Massively parallel functional analysis of missense mutations in *BRCA1* for interpreting variants of uncertain significance

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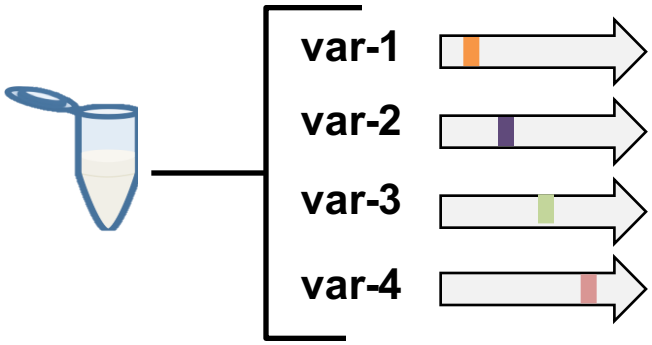
Variants of uncertain significance (VUS)

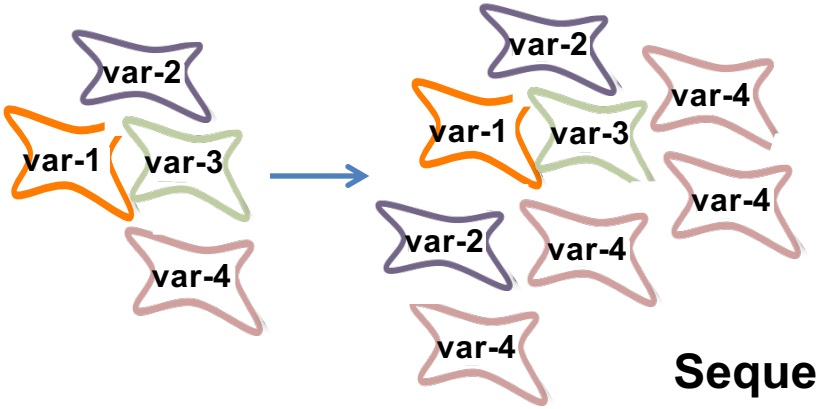
How do we interpret the impact of genetic variation at scale?

	Validity	Throughput
Genetic analysis or one-off experiments	+++	+
Computational prediction	+	+++
Massively parallel functional analysis	+++	+++

Massively parallel functional assays for assessing function of missense variants

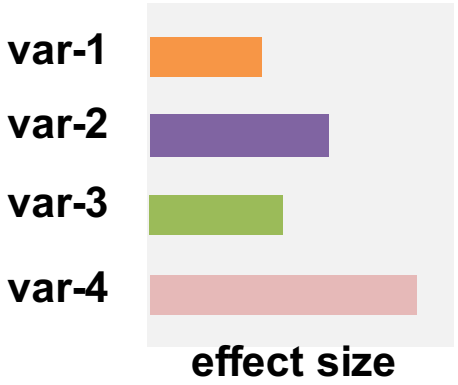


Generate a library with mutations in a sequence of interest



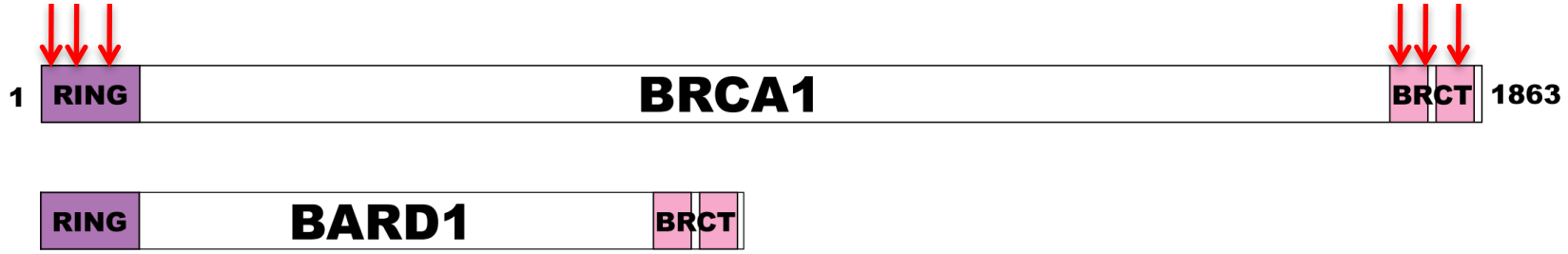
Multiplexed functional assay

Sequence variants in input and selected populations



Quantify effect sizes of individual variants

Biochemical functions of BRCA1

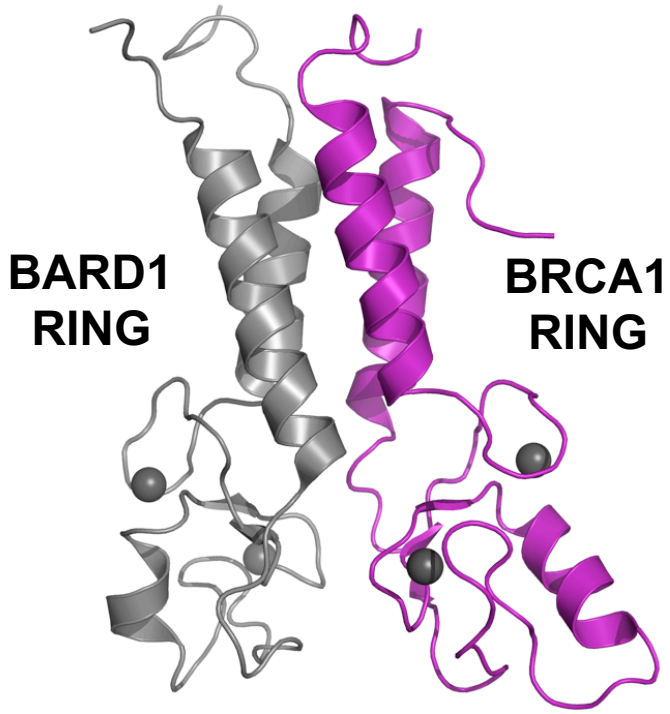


BRCA1 is required for homology-directed dsDNA break repair (HDR)

BRCA1 HDR activity is required for tumor suppression

BRCA1 must dimerize with BARD1 to function in HDR

The BRCA1:BARD1 dimer has ubiquitin ligase activity

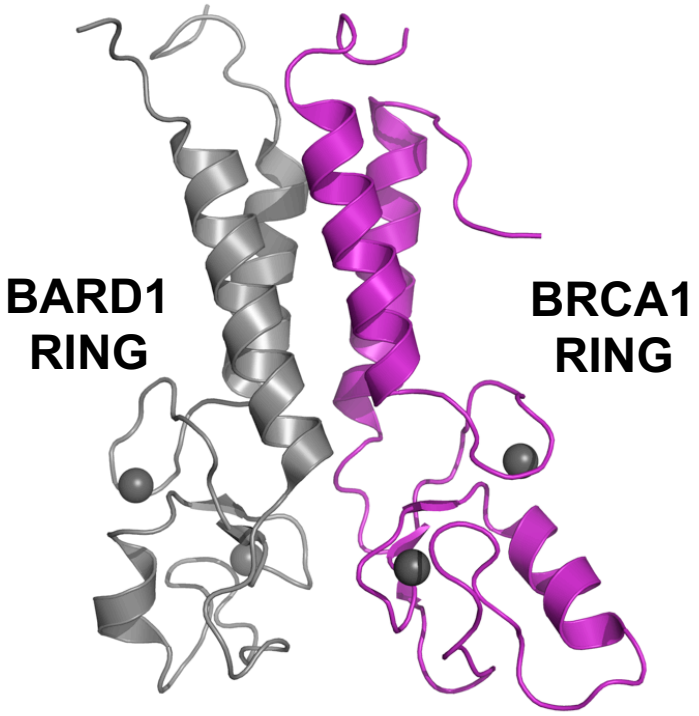


Multiplex assays for BRCA1 protein function and splicing

Experiments 1 and 2:

BARD1-BRCA1-RING E3 ligase activity

BARD1-BRCA1-RING interaction



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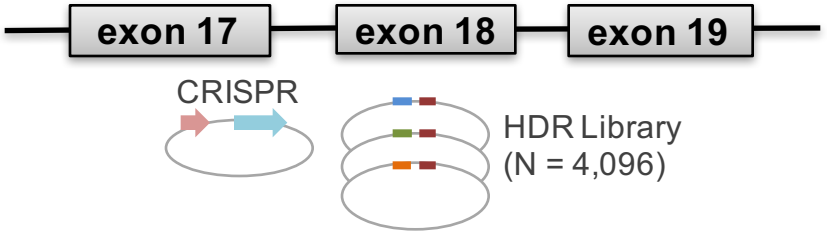
Saturation genome editing to assess the effect of SNVs on splicing.



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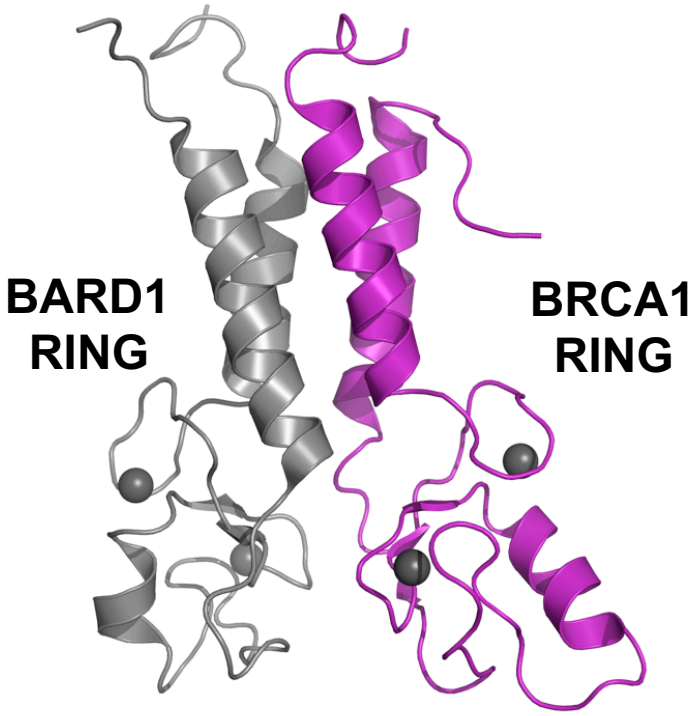

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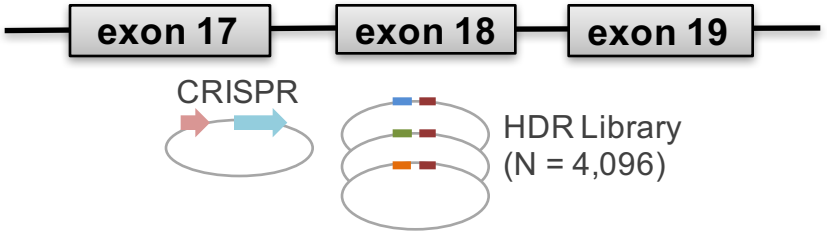
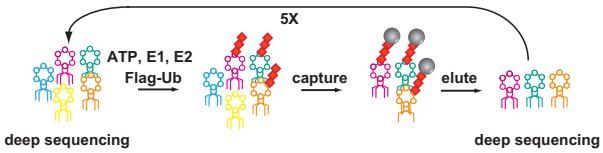
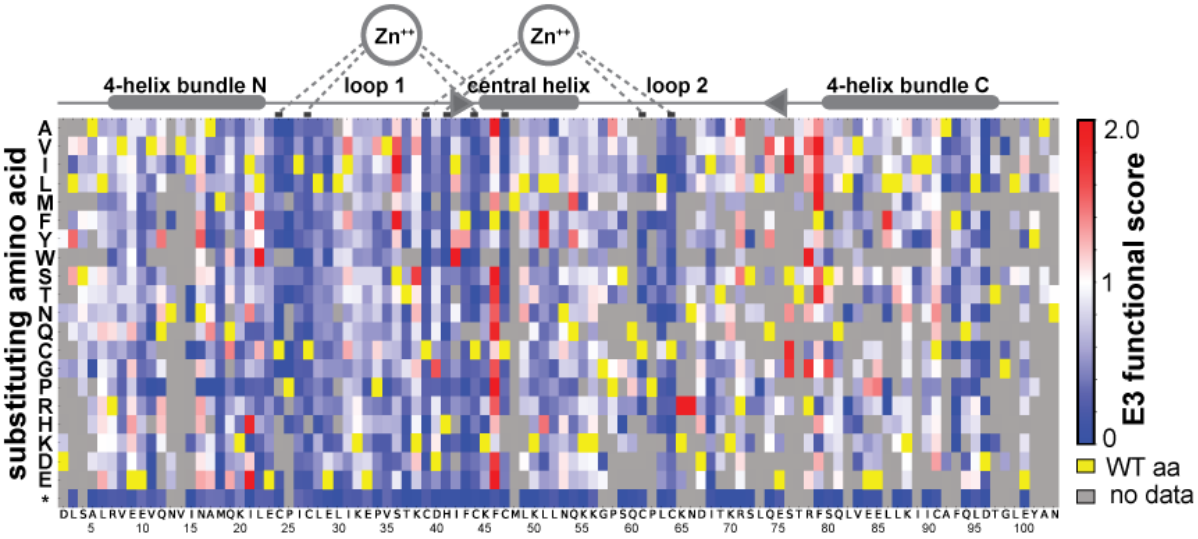


Massively parallel assays for the BRCA1-RING E3 ligase and BARD1-binding activities

E3 ligase activity





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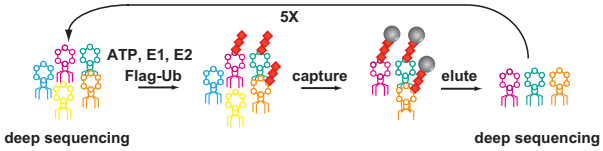
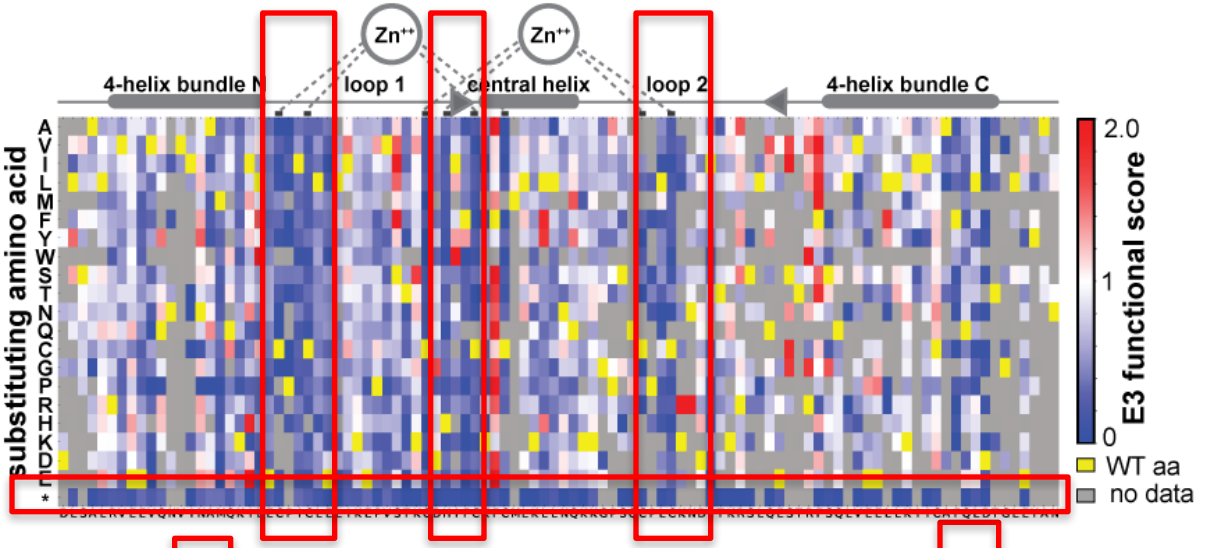
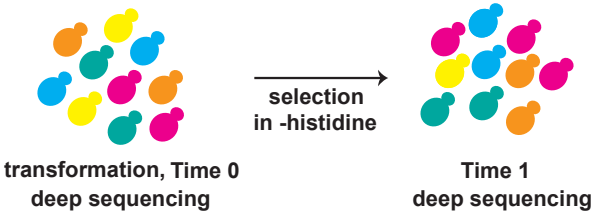
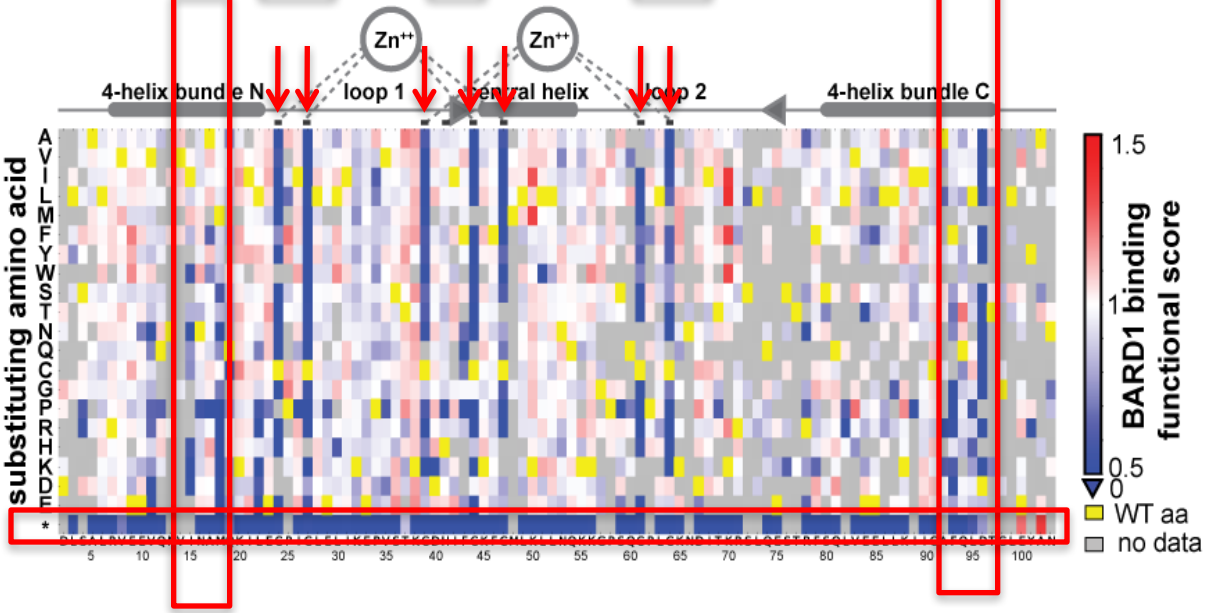


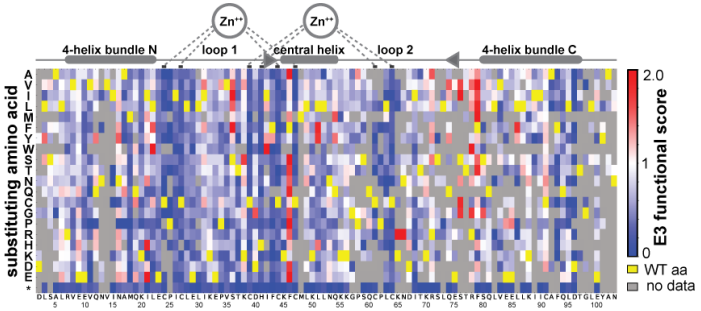


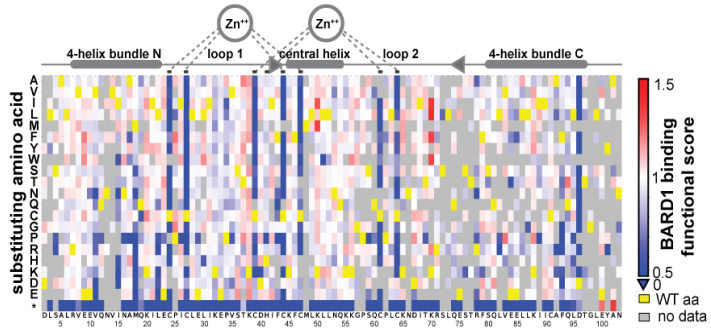
BARD1-binding activity





How can we leverage these measurements to estimate the likelihood that a BRCA1 variant would be pathogenic?

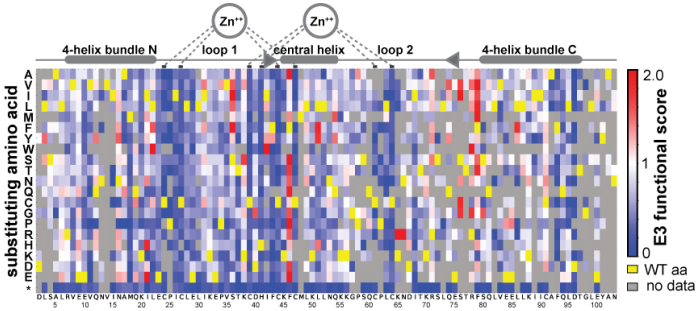


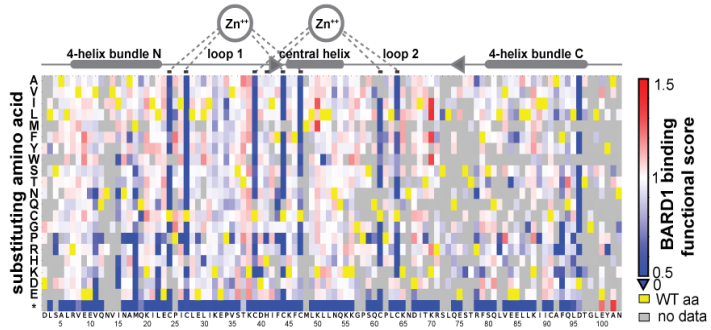


prediction model

How can we leverage these measurements to ~~estimate the likelihood that a BRCA1 variant would be pathogenic~~?

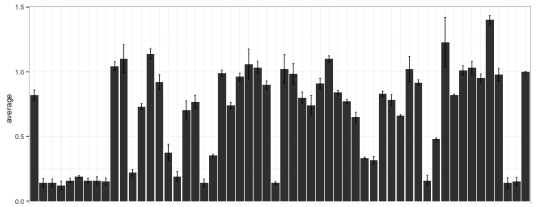
to understand the homology-directed DNA repair (HDR) function of BRCA1?





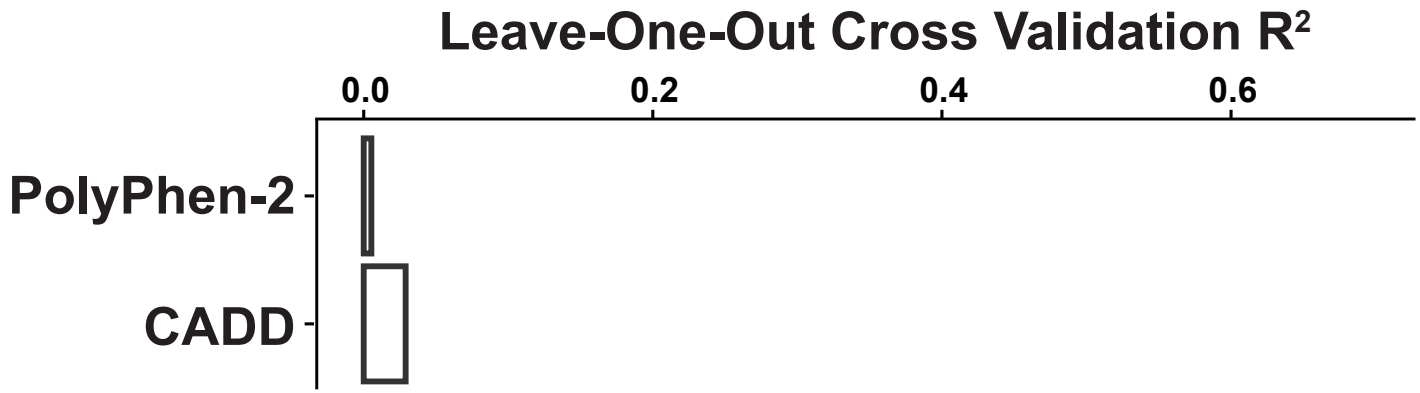
prediction model

HDR activity?

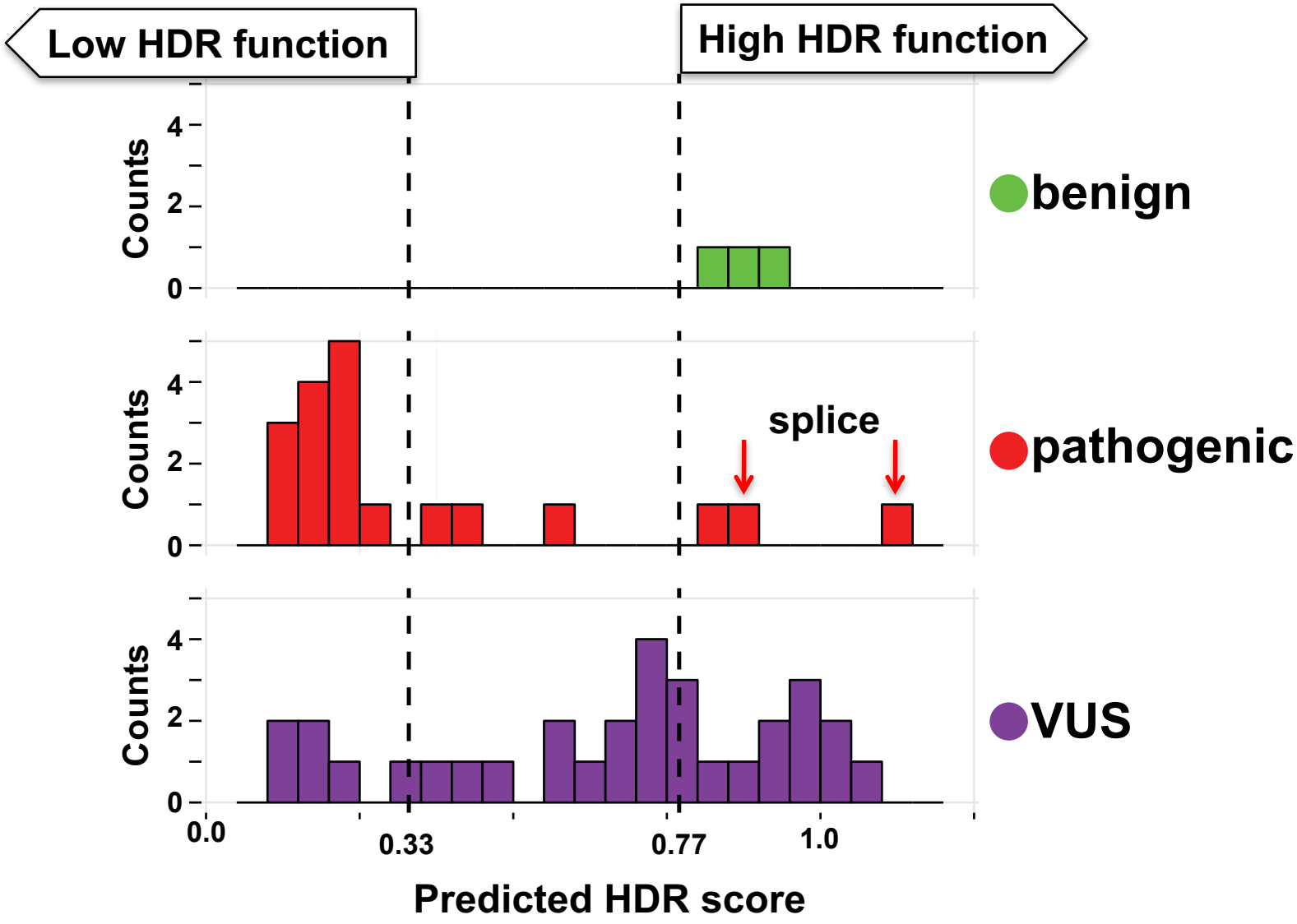


Ransburgh et al. *Cancer Research* 2010

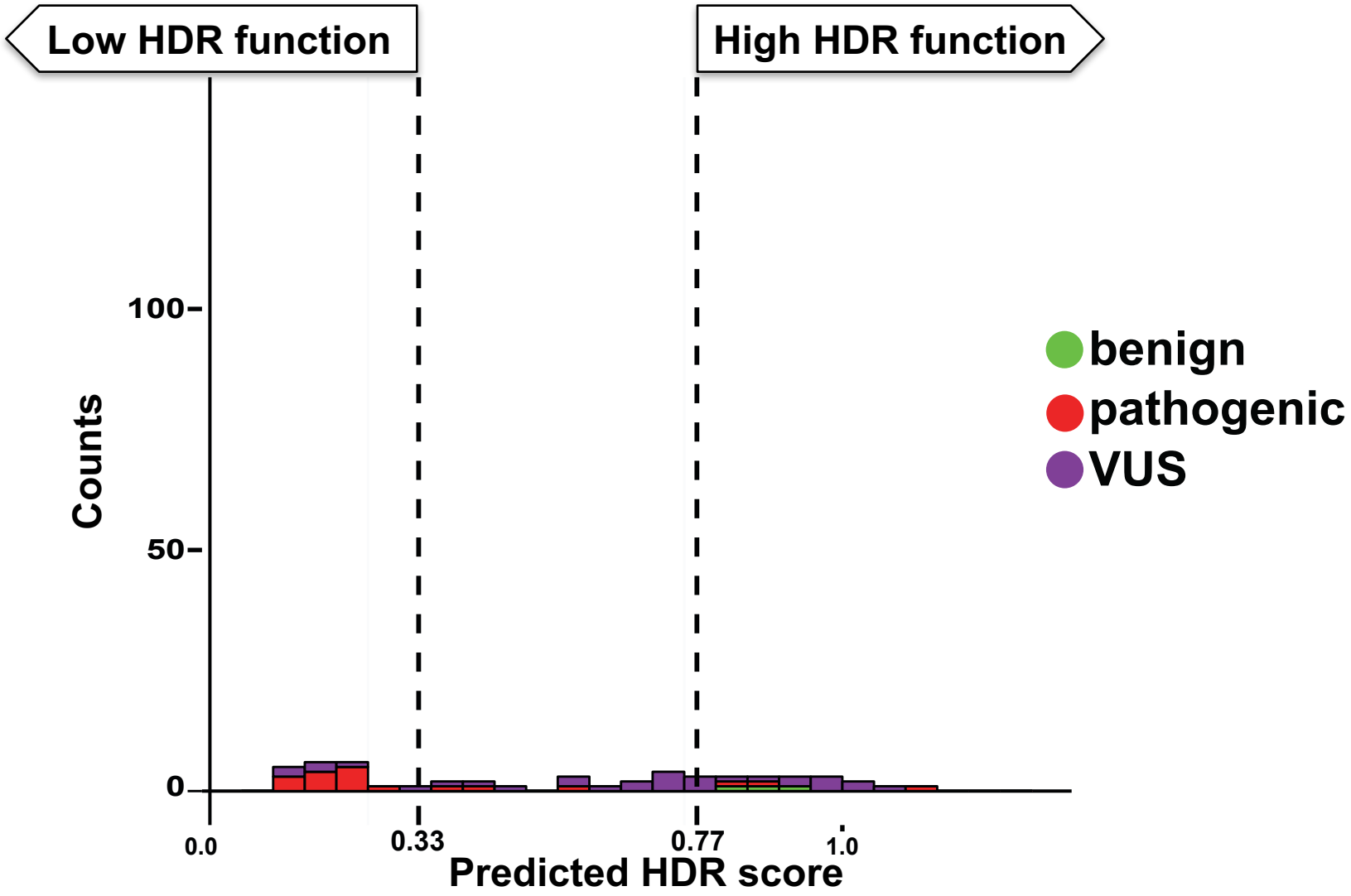
Experimental data build a better predictor of BRCA1 HDR function



HDR predictions for clinical BRCA1 variants



HDR predictions for 1,287 BRCA1 variants not yet seen in patients



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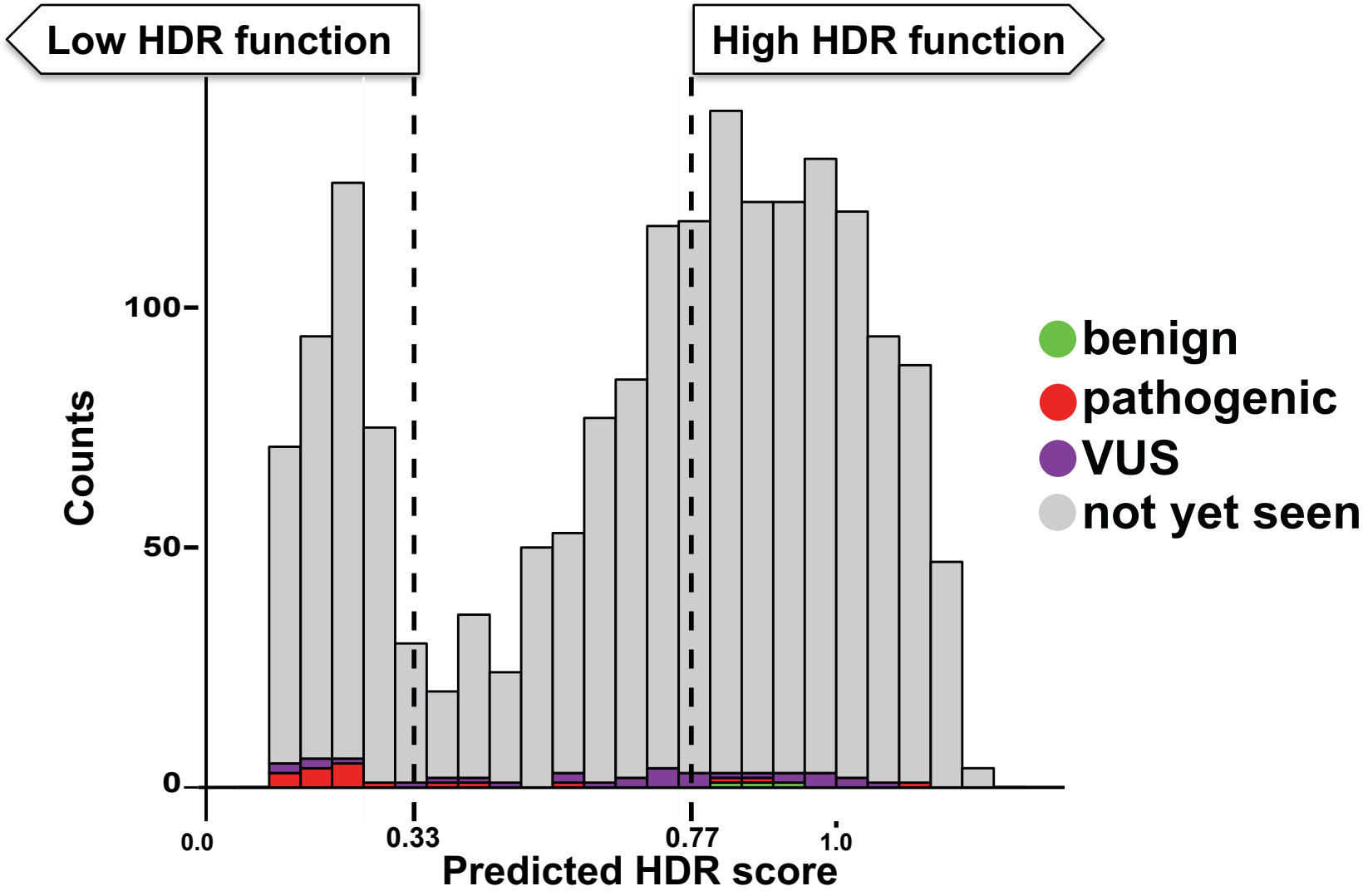

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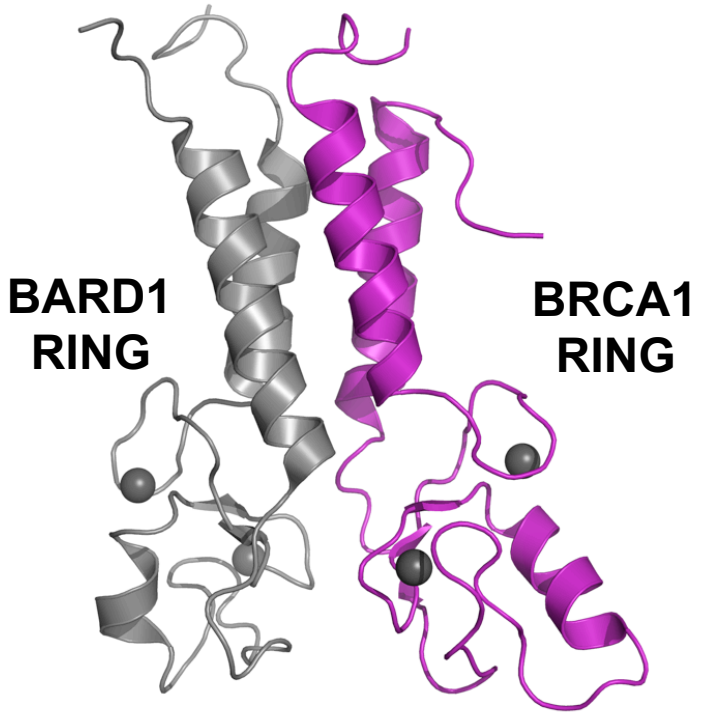

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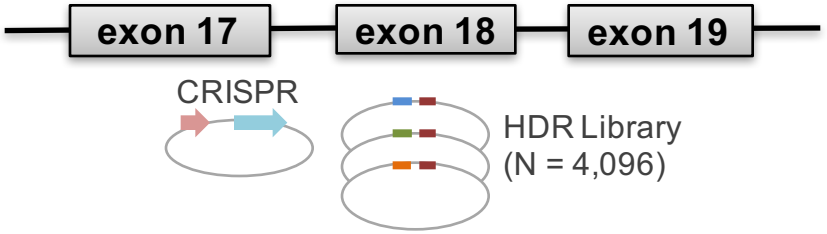
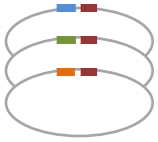


Multiplex genome editing to measure the effects of SNVs on splicing

Cas9/gRNA

Edited repair templates (N = 4,096)

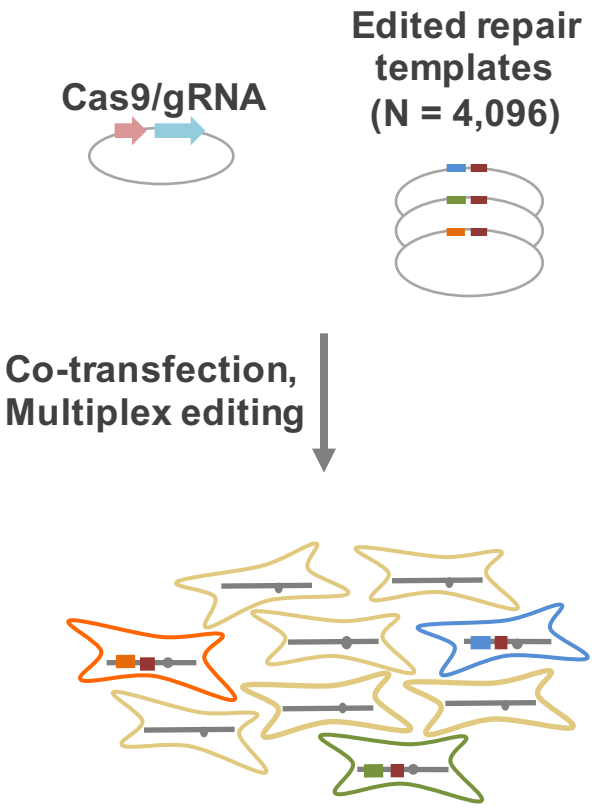


1. CRISPR-Cas9 construct targeting ***BRCA1*** exon 18

2. Repair template library to substitute **SNVs** within the exon.

Findlay, Boyle et al., *Nature* (2014).

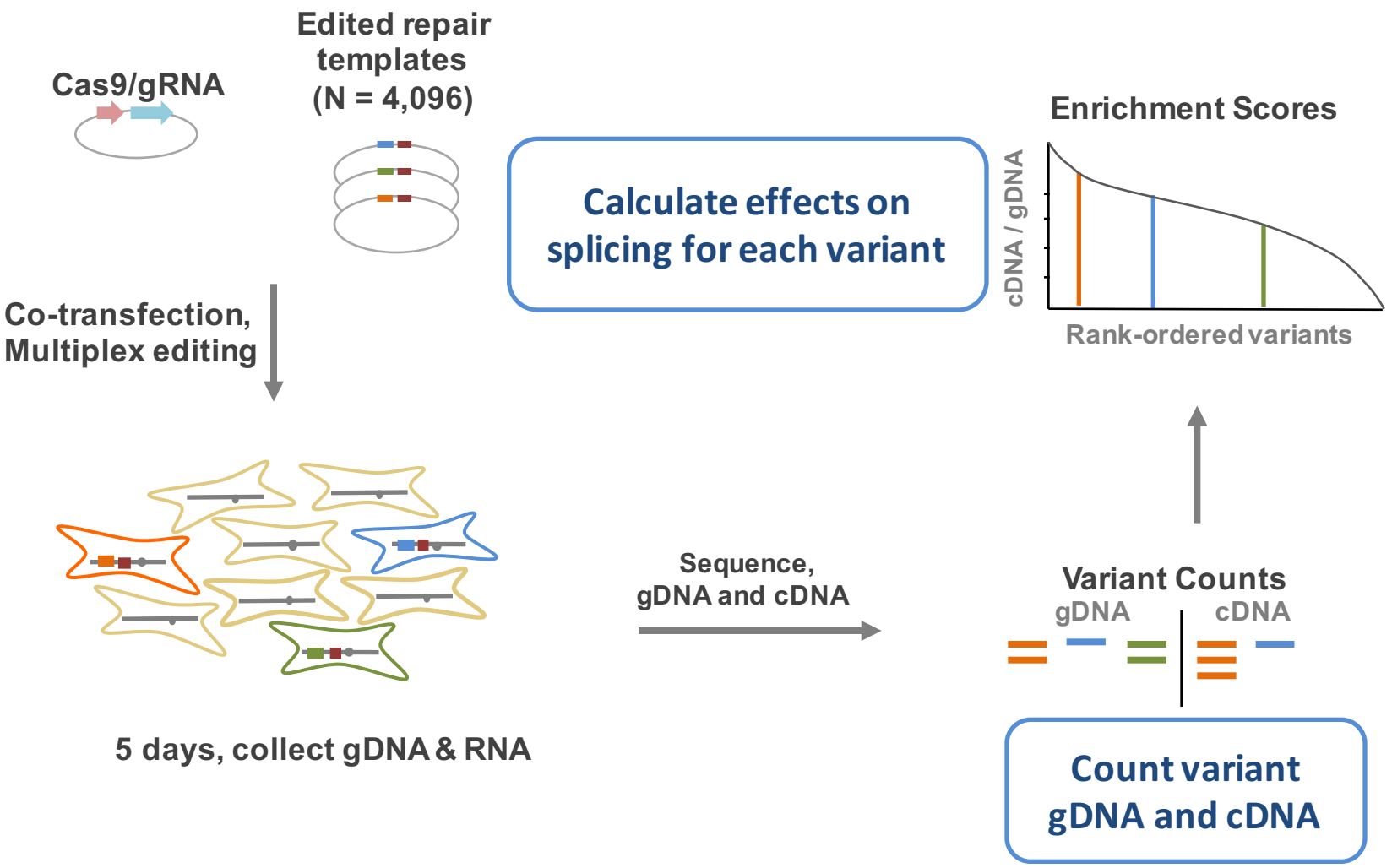
Multiplex genome editing to measure the effects of SNVs on splicing



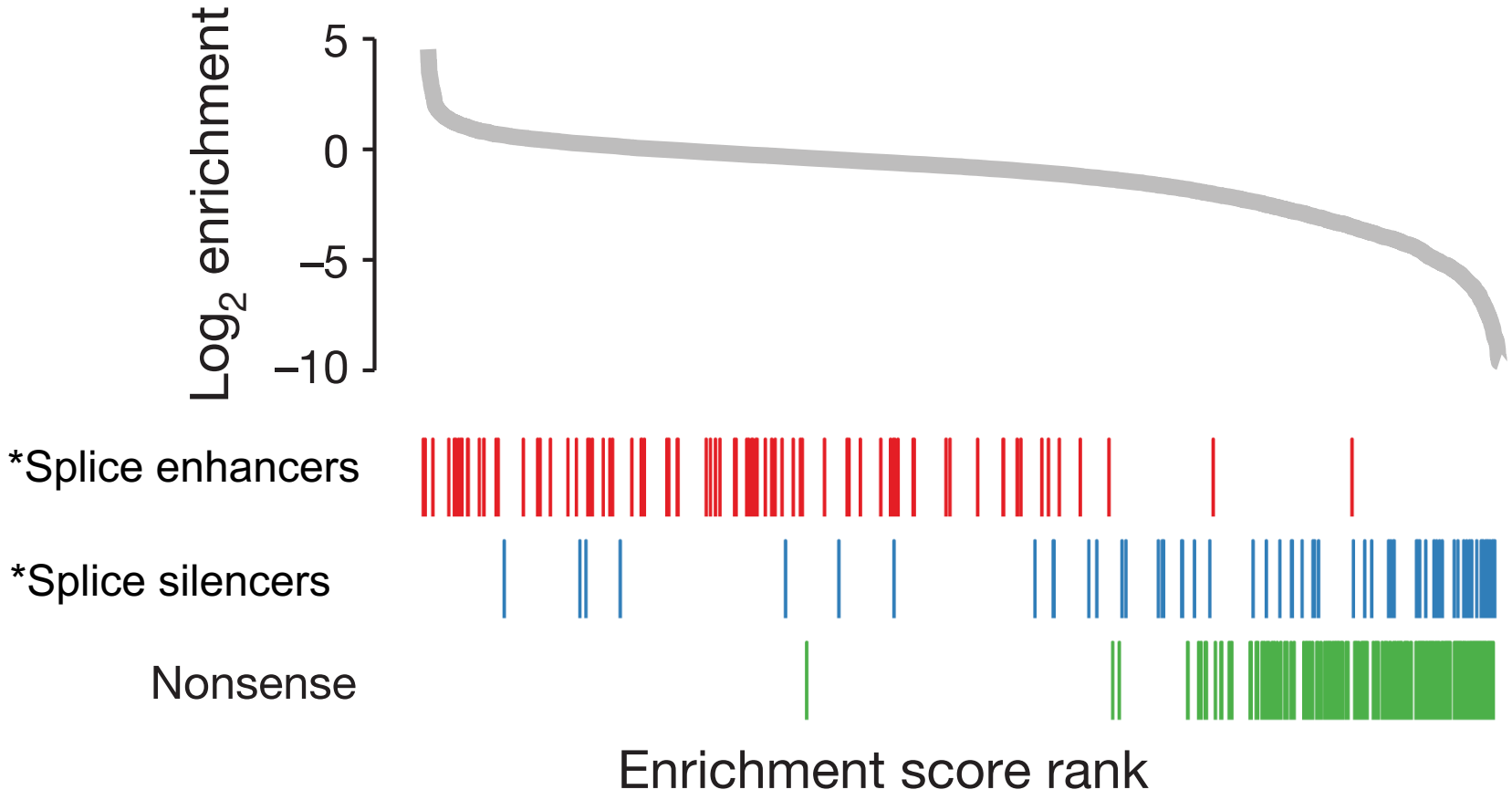
Heterogeneous population of edited cells

"Selective PCR" – only edited gDNA and cDNA

Multiplex genome editing to measure the effects of SNVs on splicing



Variants that create splice enhancers and silencers or trigger nonsense-mediated decay behave as expected



** defined from Ke et al. 2011*

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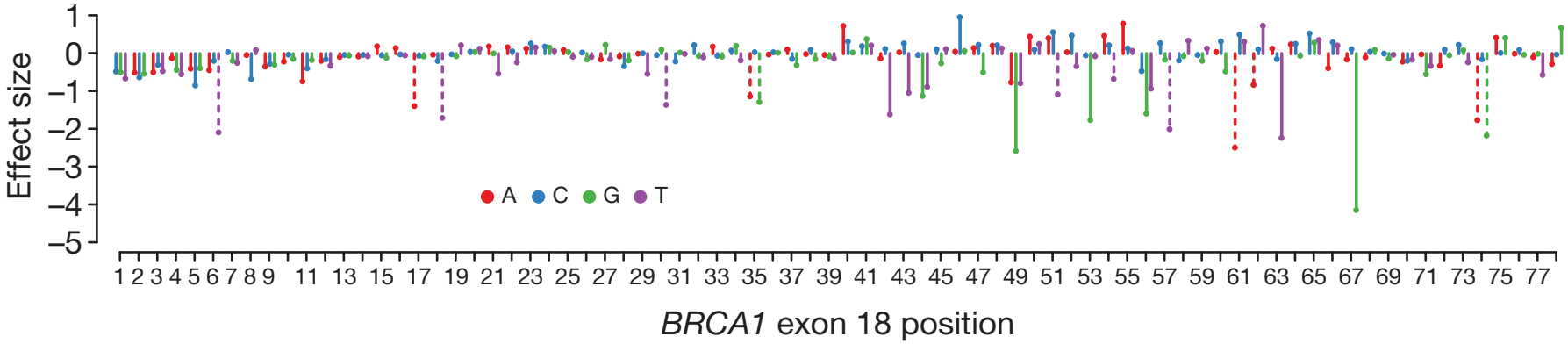
Effects of SNVs across *BRCA1* exon 18



- - - - *Dashed lines represent nonsense mutations

Findlay, Boyle et al., *Nature* (2014).

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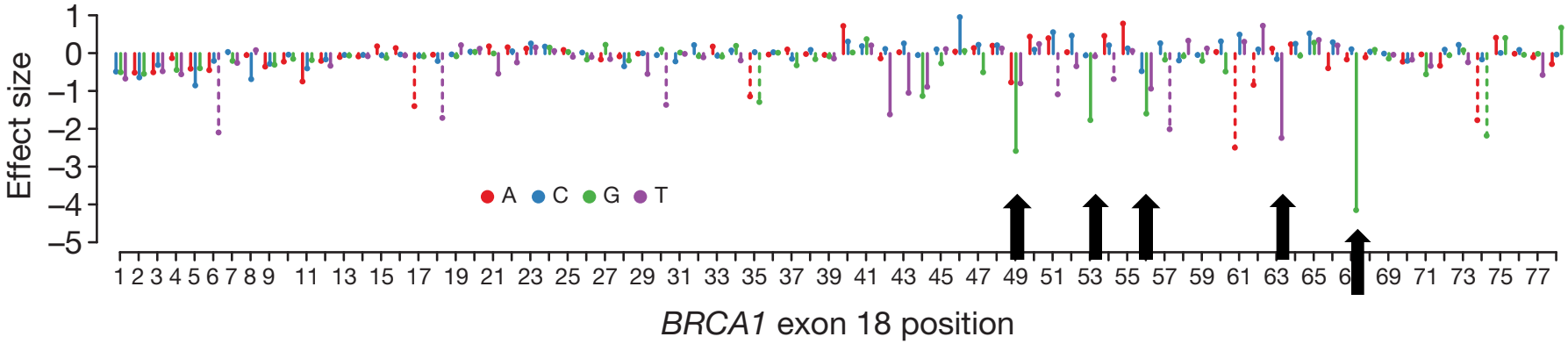


- - - - *Dashed lines represent nonsense mutations

MutPred Splice annotations:

C49G "Splice Affecting Variant"

A53G "ESE Loss / ESS Gain"

A56G "ESE Loss"

G63T "Cryptic 5' SS"

T67G "Cryptic 5' SS" **aka VUS V1714G**

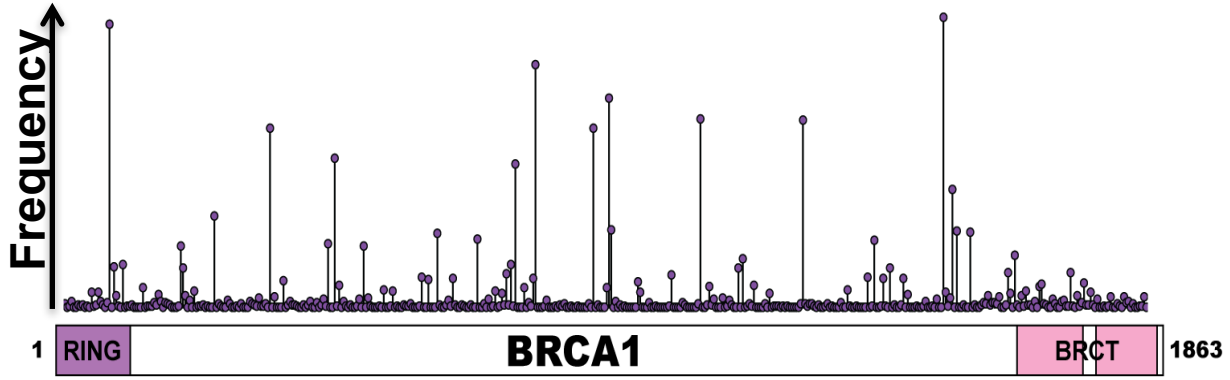
Findlay, Boyle et al., *Nature* (2014).

In summary

Parallelized assays for the protein function of the RING domain of BRCA1

Saturation genome editing to understand the effect of missense variants on splicing

Next steps…



Suggestions?

Challenges for scaling up

Library construction and variant delivery ✓

Parallelizable assays for protein function

Sequencing of variants ✓

Computational variant scoring pipeline ✓

Calculate likelihood estimates for pathogenicity

How the results from massively parallel assays could get to the bedside…

Thanks to:

Genome Sciences
UNIVERSITY OF WASHINGTON

Shendure lab

Fields lab
Dave Young
Justin Gullingsrud

Fowler lab

Parvin lab
Muhtadi Islam
The Ohio State University

Kitzman lab
University of Michigan

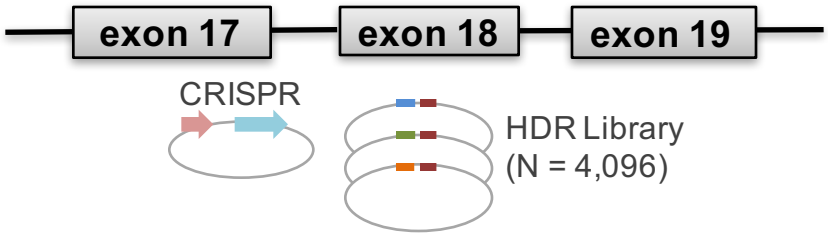
Funding from the
Yeast Resource Center
NIH P41

hhmi

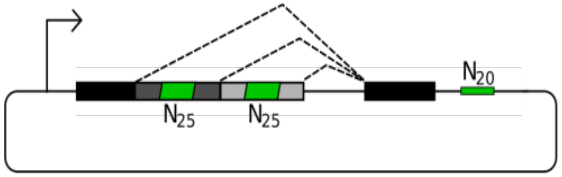
The effects of missense SNVs on splicing and protein function are difficult to predict

Stop Gain √ **Frameshift** √ **Missense** ?

Splicing effects

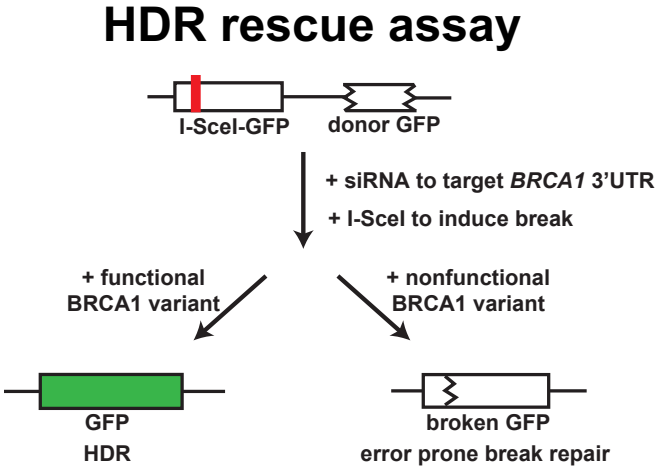


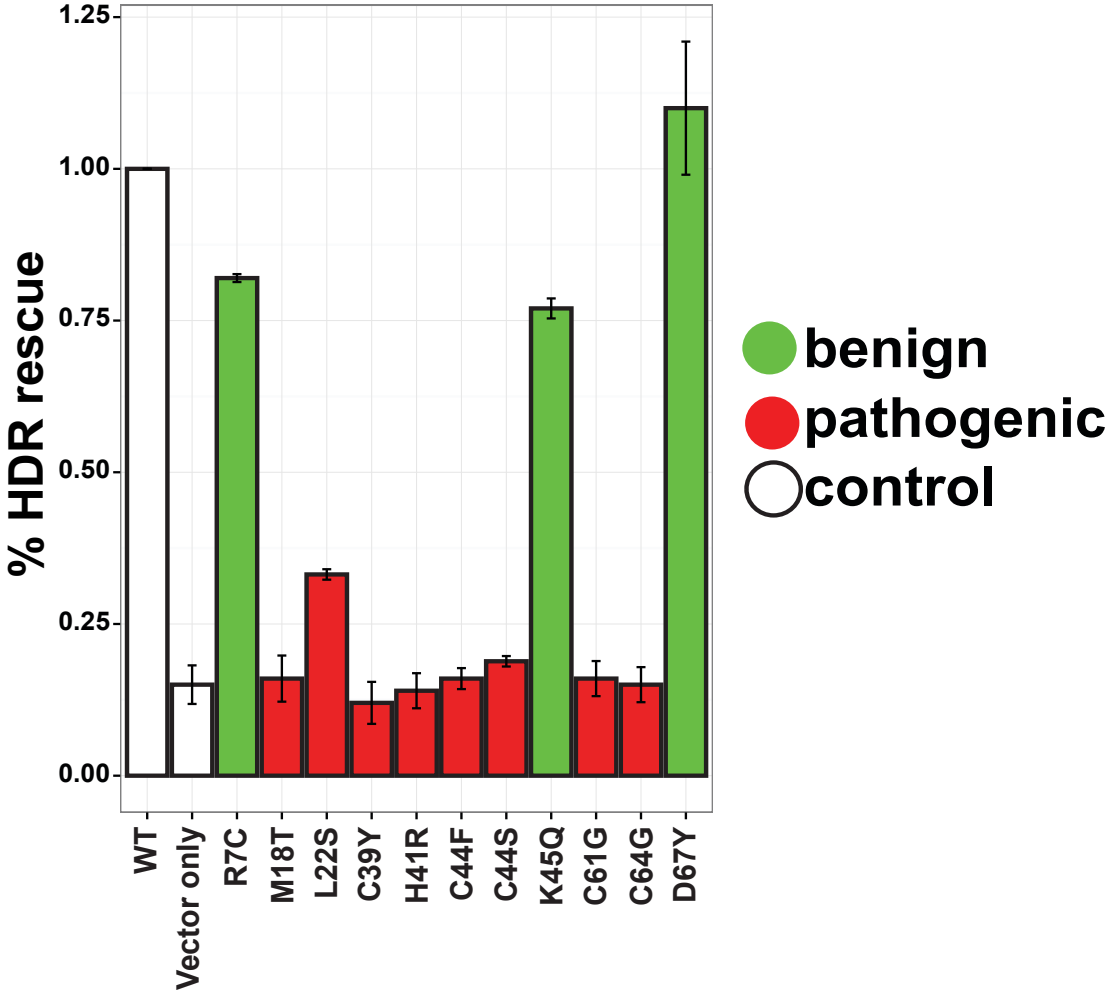
Multiplex genome editing to determine effects of SNVs on splicing of exon 18 of BRCA1
Findlay et al. Nature 2014



Learning the Sequence Determinants of Alternative Splicing from Millions of Random Sequences
Rosenberg et al. Cell 2015

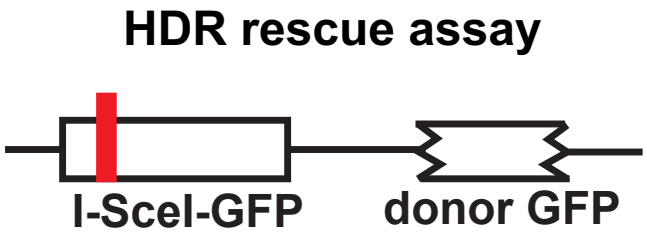
Scoring full-length BRCA1 variants for HDR function in human cells



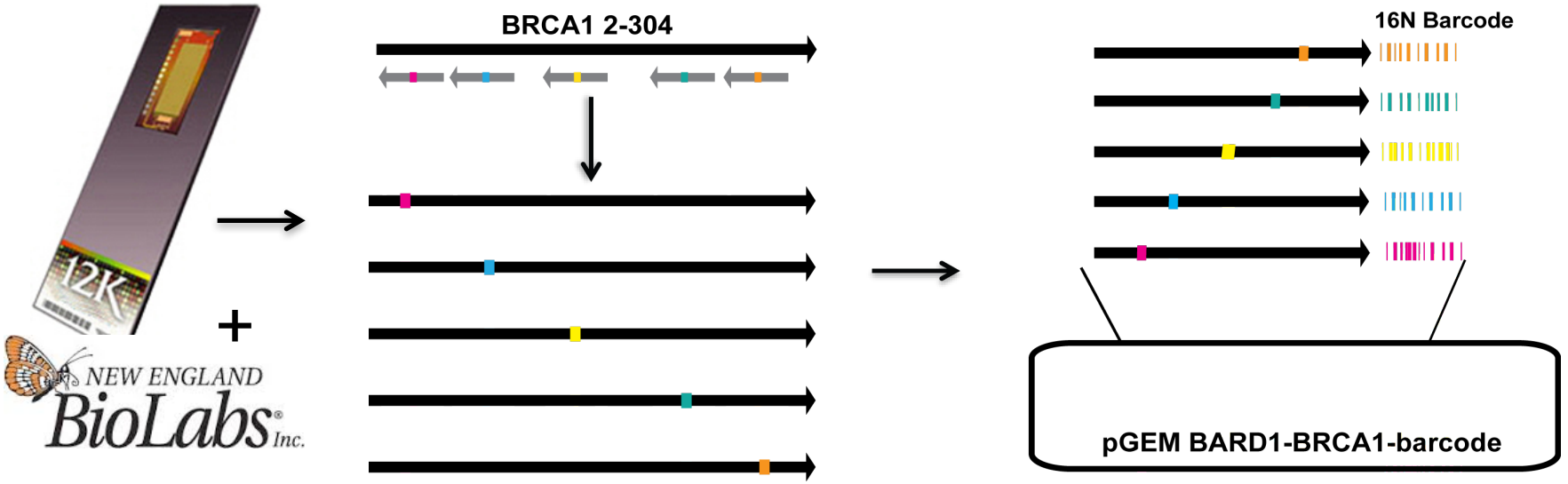


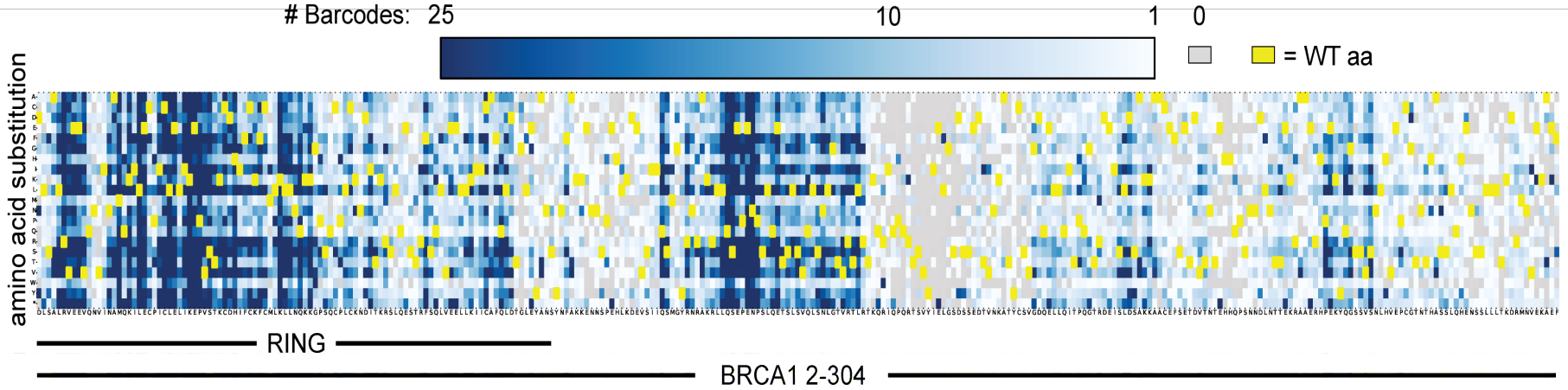
Muhtadi Islam and Jeff Parvin

Scoring full-length BRCA1 variants for HDR function in human cells



Construction of the barcoded single amino acid substitution BRCA1-RING library



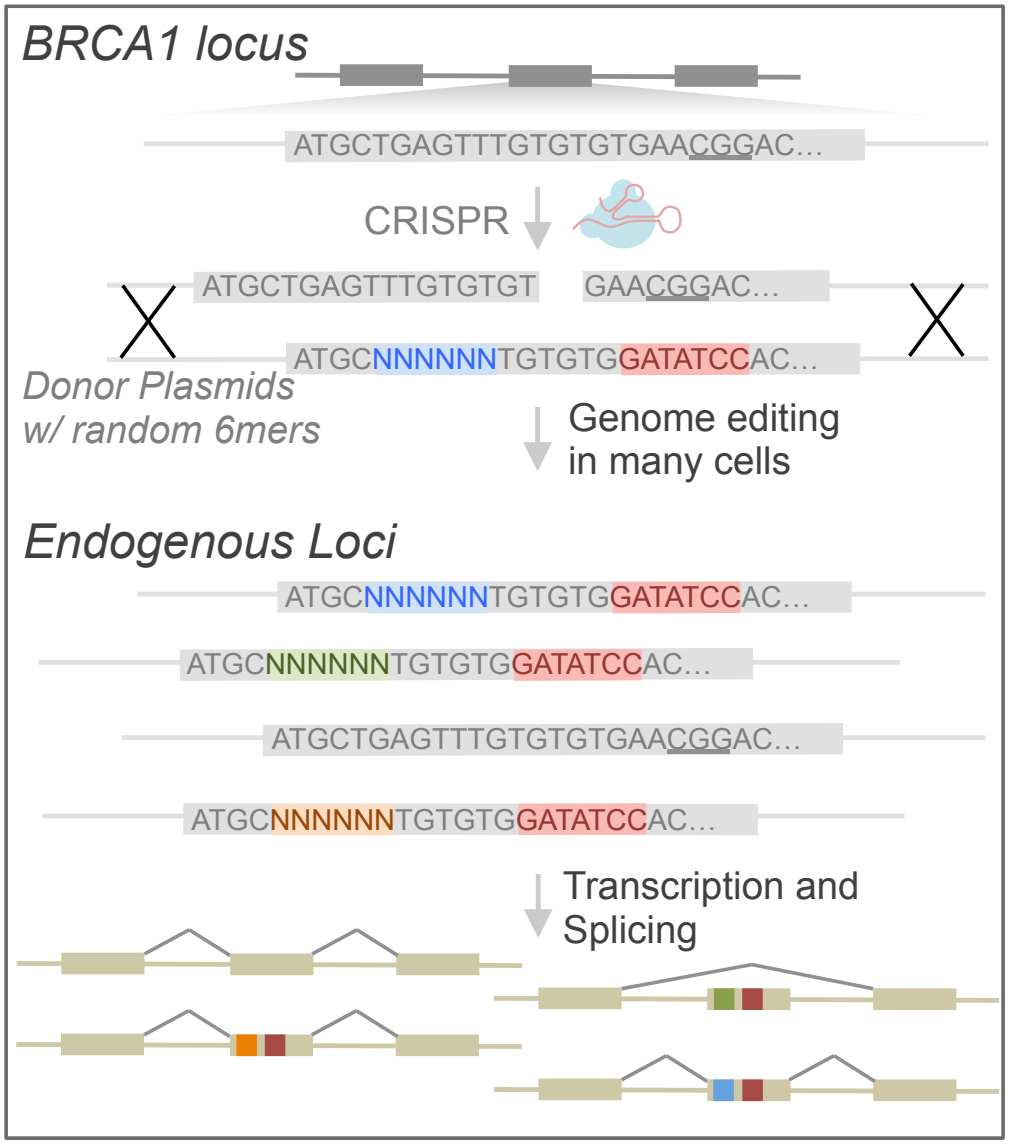


Multiplex genome editing to measure the effects of SNVs on splicing

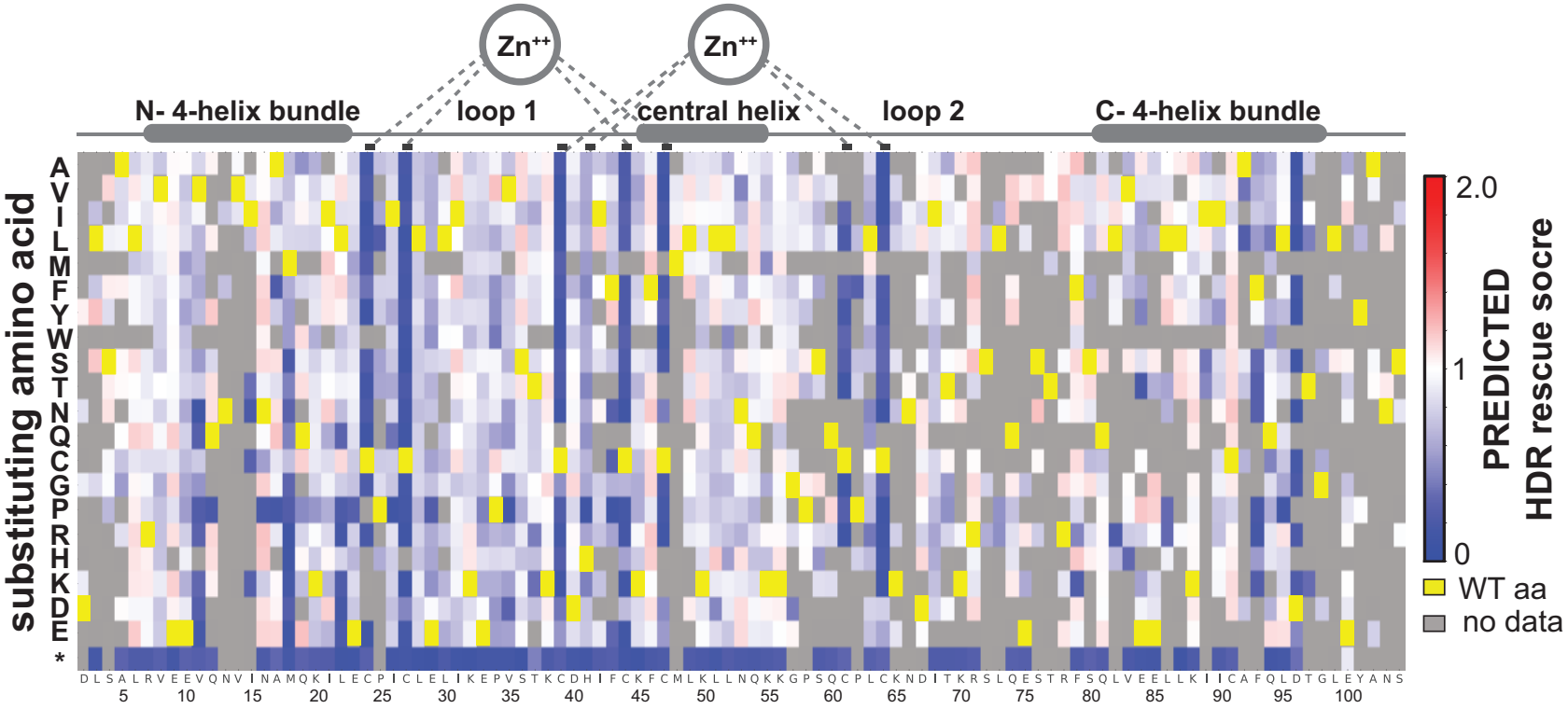
Each edited exon receives

1. **A random SNV**
2. **A fixed mutation**

3% of 10^6 cells = 30,000 events

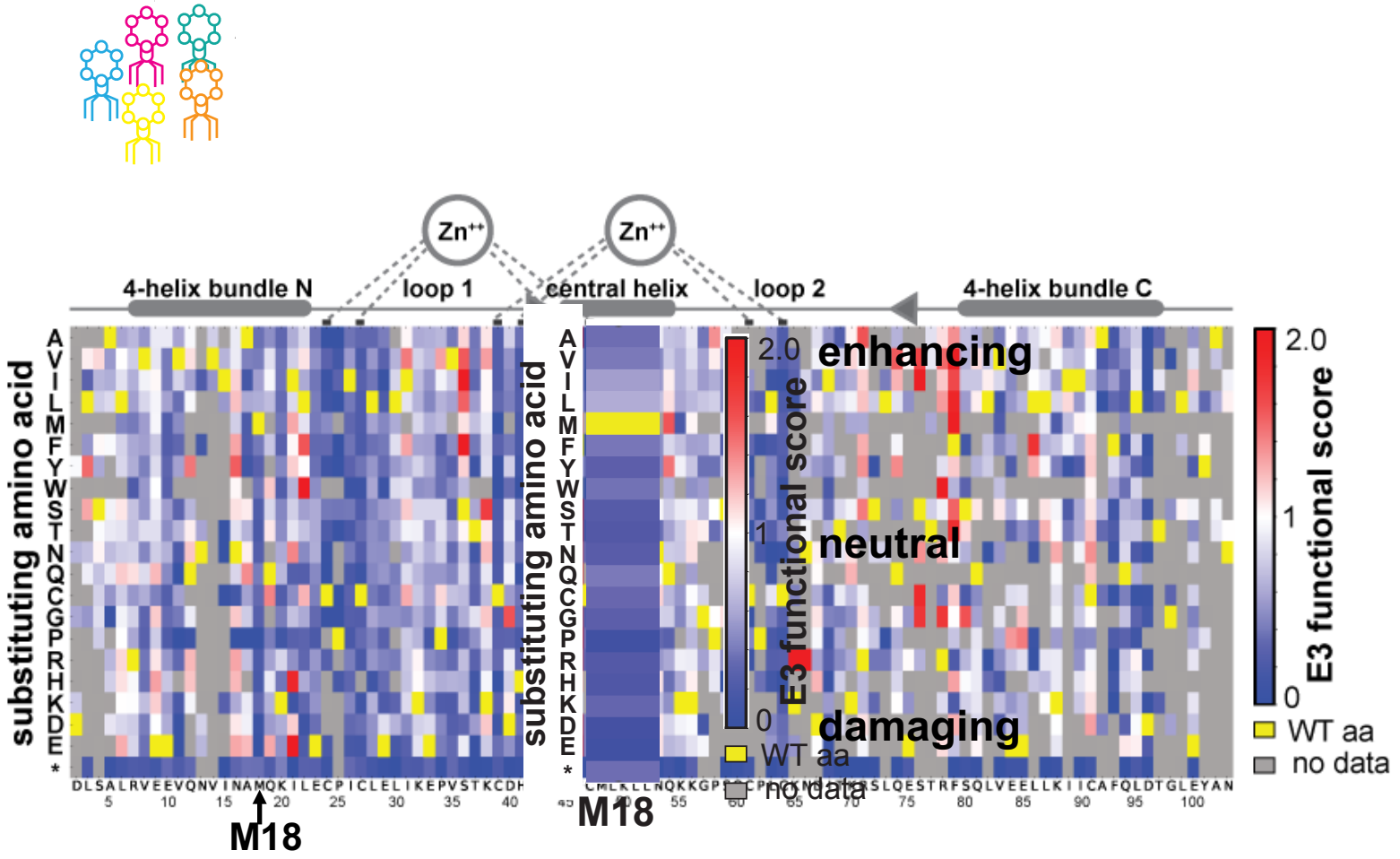


Prospective functional map for 1,287 BRCA1 RING variants



Massively parallel assays for BRCA1-RING E3 ligase activity

E3 ligase activity



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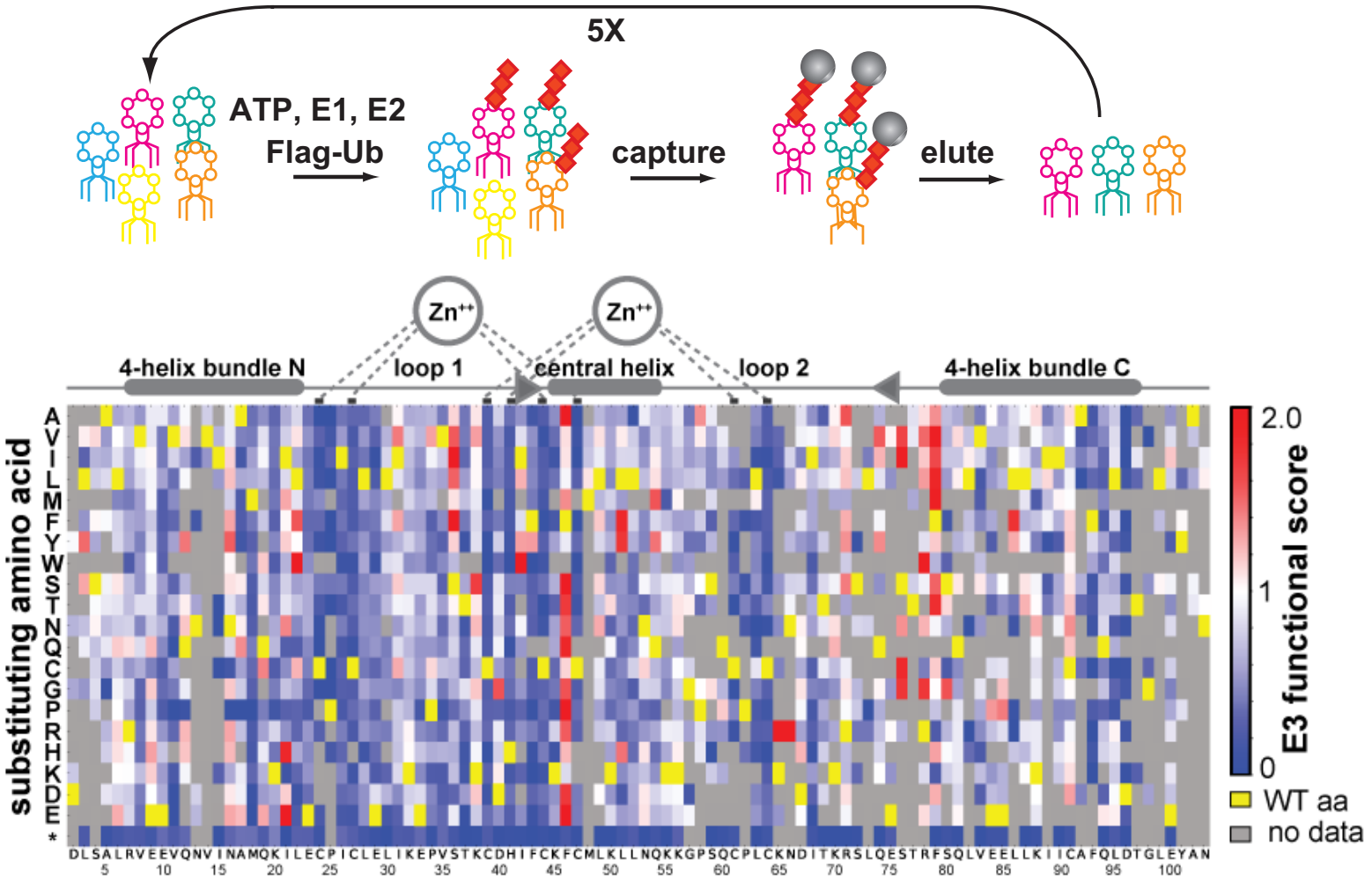

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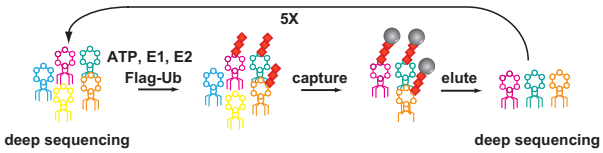

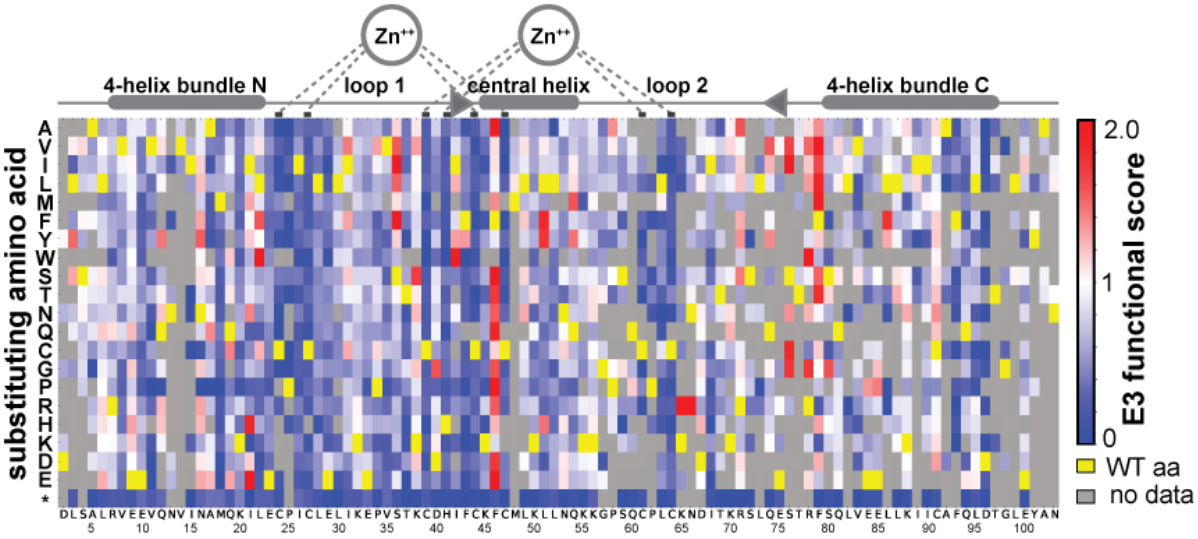

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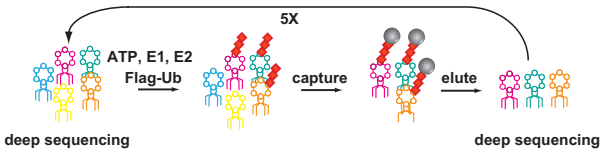
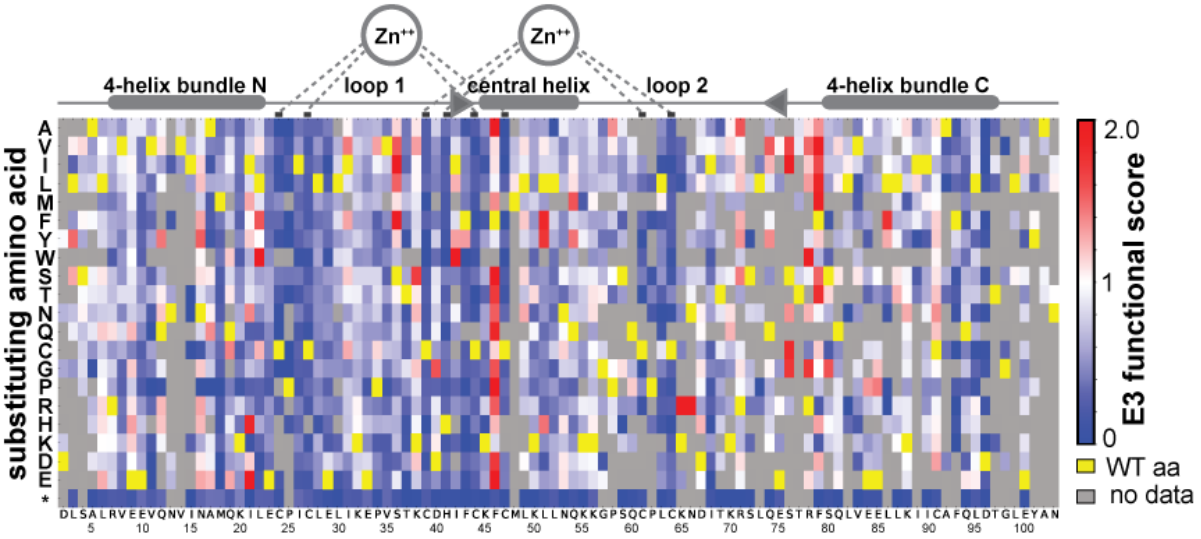
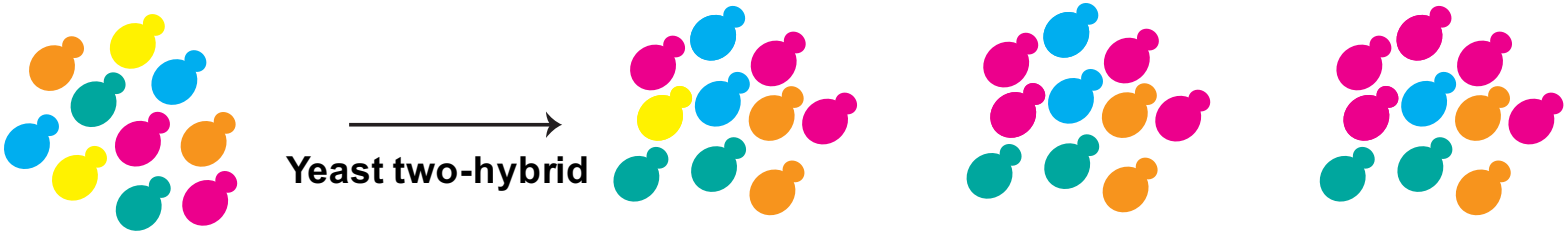




BARD1-binding activity



transformation, Time 0
deep sequencing

Time 1
deep sequencing

Time 2
deep sequencing

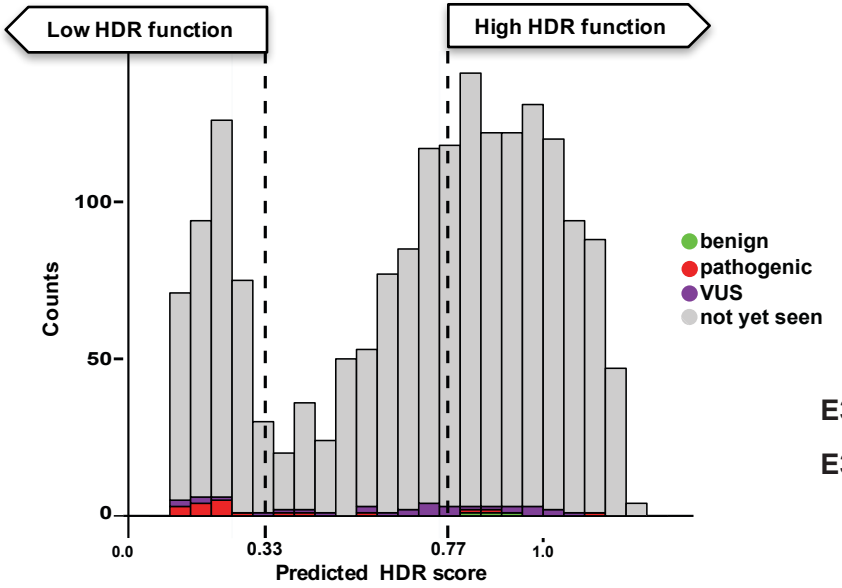
Time 3
deep sequencing

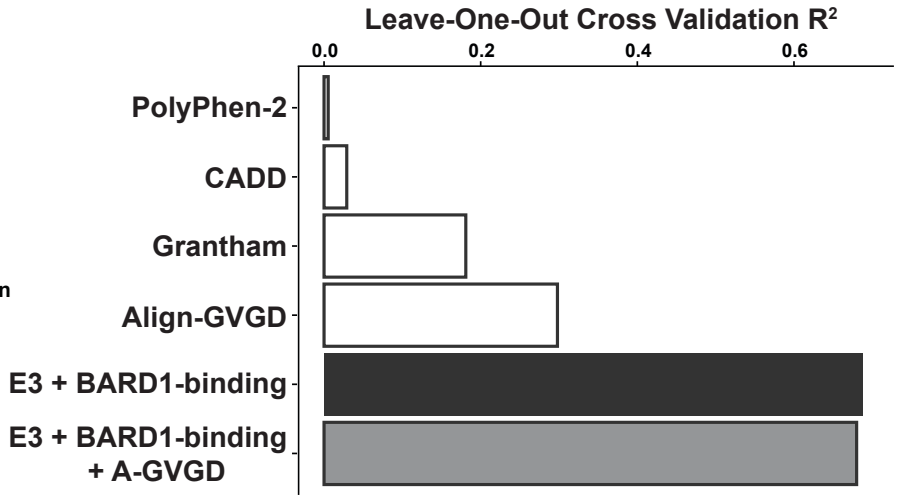
Genetic testing is big business

More companies, lower costs, more genes*

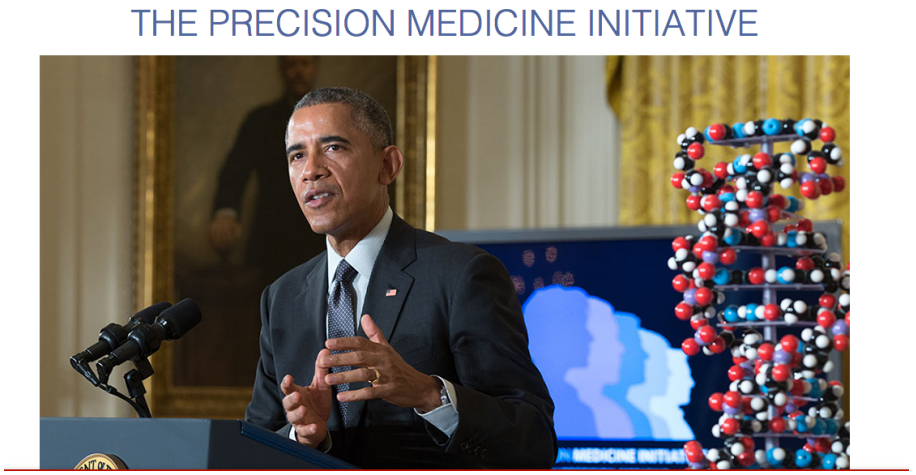
*41.7% of tests revealed a VUS in at least one gene

Tung et al. Frequency of mutations in individuals with breast cancer referred for *BRCA1* and *BRCA2* testing using next-generation sequencing with a 25-gene panel. *Cancer* 2015



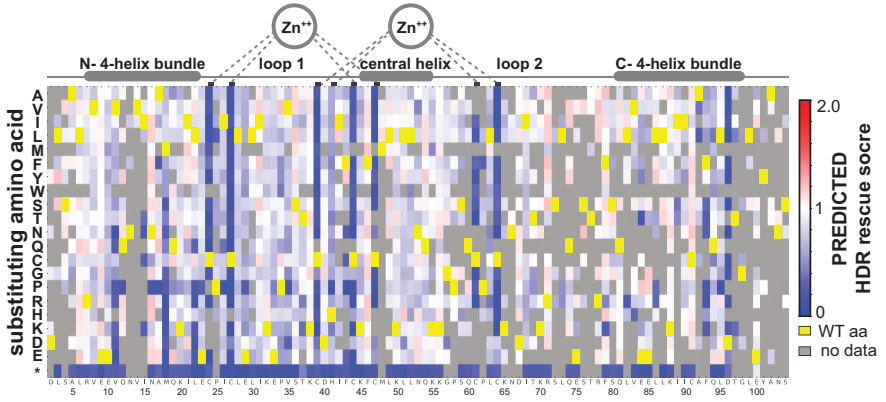


We need new technologies to deliver on the promises of genetic medicine



https://www.whitehouse.gov/precision-medicine

Massively parallel functional analyses are a possible solution



Starita et al. *Genetics*, 2015
Findlay et al. *Nature*, 2014
Rosenberg et al. *Cell*, 2015
Patwardhan et al. *Nature Biotech*, 2012
Fowler et al. *Nature Methods*, 2010
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