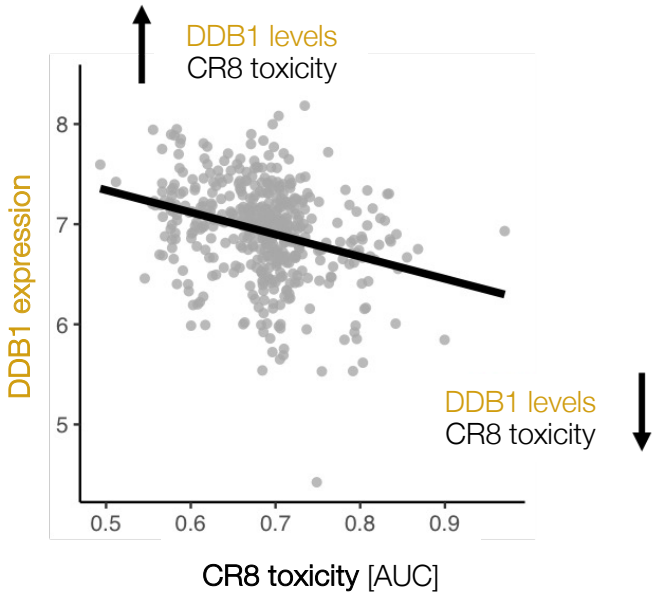


CR8
CDK inhibitor

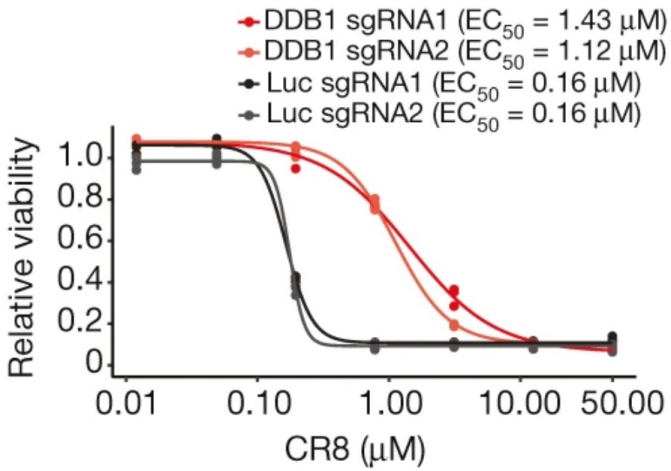
ManRos

Chapter 1: searching for molecular degraders (using DepMap)

DepMap correlation

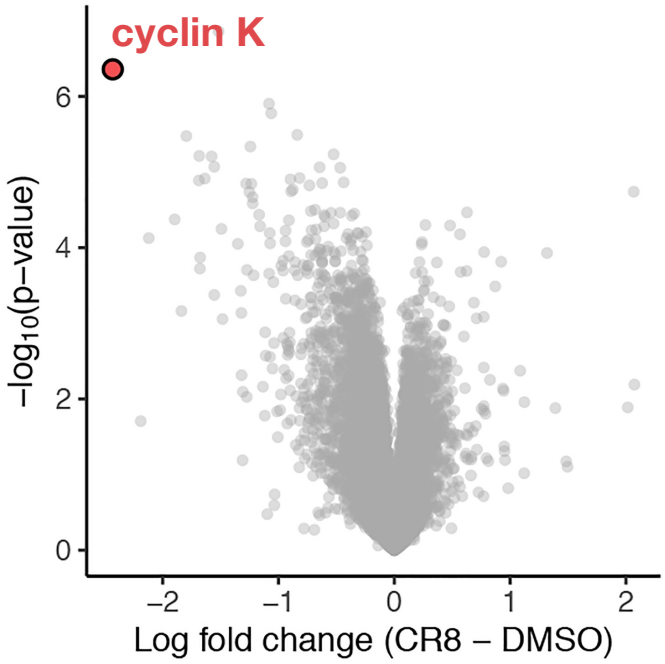


sgRNA validation

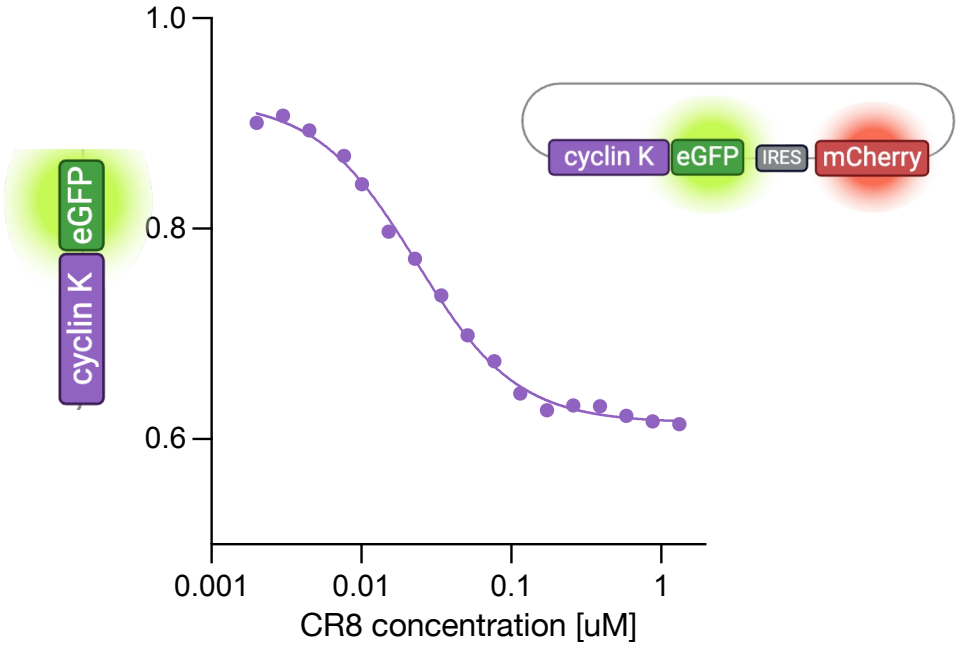


Chapter 2: identify the degradation target

global proteomics



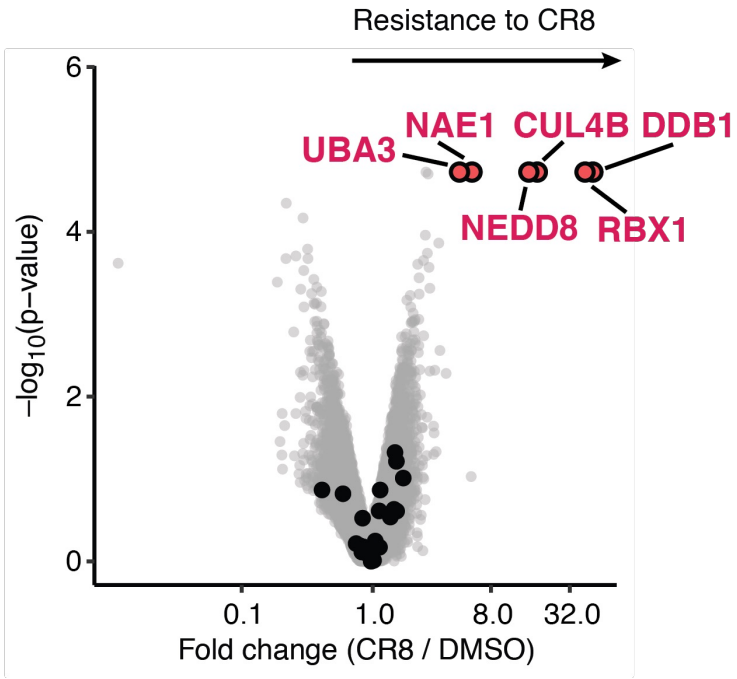
stability reporter

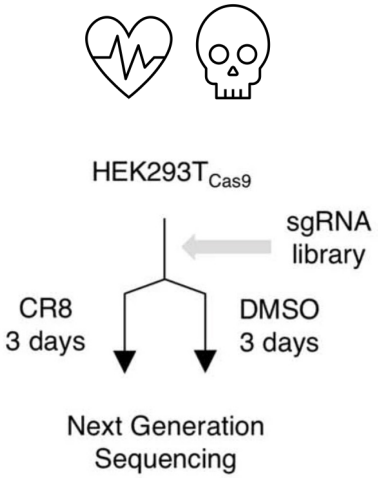


Chapter 3: mode of action deconvolution – **CRISPR screens** (!)

genetic screens

- resistance screen
- genome-wide library

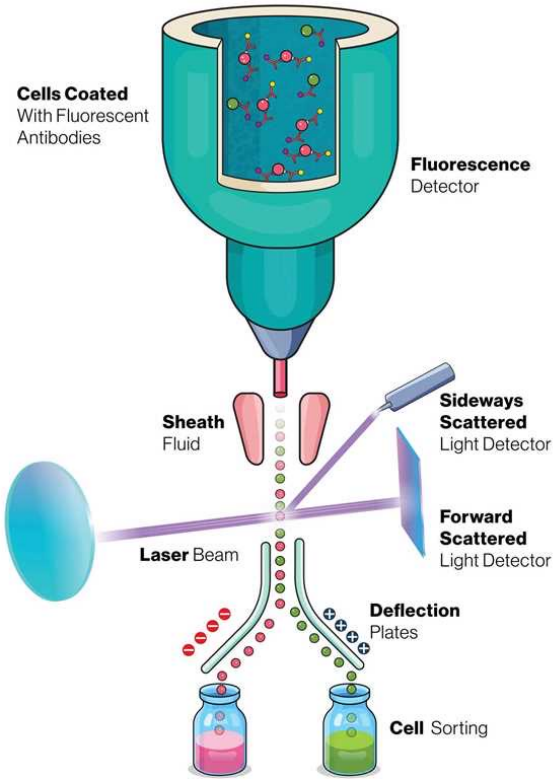




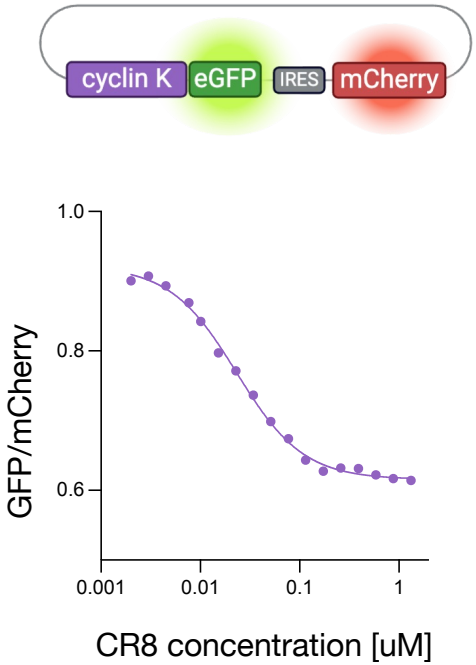
genome-wide CRISPR screen for CR8 resistance

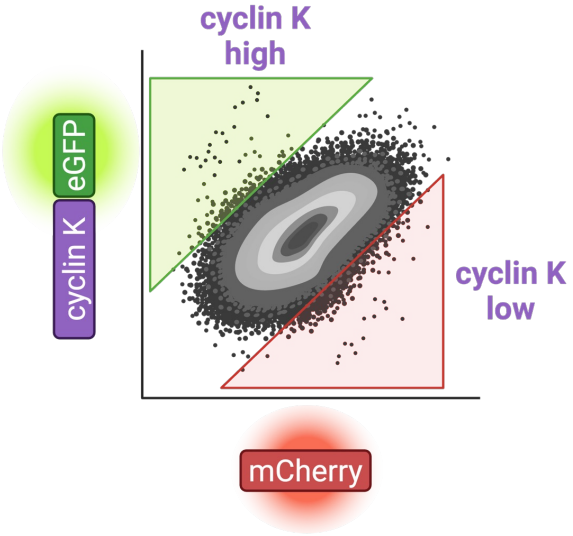
Five per cent (v/v) of the human genome-wide CRISPR-KO Brunello library with 0.4 µl polybrene ml^{-1} (stock of 8 mg ml^{-1}) was added to 1.5 × 10^{8} HEK293T-Cas9 cells in 75 ml medium and transduced (2,400 rpm, 2 h, 37°C). Twenty-four hours after infection, sgRNA-transduced cells were selected with 2 µg ml^{-1} puromycin for two days. On the ninth day after infection, cells were treated with either DMSO (n = 1) or 1 µM CR8 (n = 1) and cultured for an additional three days. ***Resistant*** *live cells were selected by gently washing away detached* ***dead*** *cells from the medium.*

Fluorescence Activated Cell Sorting (FACS)-based genetic screens



readout: fluorescence!
sort cells based on GFP/mCherry ratio

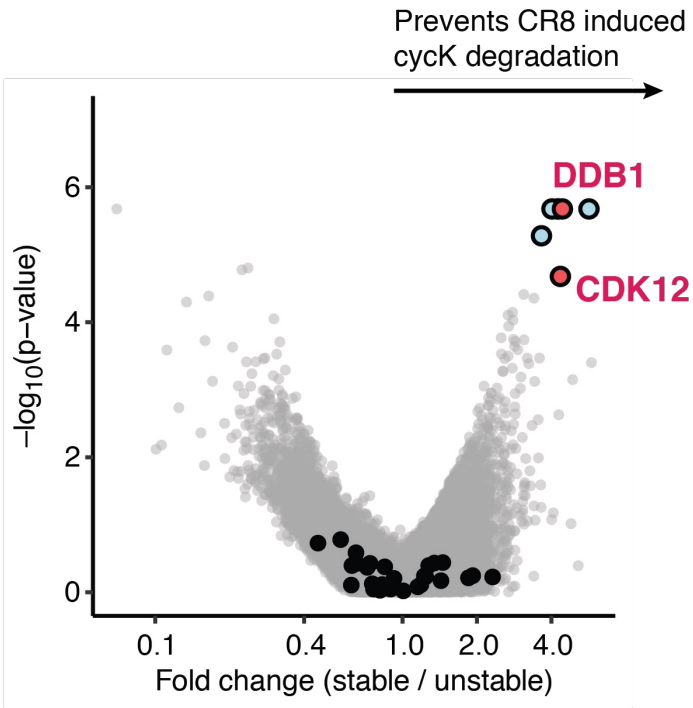


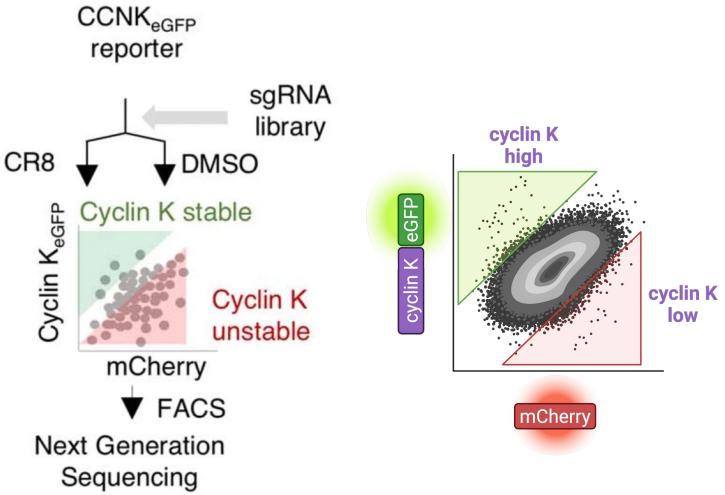


Chapter 3: mode of action deconvolution – **CRISPR screens** (!)

genetic screens

- flow cytometry-based reporter screen
- genome-wide library





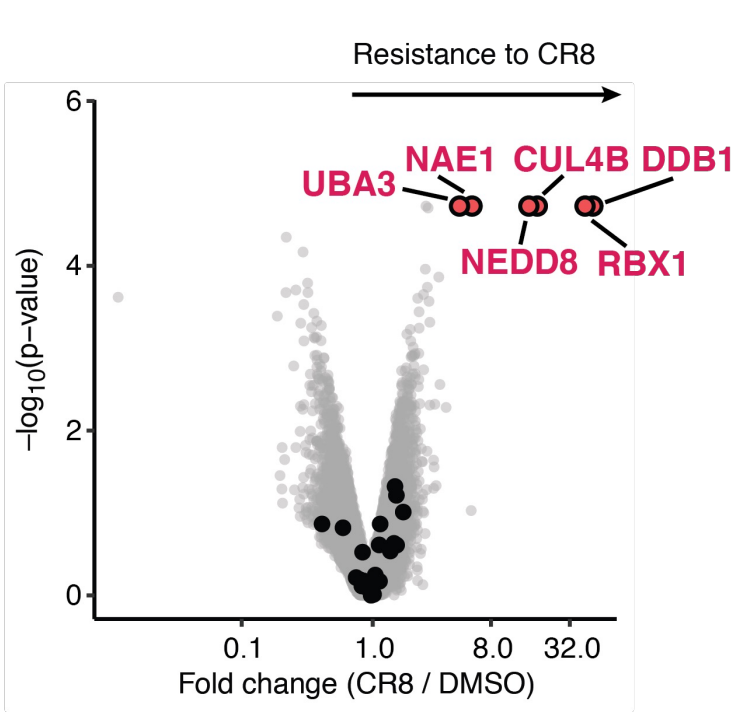
genome-wide CRISPR screen for reporter stability

*A single clone of cyclin K$_{eGFP}$ HEK293T-Cas9 was transduced with the genome-wide Brunello library as described above with the following modification: 4.5 × 10^8 cyclin K$_{eGFP}$ HEK293T-Cas9 cells in 225 ml medium. Nine days later, cells were treated with CR8 (n = 3) or DMSO (n = 3) for at least 2 h and the cyclin K stable population was separated using fluorescence-activated cell sorting (FACS). Four populations were collected (**top** 5%, top 5–15%, lowest 5–15% and **lowest** 5%) on the basis of the **cyclin K$_{eGFP}$ to mCherry** mean fluorescent intensity (MFI) **ratio** on an MA900 Cell Sorter (Sony).*

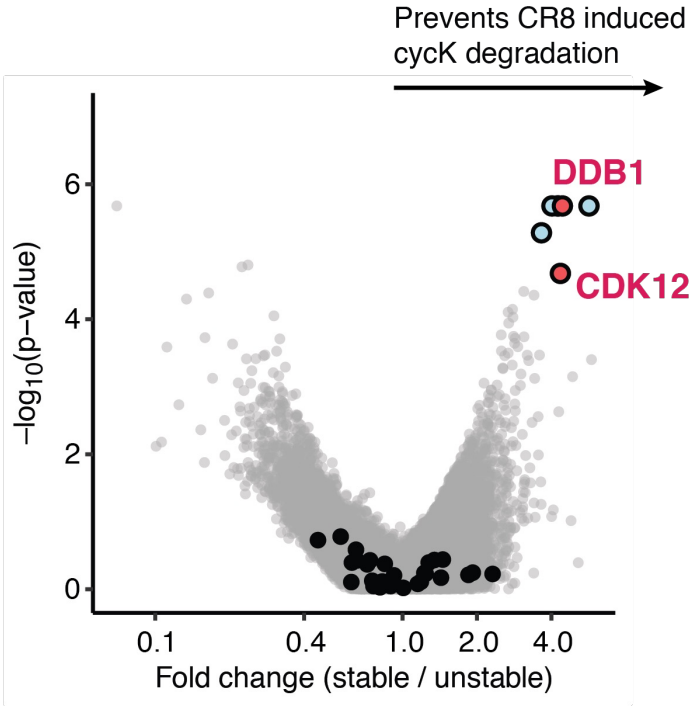
Chapter 3: mode of action deconvolution – **CRISPR screens** (!)

genetic screens

- resistance screen
- genome-wide library

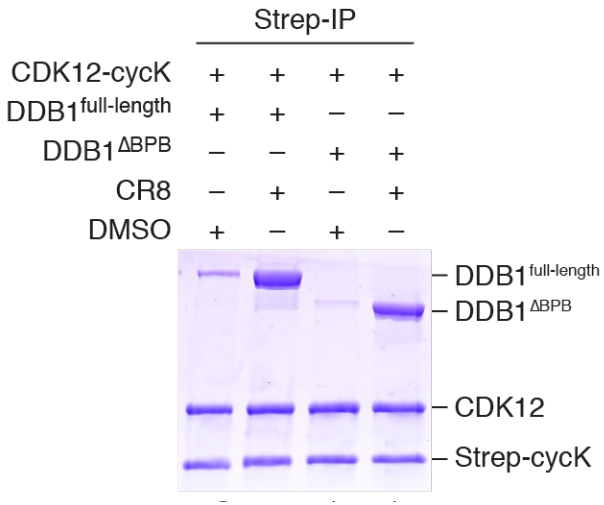


- flow cytometry-based reporter screen
- genome-wide library

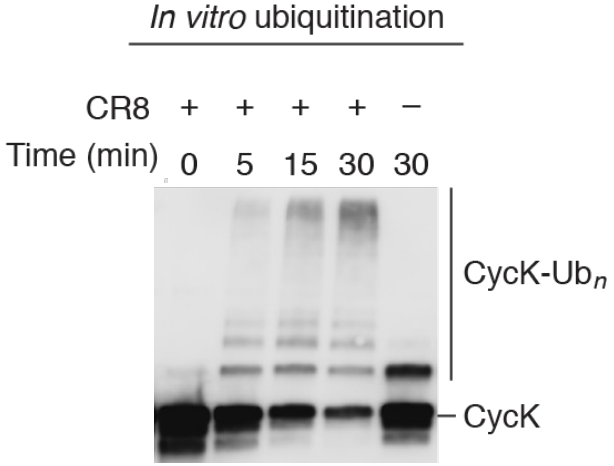


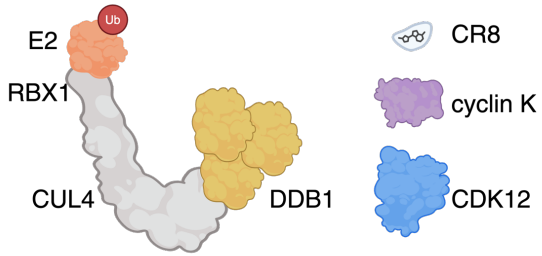
Chapter 4: in vitro reconstitution

pulldown

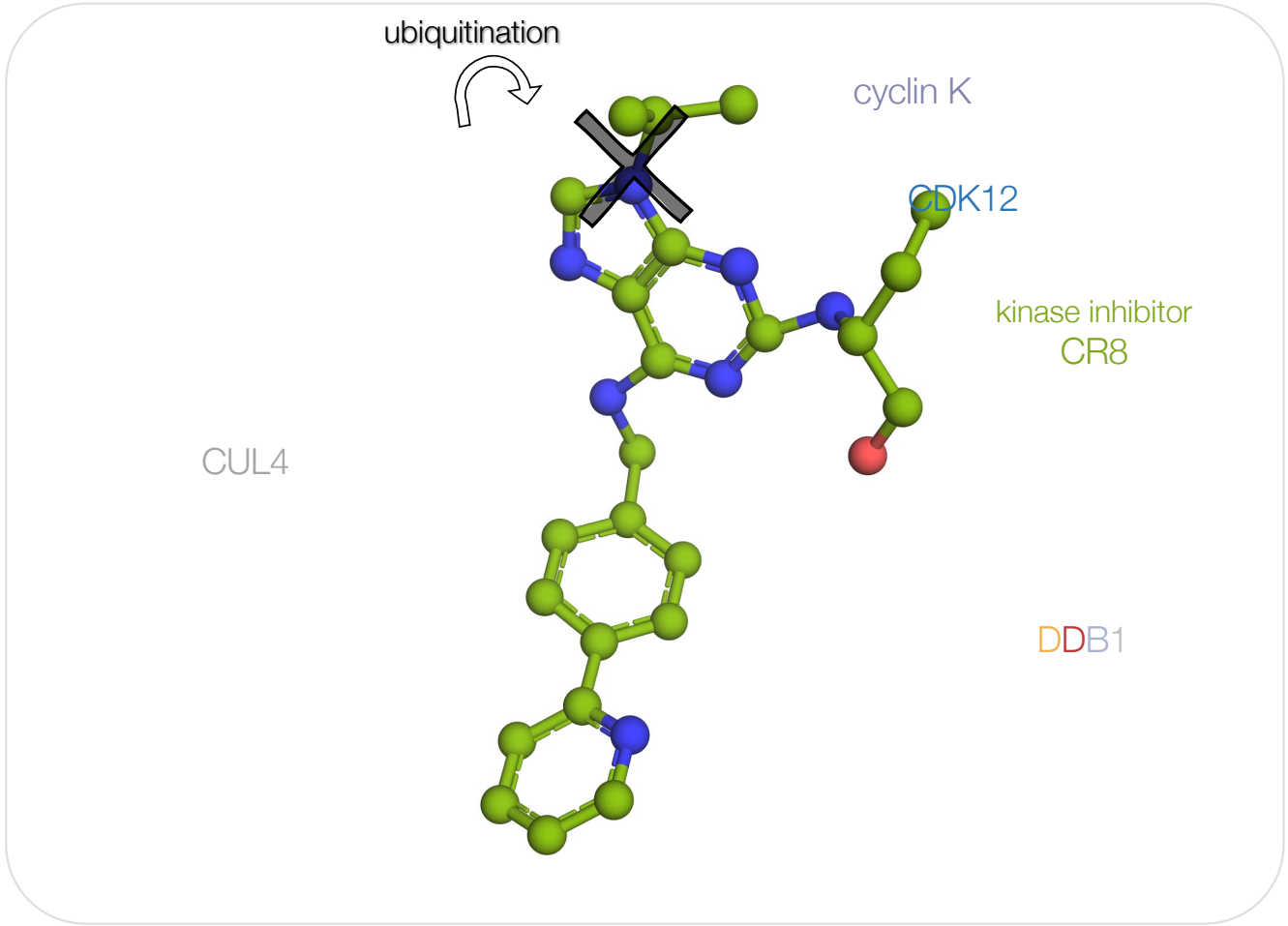


in vitro ubiquitination



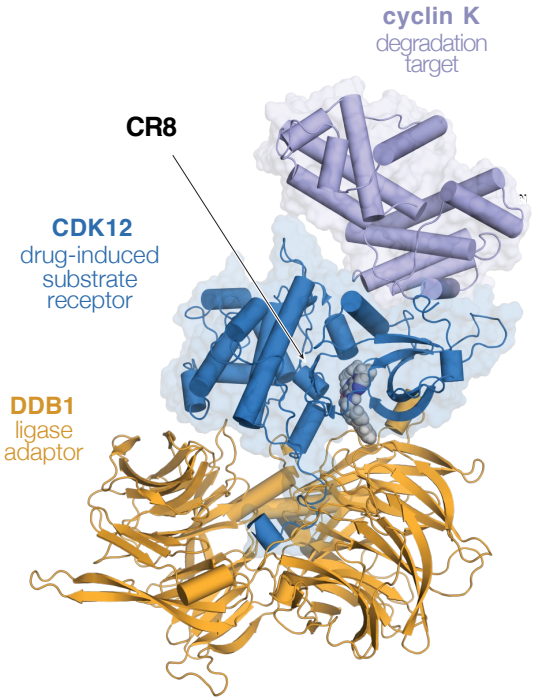


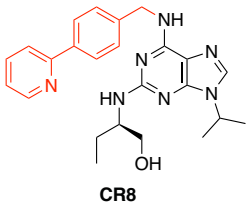
Chapter 5: structural studies



Słabicki*, Kozicka*, Petzold* *et al*. (2020): https://www.nature.com/articles/s41586-020-2374-x

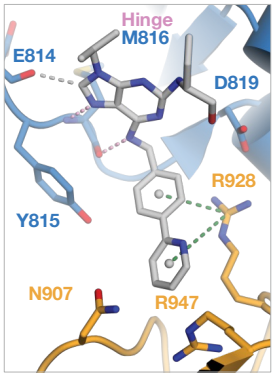
Chapter 5: structural studies → many many chapters later: clinical trials





CR8

CDK12



DDB1

A Modular Phase 1/2 Study with CT7439 in Participants with Solid Malignancies

ClinicalTrials.gov ID ⓘ NCT06600789

Program	Cancer Indications	Trial	Stage of Development
CT7439 Cyclin Dependent Kinase 12/13 Inhibitor (CDK12/13i)/ Cyclin K Degrader	N/A	Monotherapy	Phase 1
	Ovarian	PARP inhibitor combination expansion	Phase 1
	Ewing's Sarcoma	Monotherapy expansion	Phase 1

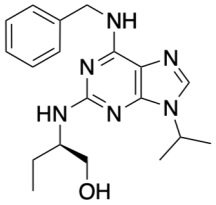
Stage of Development: Discovery | Preclinical | Phase 1 | Phase 2 | Phase 3

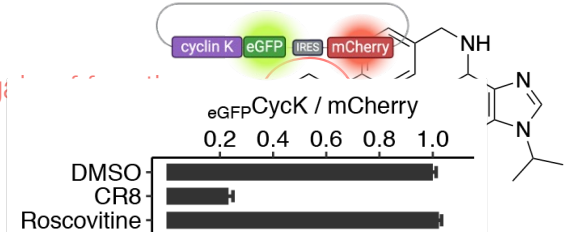
Case study: CRISPR screen to understand drug MoA – how does the compound degrade my target? (2)

Brd4 degraders

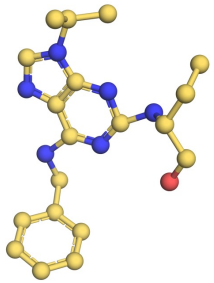
Preface: a fishing expedition inspired by cyclin K degraders

kinase inhibitor

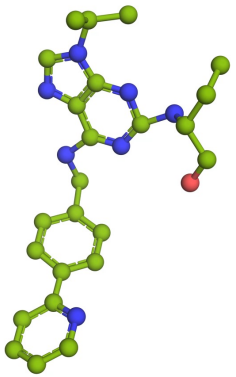




cyclin K degrader



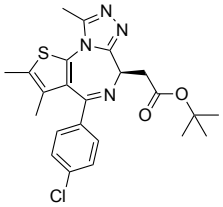
roscovitine



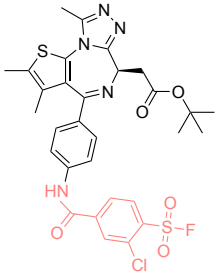
CR8

Chapter 1: derivatise Brd4 binders

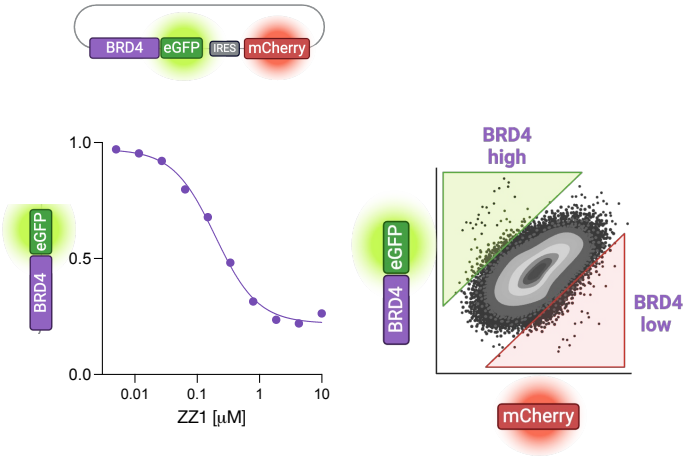
JQ1
BRD4 inhibitor



gain of function



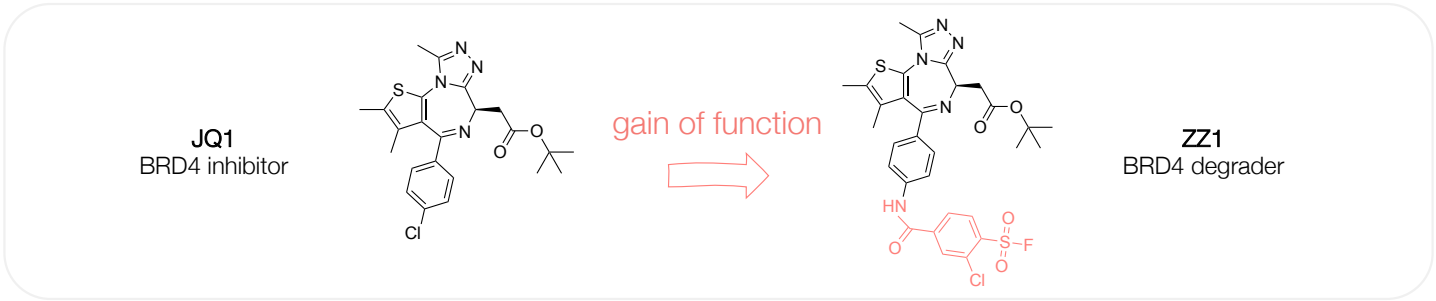
ZZ1
BRD4 degrader

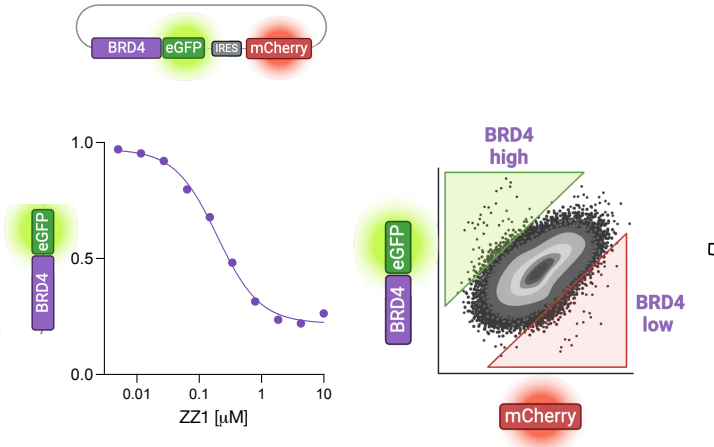


stability reporter

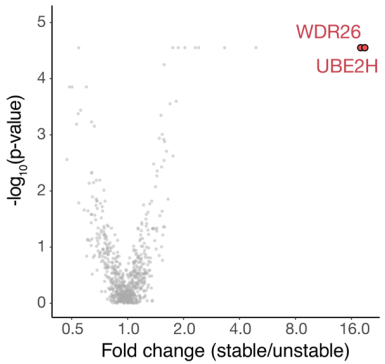
Zhe, Byun, Chrustowicz, Kozicka *et al.* Biorxiv (2024)

Chapter 2: mode of action deconvolution – **CRISPR screens** (!)





stability reporter



genetic screen

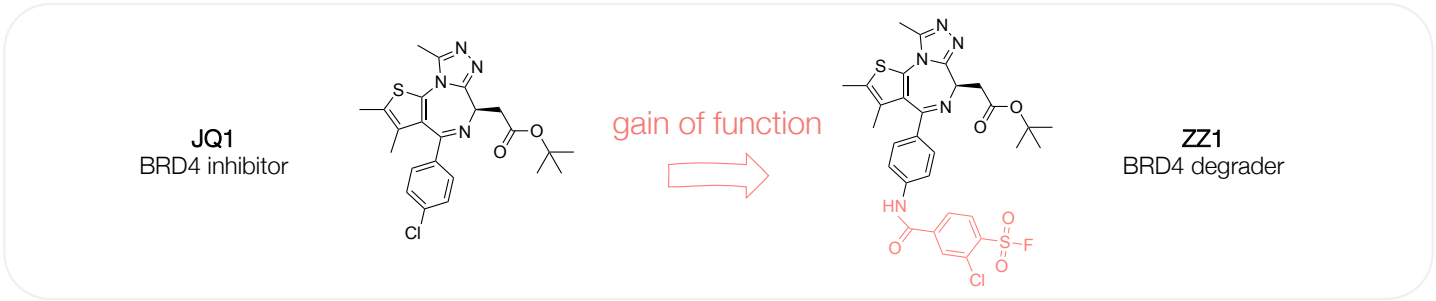
- flow cytometry-based reporter screen
- subgenome library targeting 713 genes related to protein degradation

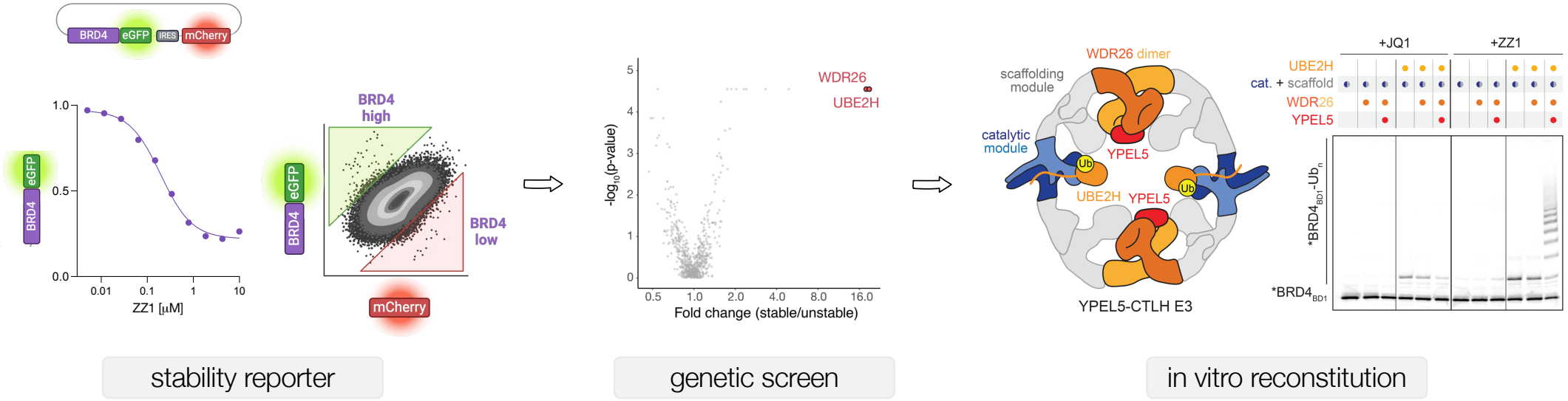
we are looking specifically for a ubiquitin ligase responsible for drug-induced degradation

more efficient, faster, cheaper than genome-wide BUT introduces bias

Zhe, Byun, Chrustowicz, Kozicka *et al.* Biorxiv (2024)

Chapter 3: in vitro reconstitution

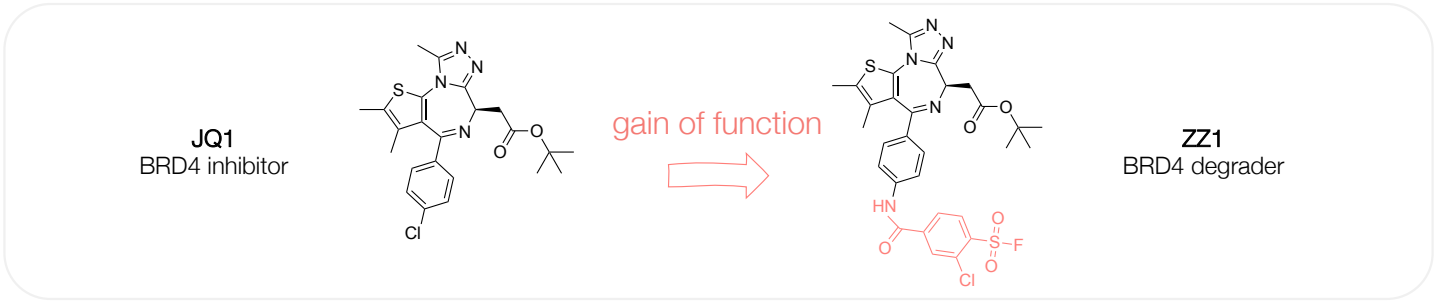


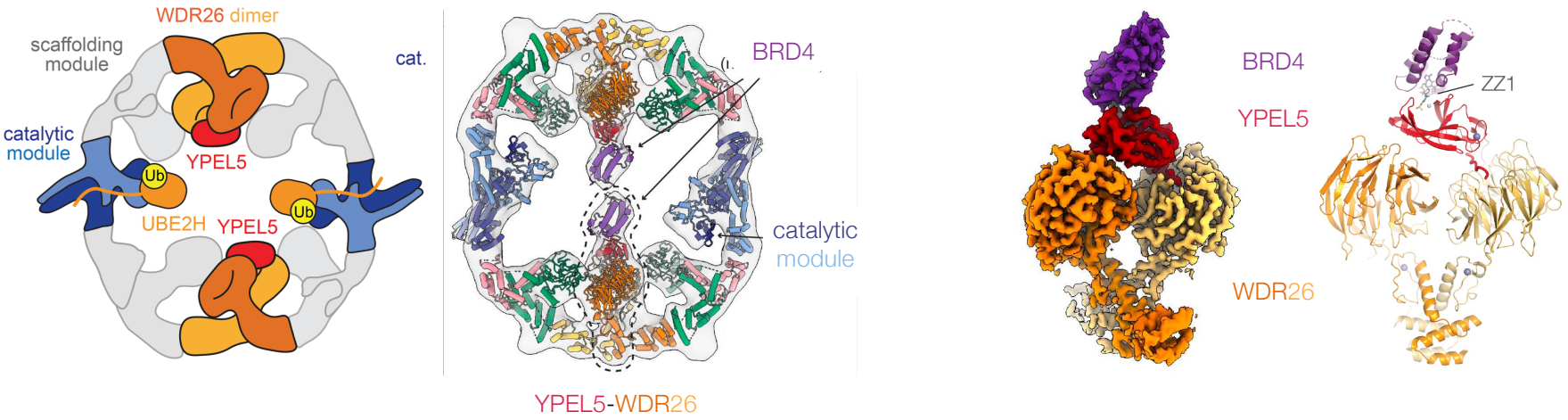


stability reporter | genetic screen | in vitro reconstitution

Zhe, Byun, Chrustowicz, Kozicka *et al.* Biorxiv (2024)

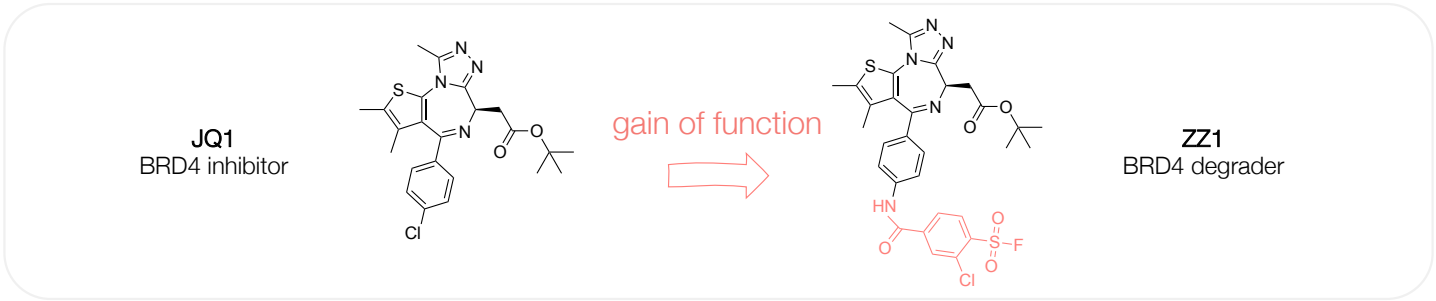
Chapter 4: structural studies

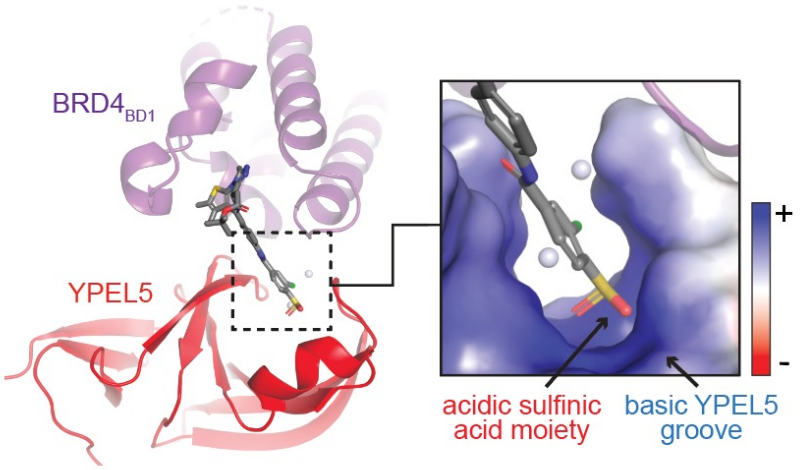


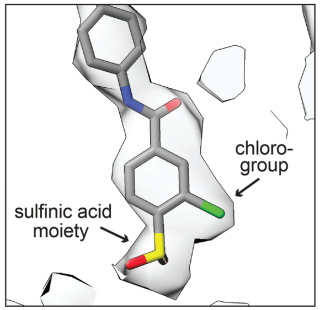


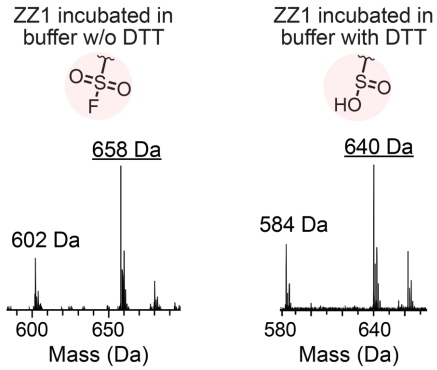
-> molecular glue ZZ1 destines Brd4 for degradation via the YPEL5-CTLH E3 ligase

Chapter 5: something does not add up… Is the compound not what we think it is?

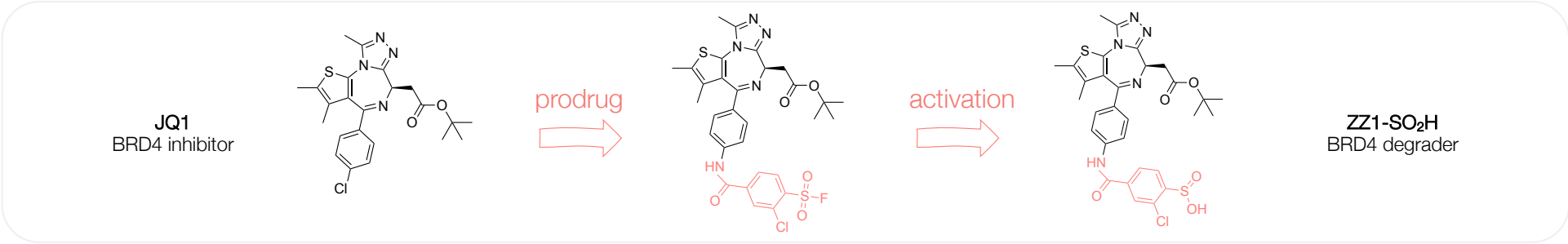


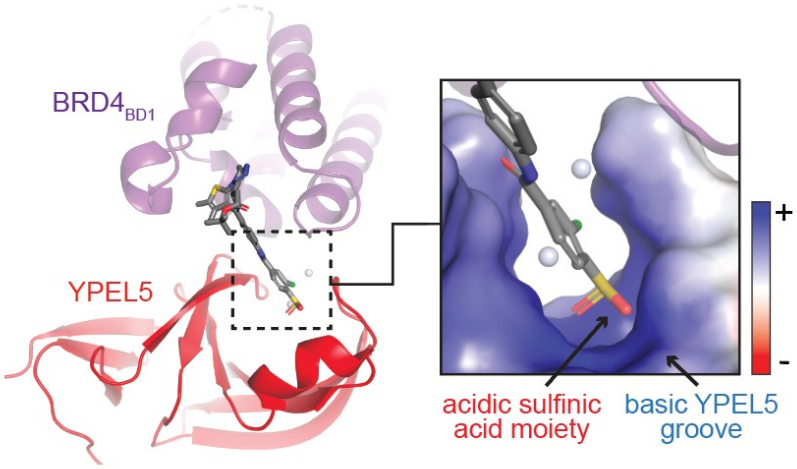


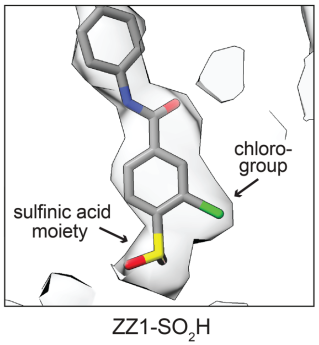


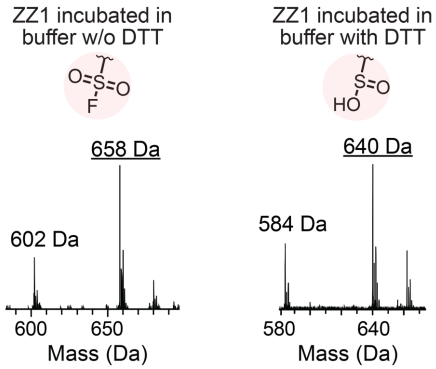


Corrected: ZZ1 is actually a prodrug of a charged molecular glue degrader



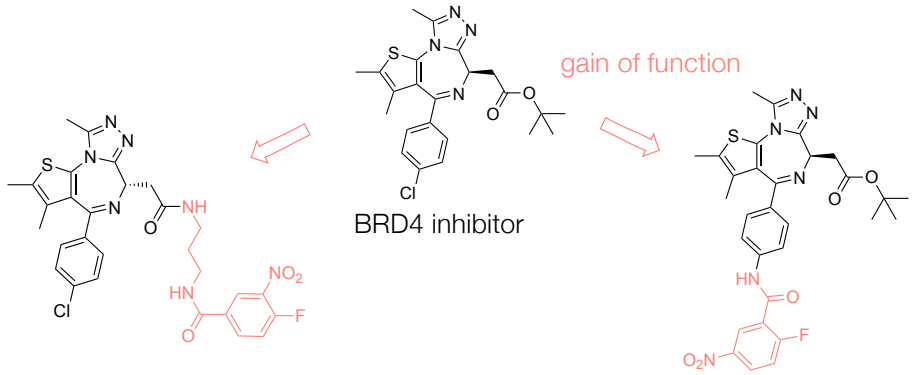


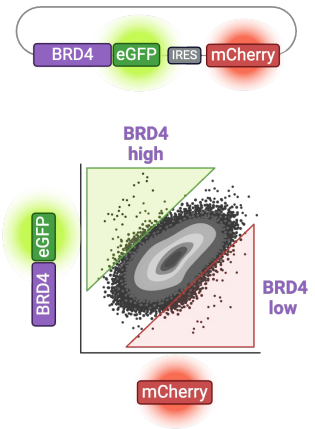




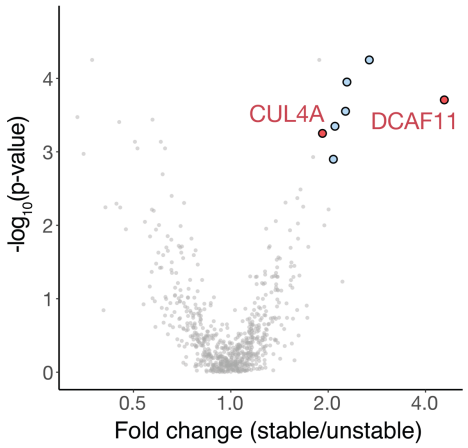
More Brd4 degraders: even with the same warhead can get different mechanisms

these two only differ in the **attachment point of this** "gluing moiety" !

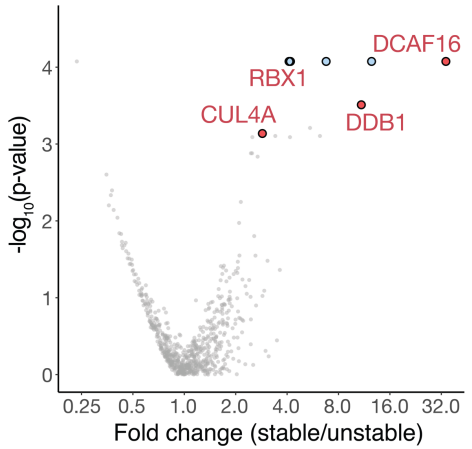




BRD4 degrader 1
via $CRL4^{DCAF11}$



BRD4 degrader 2
via $CRL4^{DCAF16}$

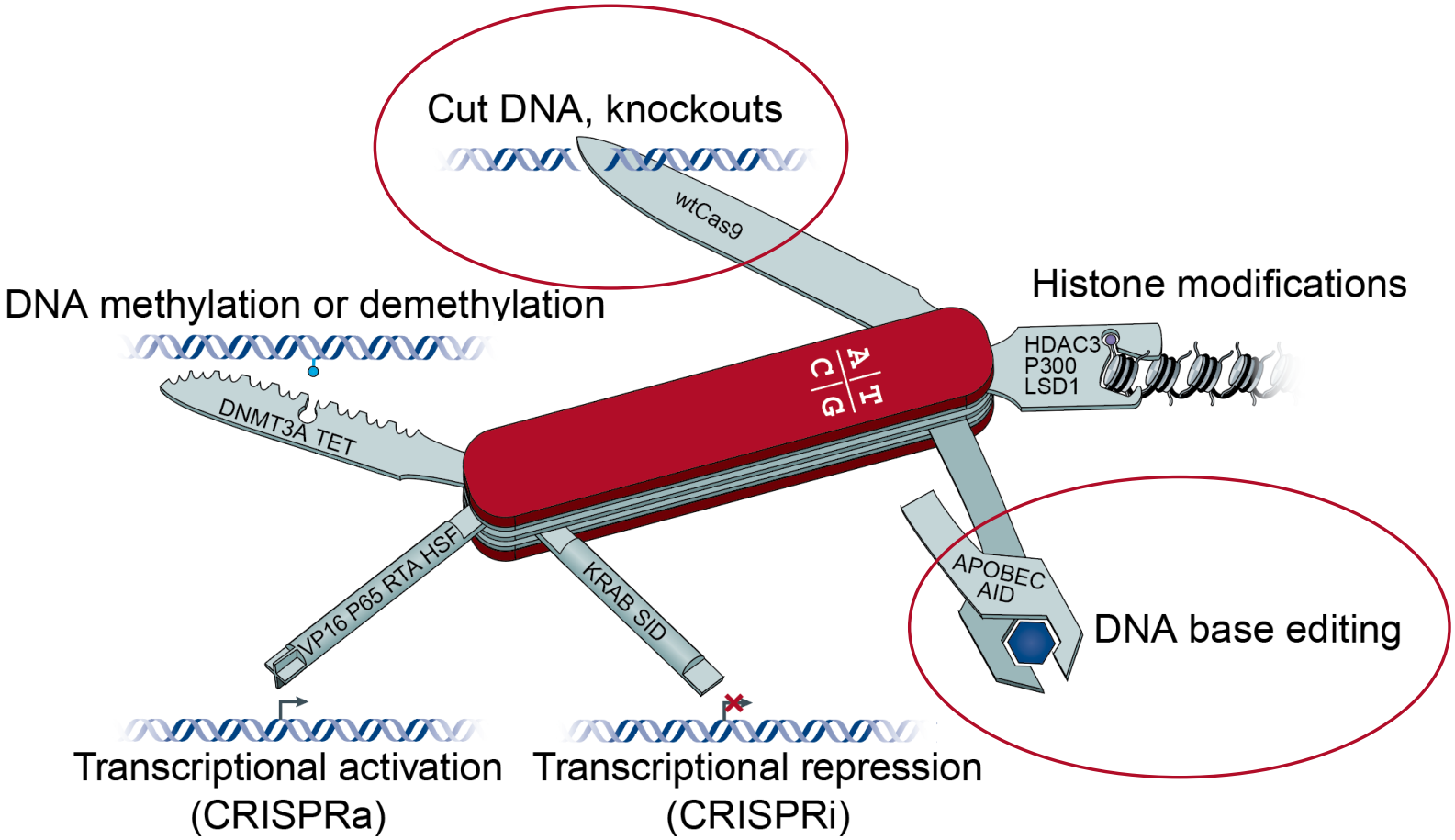


- flow cytometry-based reporter screen
- subgenome library targeting 713 genes related to protein degradation

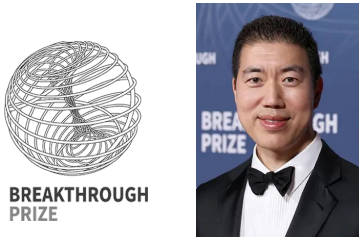
Zhe, Byun, Kozicka *et al.,* ChemRxiv (2024)

Bonus section: base editing

Beyond KOs: CRISPR enables diverse genomic manipulations



Base editing



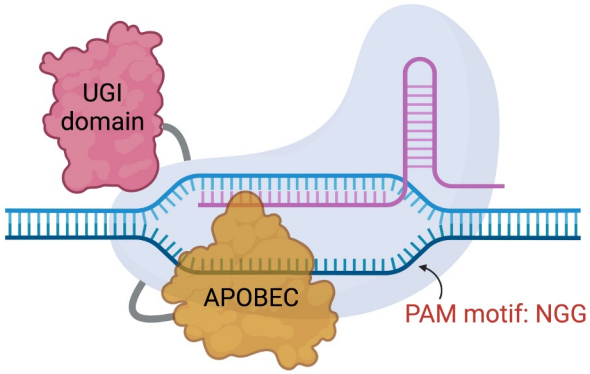
David Liu
(Broad Institute)

- use mutated Cas9 – no cut, just target DNA recognition
- bring in an enzyme – e.g. a cytydine deaminase for a precise C-to-T edit

C-to-T editors (2016)

Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage

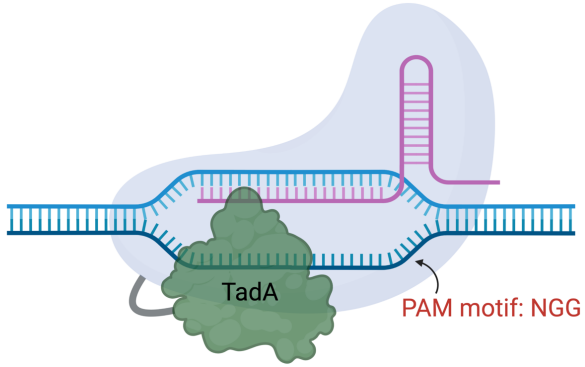
Alexis C. Komor[1,2], Yongjoo B. Kim[1,2], Michael S. Packer[1,2], John A. Zuris[1,2] & David R. Liu[1,2]



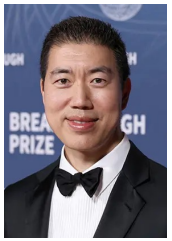
A-to-G editors (2017)

Programmable base editing of A·T to G·C in genomic DNA without DNA cleavage

Nicole M. Gaudelli[1,2,3], Alexis C. Komor[1,2,3]†, Holly A. Rees[1,2,3], Michael S. Packer[1,2,3]†, Ahmed H. Badran[1,2,3], David I. Bryson[1,2,3]† & David R. Liu[1,2,3]



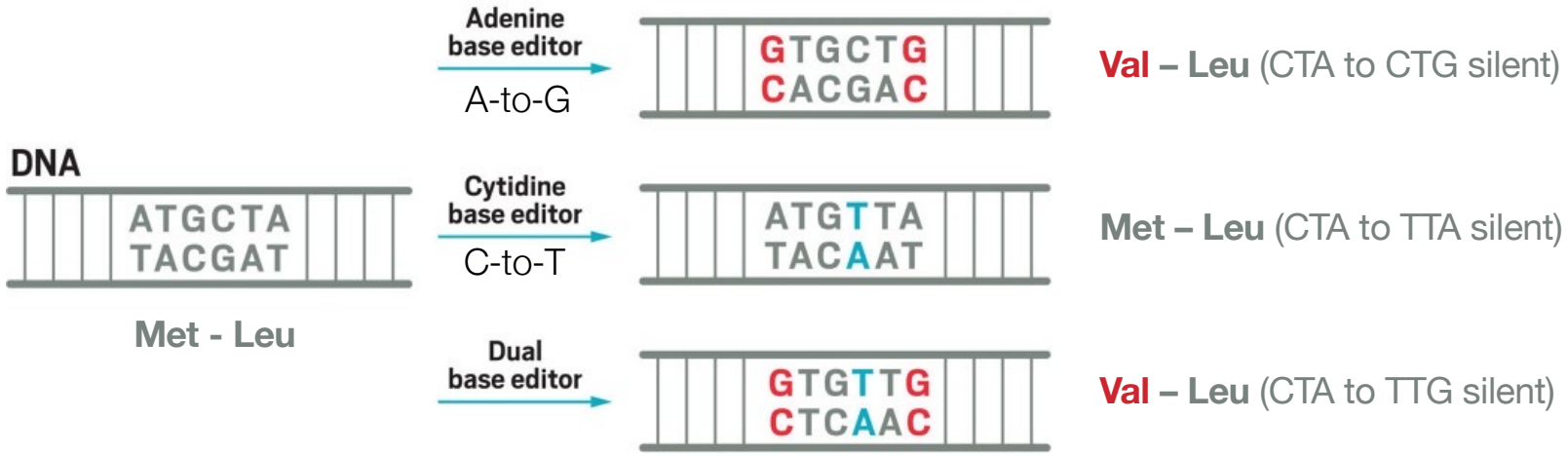
Base editing



David Liu
(Broad Institute)

- use mutated Cas9 – no cut, just target DNA recognition
- bring in an enzyme – e.g. a cytydine deaminase for a precise C-to-T edit

→ **important caveat:** only some edits are consequential, not all changes are feasible



https://www.genscript.com/tools/codon-frequency-table

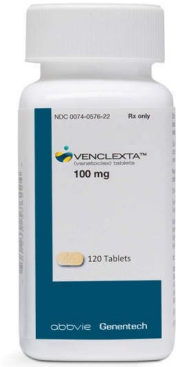
Case study: base editor screen to understand drug-target interactions

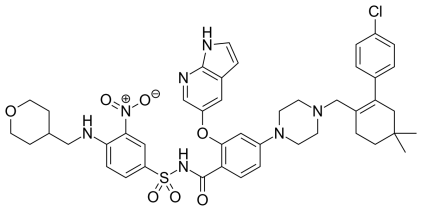
venetoclax and BCL2

Adapted from Sangree et al, 2022

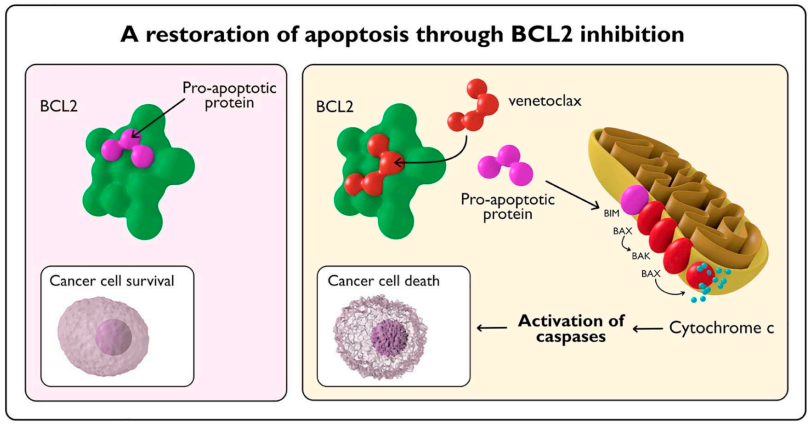
https://doi.org/10.1038/s41467-022-28884-7

Venetoclax is a BCL2 inhibitor





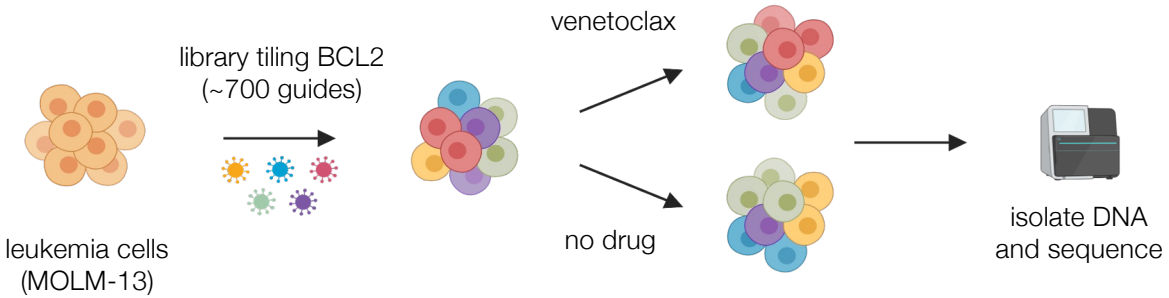
Venetoclax

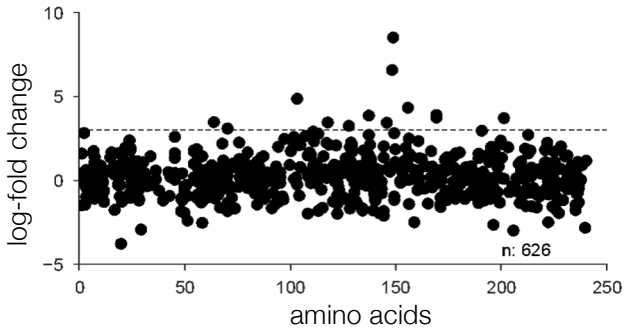


Mihalyova et al, 2018

- Venetoclax is a BH3 mimetic that inhibits proapoptotic protein BCL2
- Approved for clinical use in 2016 to treat various leukemias
- BUT resistance arises… How?
- Perform base editor tiling screen to understand which mutations drive resistance

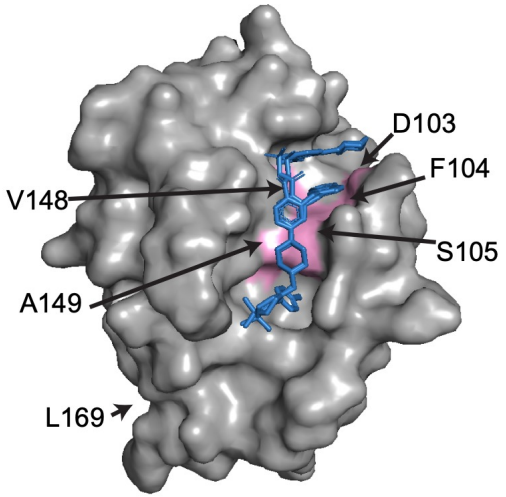
BCL2 tiling screen





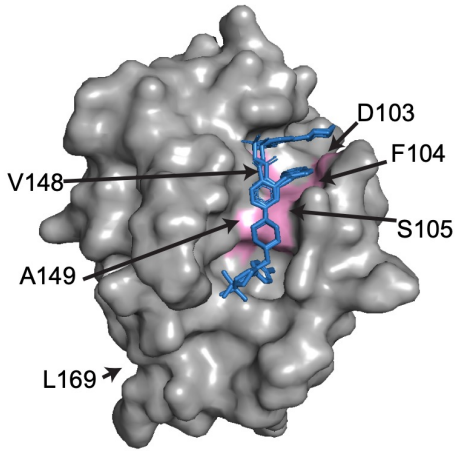
resistance mutations enrich

loss-of-function mutations deplete



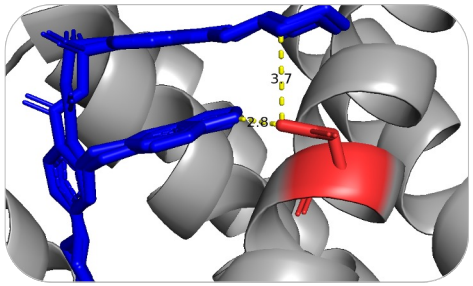
Adapted from Sangree et al, 2022

Mutations abolish protein-ligand interactions

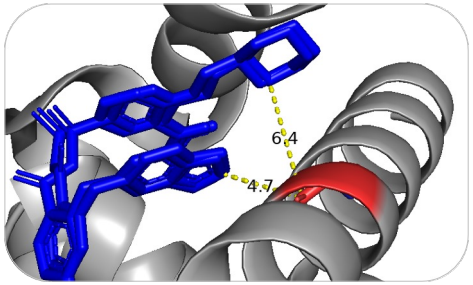


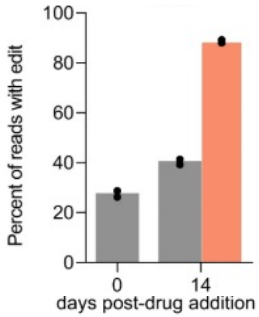
- Resistance mutations cluster around binding pocket
- D103 lines the pocket and makes a hydrogen bond with drug
- F104 makes a stacking interaction with the drug

D103

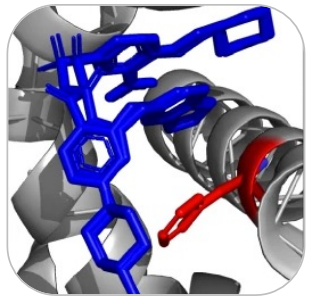


D103G

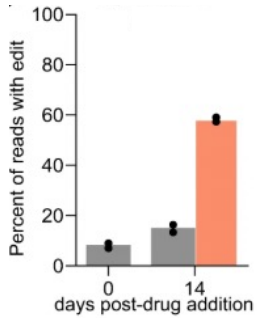




F104



F104L



Adapted from Sangree et al, 2022

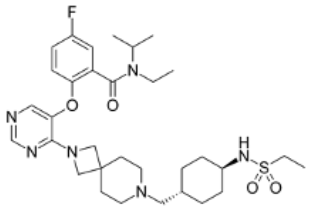
Case study: base editor screen to understand drug resistance

menin (MEN1) inhibitors

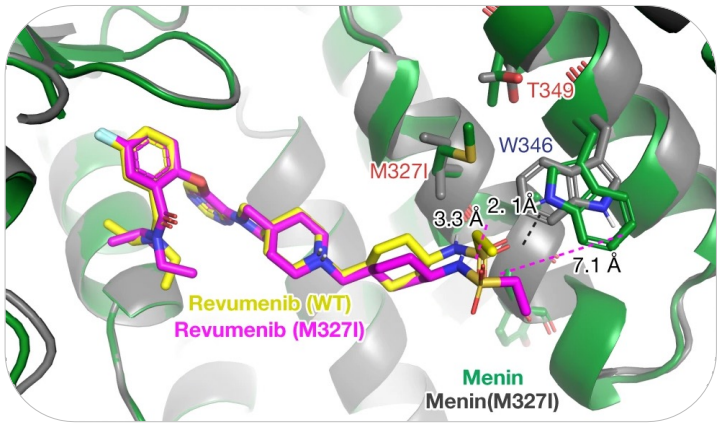
Adapted from Perner et al, 2023

https://doi.org/10.1038/s41586-023-05755-9

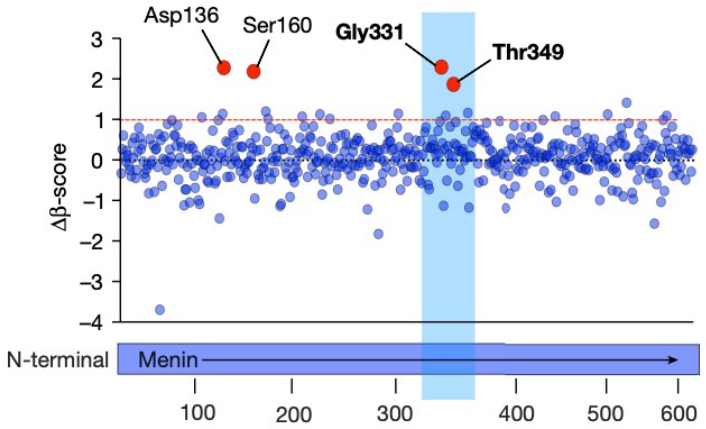
Base editor screen identifies MEN1 resistance mutations - same as concurrent clinical trial



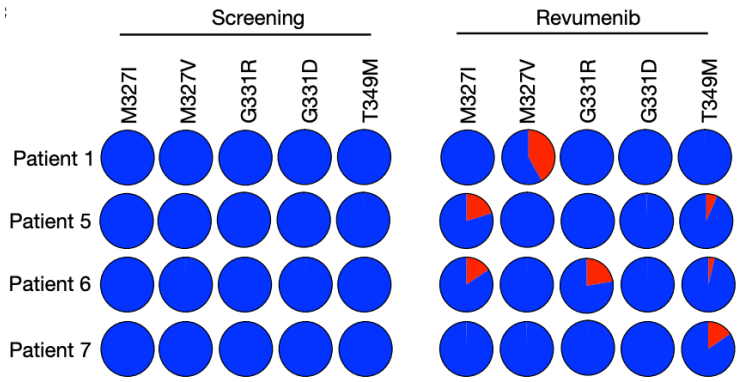
Revumenib
(leukemia drug)



screen:

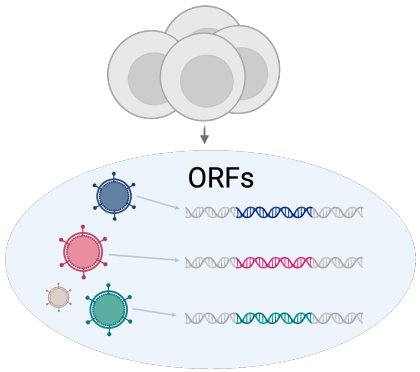
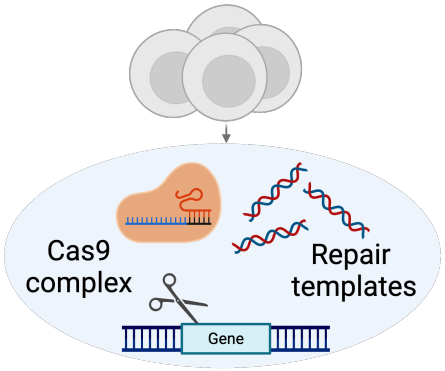
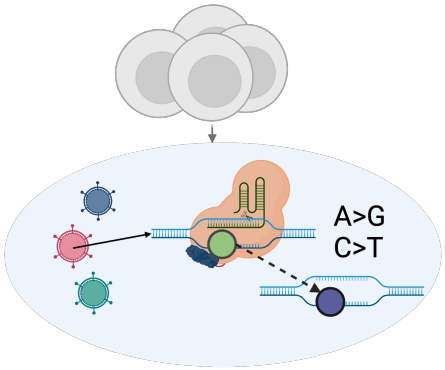
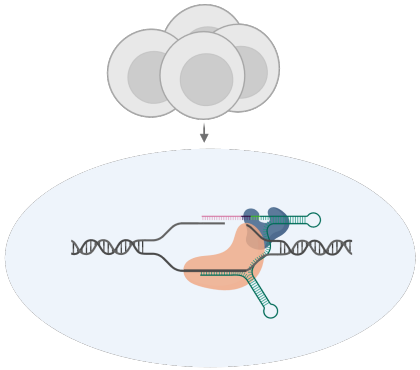


patients:



Adapted from Perner et al, 2023

Alternative methods for installing mutations

	Deep Mutational Scanning	Saturation Genome Editing	Base Editing	Prime Editing
				
mechanism	variant ORF pool overexpression	HDR of exogenous oligos	sgRNA library	pegRNA library
resolution	precise nucleotide resolution	precise nucleotide resolution	imprecise nucleotide resolution	precise nucleotide resolution
% possible edits	100%	100%	~70% (edits limited to A>G or C>T)	100%
throughput	medium	very low	high	medium
edit location	exogenous	endogenous	endogenous	endogenous
limitations	costly library construction, cell line engineering required	haploid cell lines only	editing uncertainty	lower efficiency; requires pegRNA optimization; haploid cell lines only

Alternative methods for installing mutations – further reading

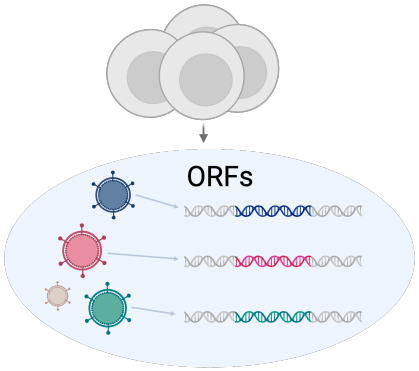
Saturation editing of genomic regions by multiplex homology-directed repair

Gregory M Findlay [1], Evan A Boyle [1], Ronald J Hause [2], Jason C Klein [2], Jay Shendure [2]

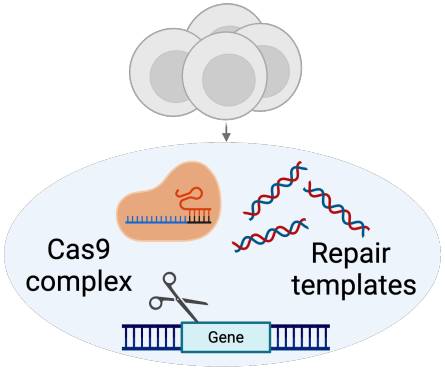
Search-and-replace genome editing without double-strand breaks or donor DNA

Andrew V. Anzalone, Peyton B. Randolph, Jessie R. Davis, Alexander A. Sousa, Luke W. Koblan, Jonathan M. Levy, Peter J. Chen, Christopher Wilson, Gregory A. Newby, Aditya Raguram & David R. Liu

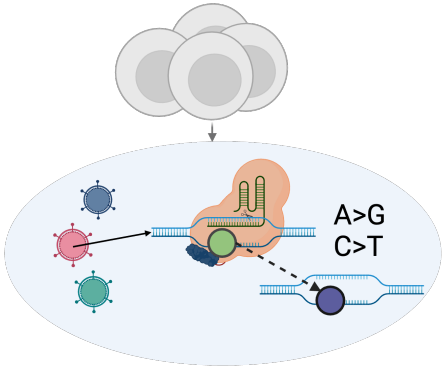
Deep Mutational Scanning



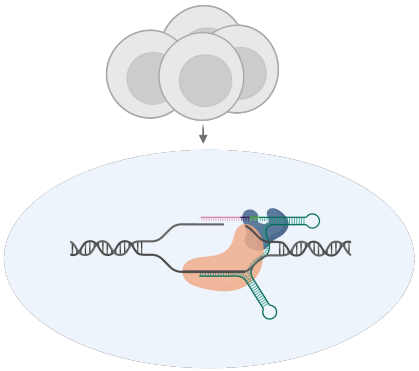
Saturation Genome Editing



Base Editing



Prime Editing



Deep mutational scanning: assessing protein function on a massive scale

Carlos L Araya [1], Douglas M Fowler

Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage

Nicole M. Gaudelli, Alexis C. Komor, Holly A. Rees, Michael S. Packer, Ahmed H. Badran, David I. Bryson & David R. Liu

What else can you do with a base editing screen?

- Uncover drug resistance mutations (BCL2, MEN1)
- Identify loss-of-function mutations and endogenous regulatory elements
  - BRCA1, TP53
- Confirm on-target activity of a less-characterized small molecule
- Map the functional landscape of an uncharacterized gene

Functional interrogation of DNA damage response variants with base editing screens

Raquel Cuella-Martin,[1] Samuel B. Hayward,[1,5] Xiao Fan,[2,3,5] Xiao Chen,[1] Jen-Wei Huang,[1] Angelo Taglialatela,[1] Giuseppe Leuzzi,[1] Junfei Zhao,[2,3,4] Raul Rabadan,[2,3,4] Chao Lu,[1] Yufeng Shen,[2,3] and Alberto Ciccia[1,6,*]

Article

Base editing screens map mutations affecting interferon-γ signaling in cancer

Matthew A. Coelho [1 8], Sarah Cooper [2 8], Magdalena E. Strauss [9], Emre Karakoc [1 8], Shriram Bhosle [1], Emanuel Gonçalves [1 7], Gabriele Picco [1 8], Thomas Burgold [2], Chiara M. Cattaneo [5 8], Vivien Veninga [5 8], Sarah Consonni [1 8], Cansu Dinçer [1], Sara F. Vieira [1 8], Freddy Gibson [1], Syd Barthorpe [1], Claire Hardy [3], Joel Rein [4], Mark Thomas [4], John Marioni [9], Emile E. Voest [5 6 8]...Mathew J. Garnett [1 8 10]

Base editor scanning charts the DNMT3A activity landscape

Nicholas Z. Lue, Emma M. Garcia, Kevin C. Ngan, Ceejay Lee, John G. Doench & Brian B. Liau

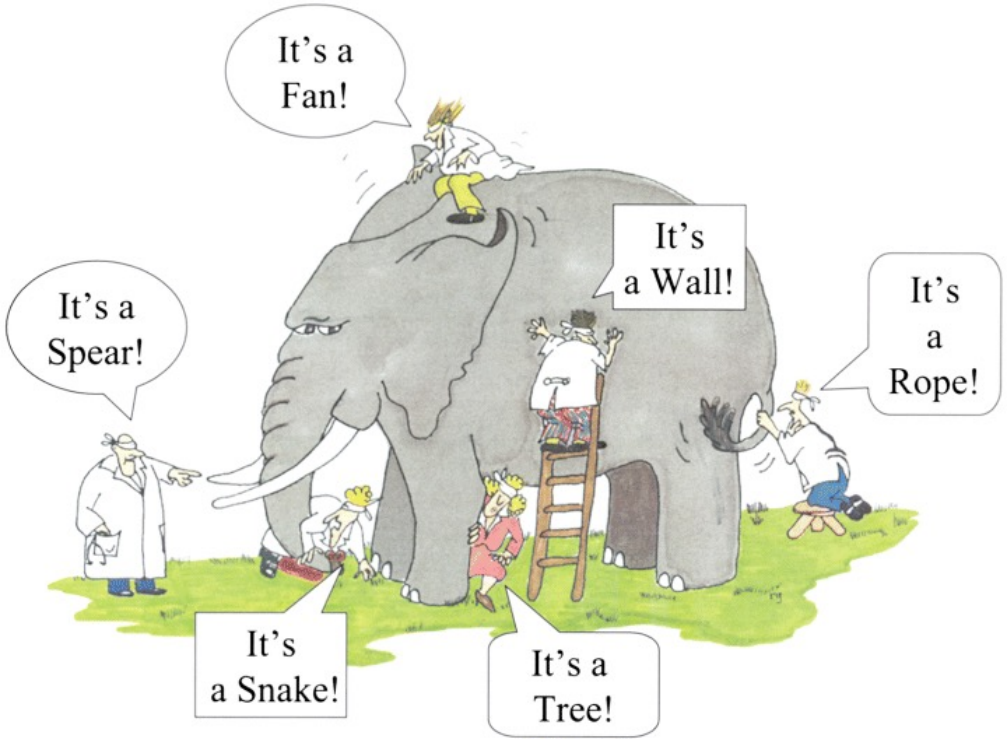
Nature Chemical Biology **19**, 176–186 (2023) | Cite this article

3516 Accesses | **40** Altmetric | Metrics

Final message: find a good model system and screen, screen, screen!!!

- RNAi
- CRISPR knockout
- Base editing
- CRISPRa
- CRISPRi
- …

…new things can be learned each time!



the blind men and the elephant