Saturation Genome Editing to Understand Genetic Variation in *BRCA1*

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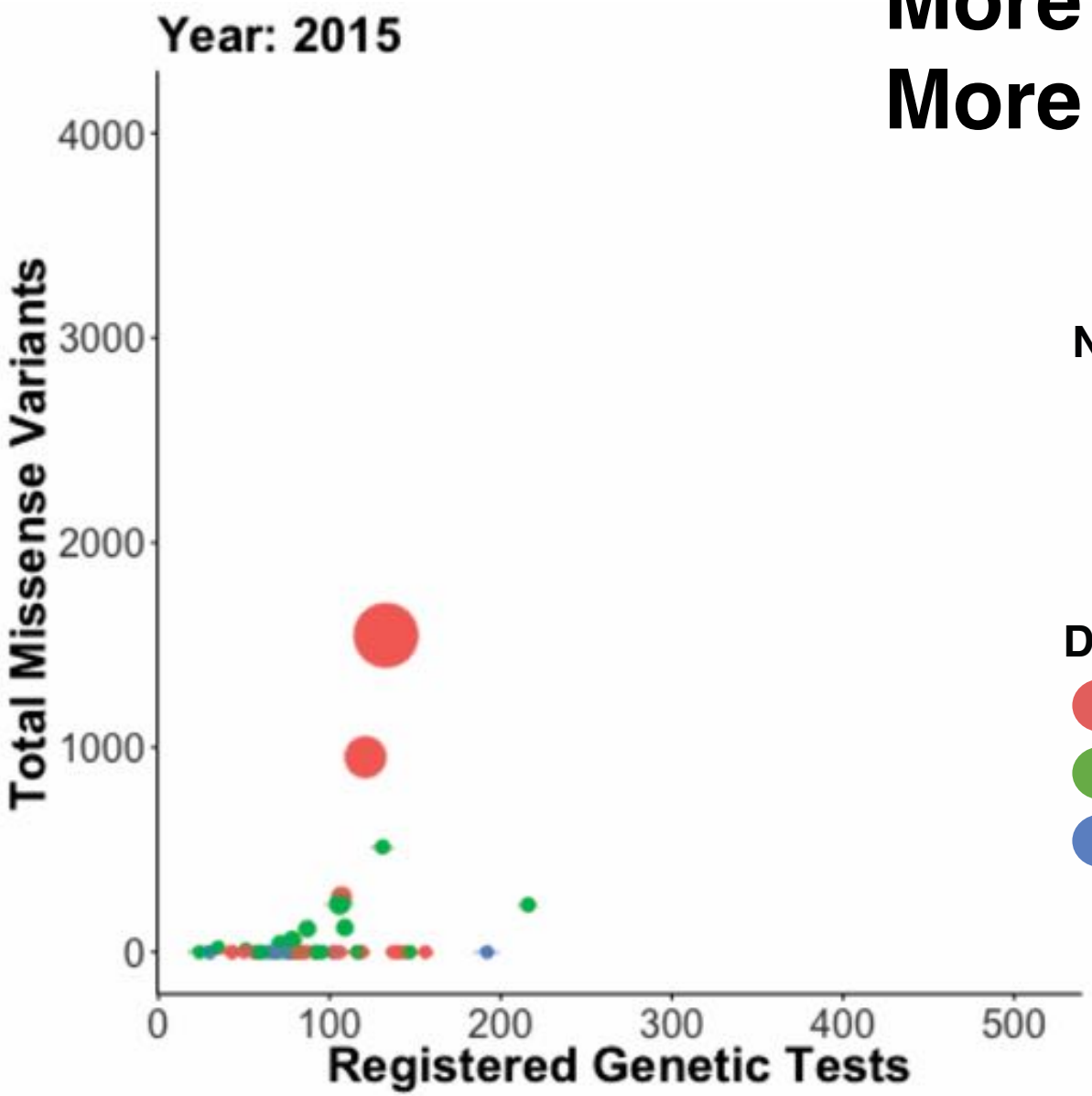
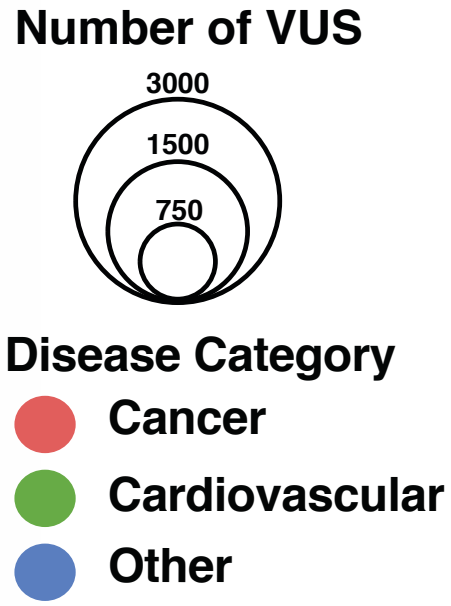
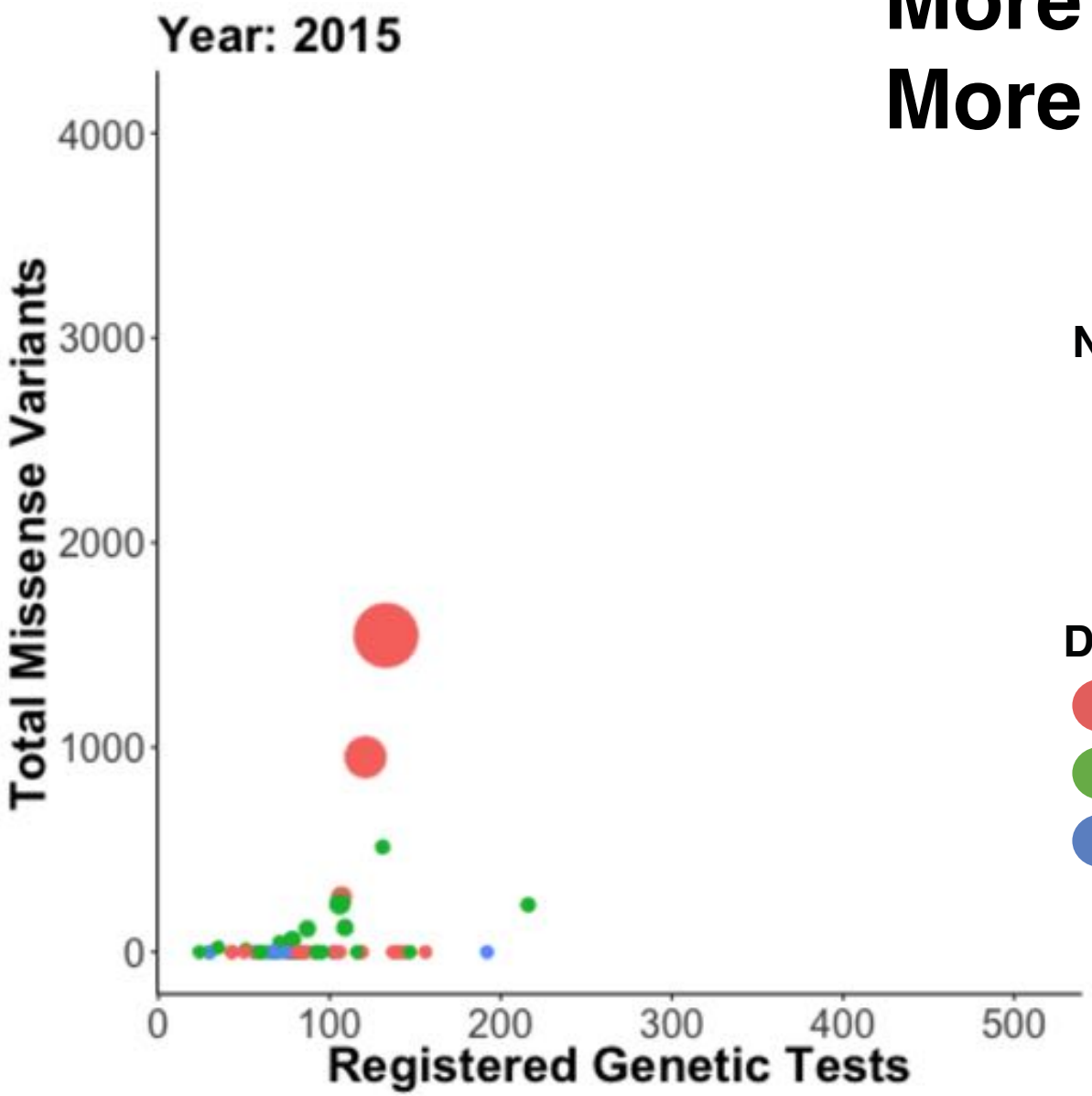
BROTMAN BATY
INSTITUTE

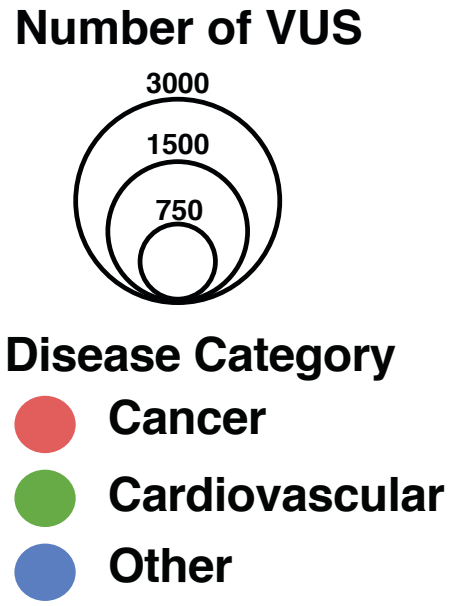
More sequencing, More VUS

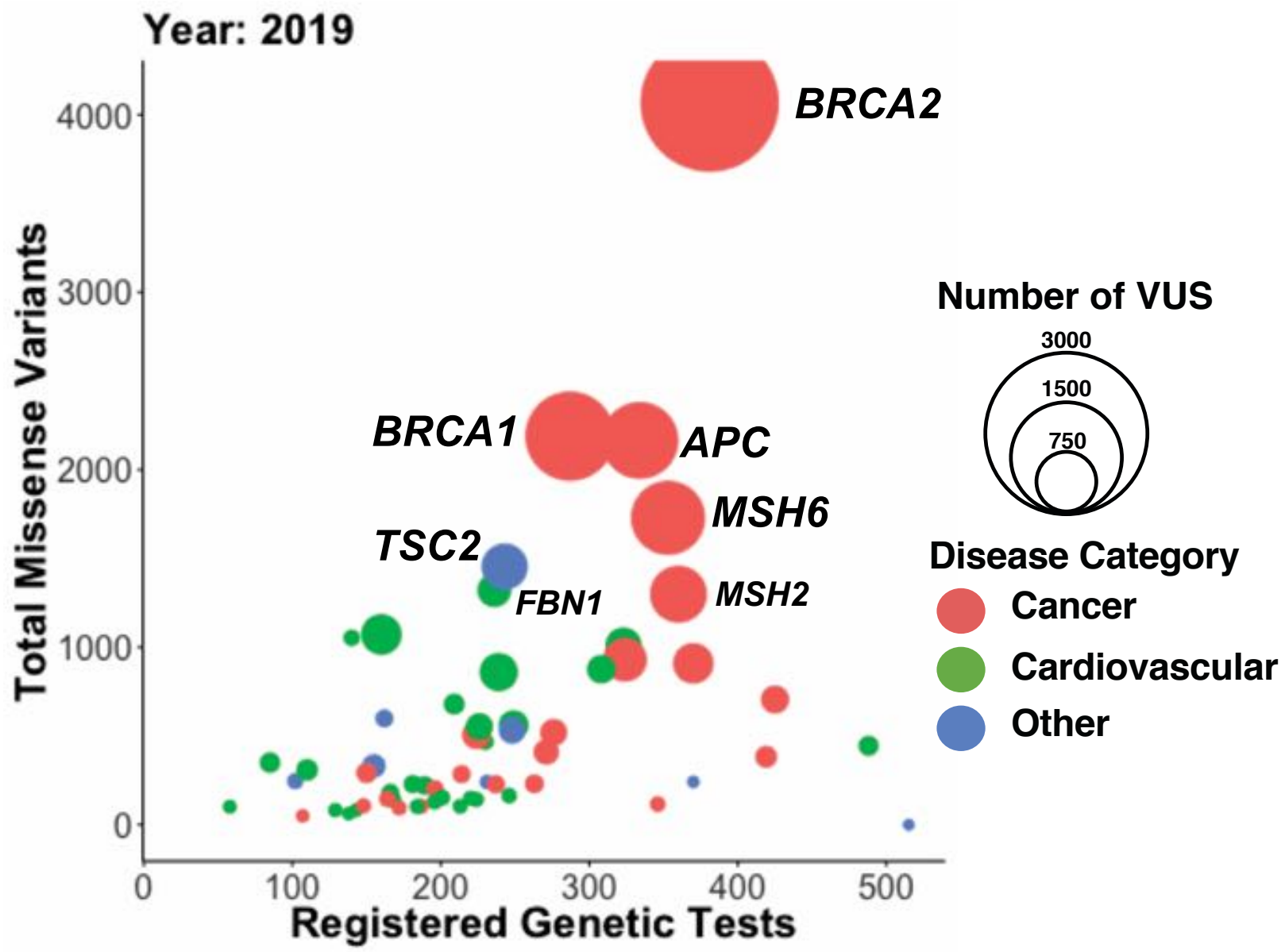




More sequencing, More VUS

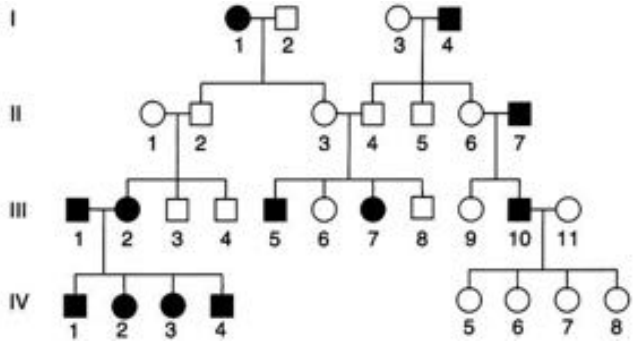





Year: 2019
Total Missense Variants
4000
3000
2000
1000
0
0
100
200
300
400
500
Registered Genetic Tests
BRCA2
BRCA1
APC
MSH6
TSC2
FBN1
MSH2
Number of VUS
3000
1500
750
Disease Category
Cancer
Cardiovascular
Other

Current strategies for understanding the effect of coding variation

Families



FindMy VARIANT

23andMe®

Data sharing

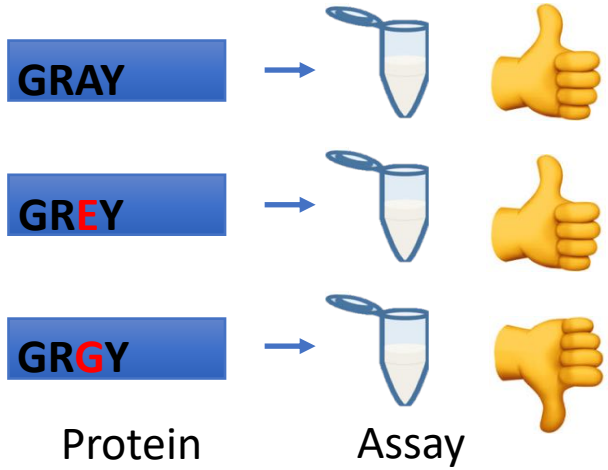
gnomAD
genome aggregation database

Brav

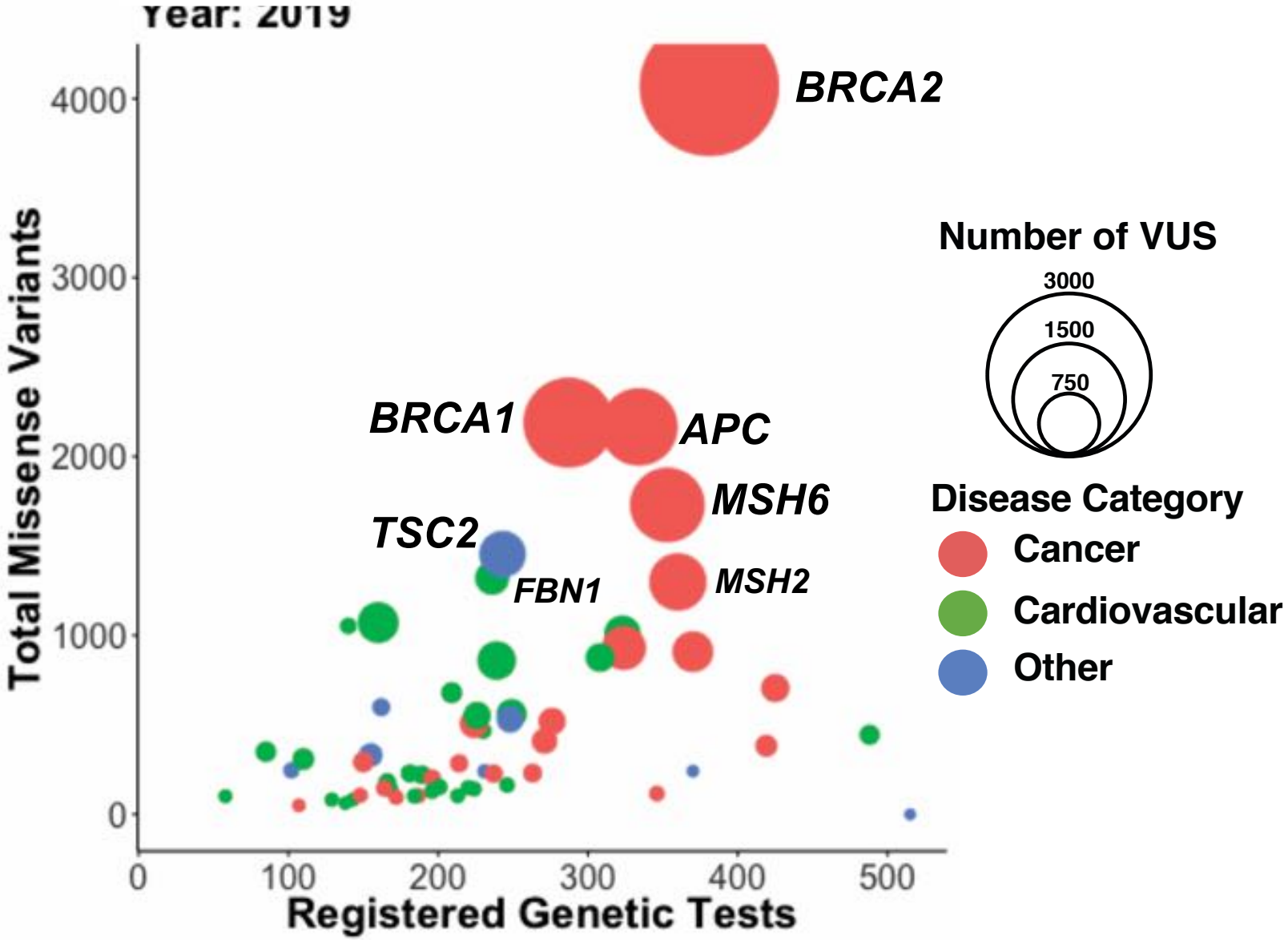
ClinGen
Clinical Genome Resource

ClinVar
Clinically relevant variation

Functional assays



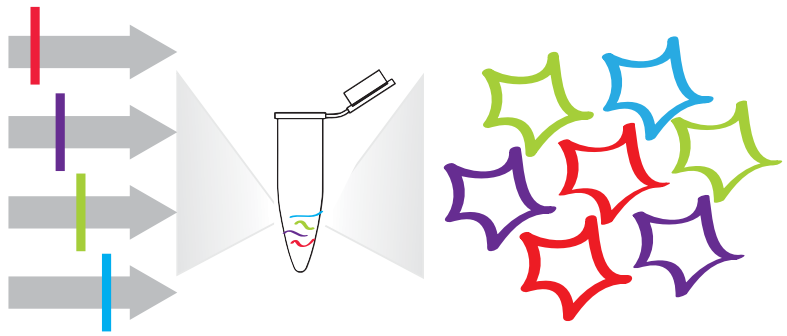
How to scale functional assays to the variant interpretation problem?



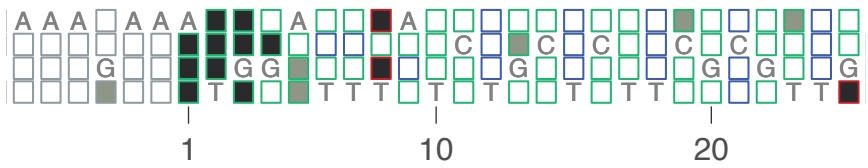
Outline

- **What is a multiplexed functional assay?**
- **Example of multiplexed functional assays for *BRCA1***
- **Benefits and limitations for variant interpretation**

Multiplexed functional assays test thousands of variants simultaneously



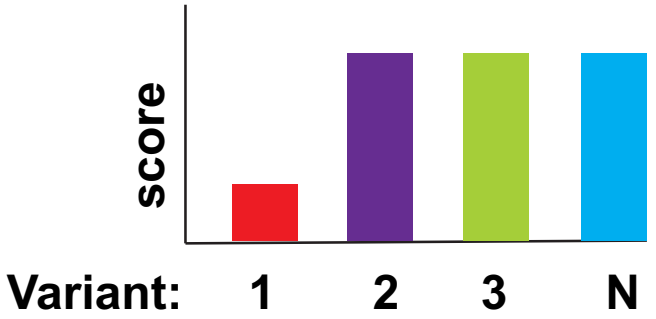
Functional Assay



Sequence–function map

Calculate scores for variants

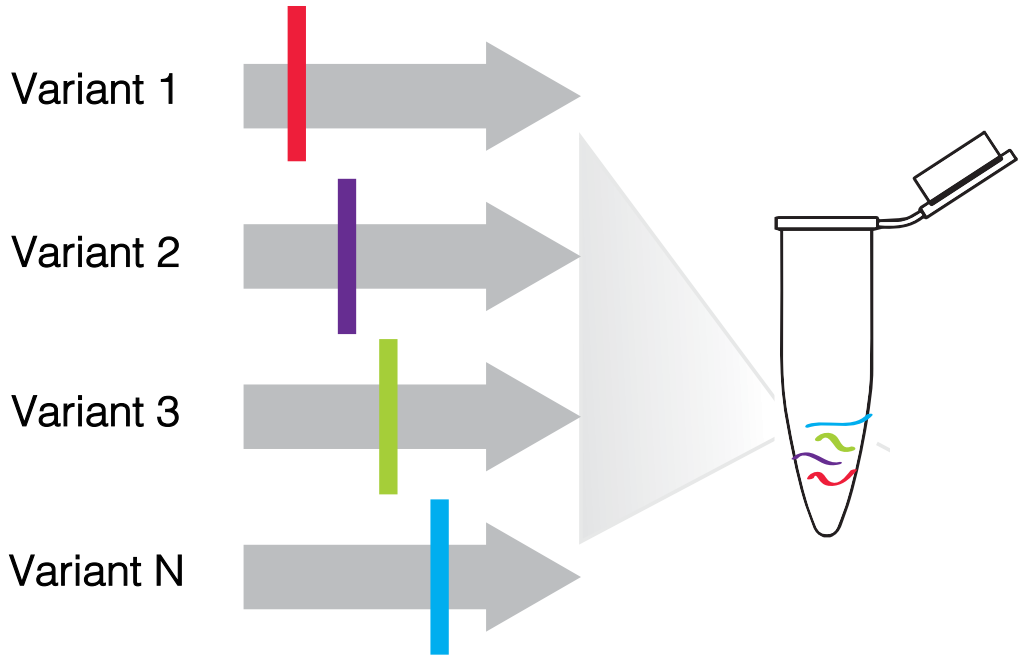
After / Before



We make a library of all possible variants

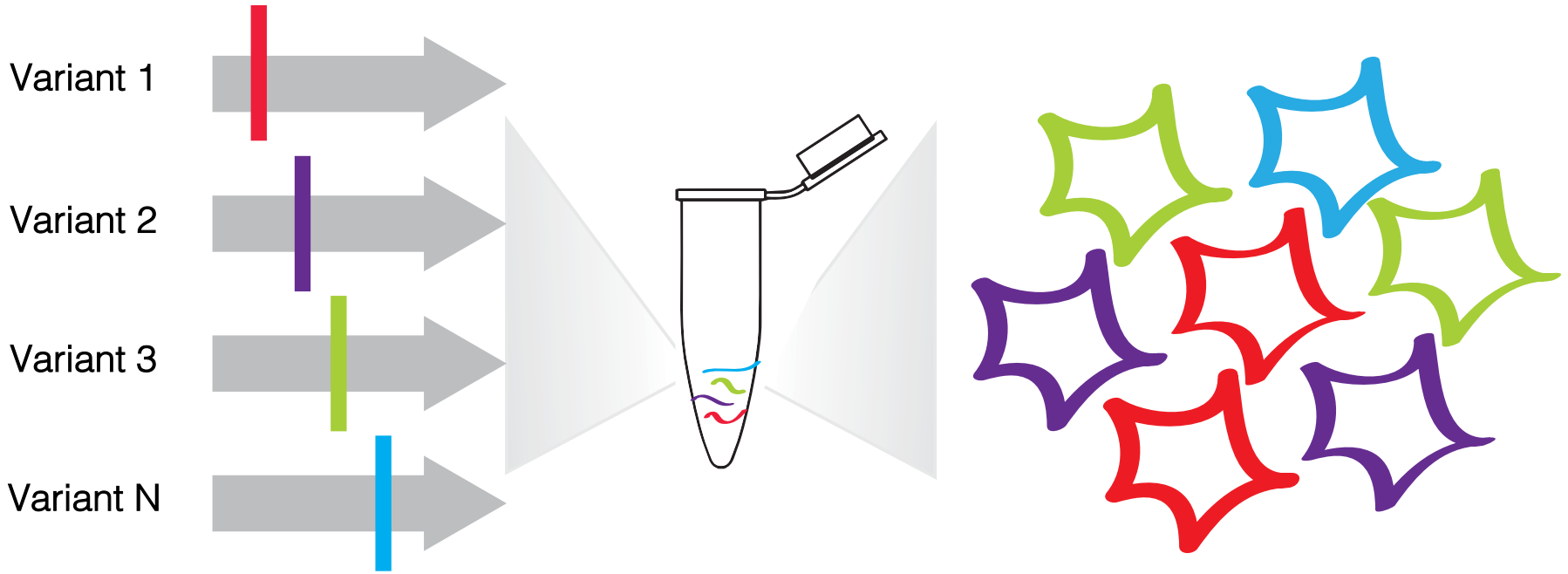
Gasperini, Starita, and Shendure (2016) Nature Protocols.

We make a library of all possible variants in a single pool



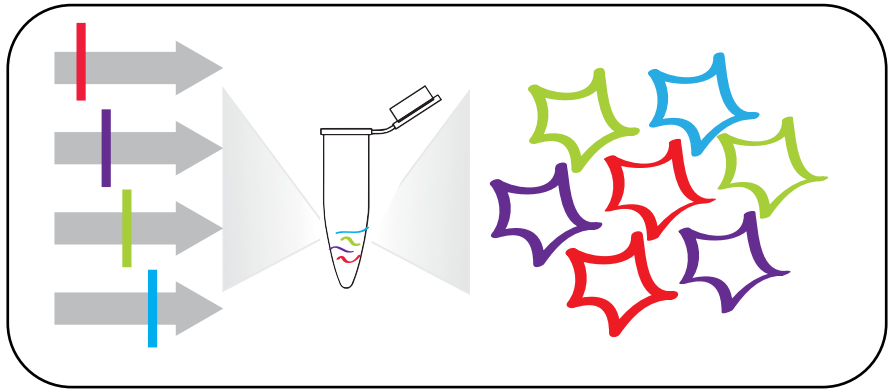
Gasperini, Starita, and Shendure (2016) Nature Protocols.

The variants are introduced into cells



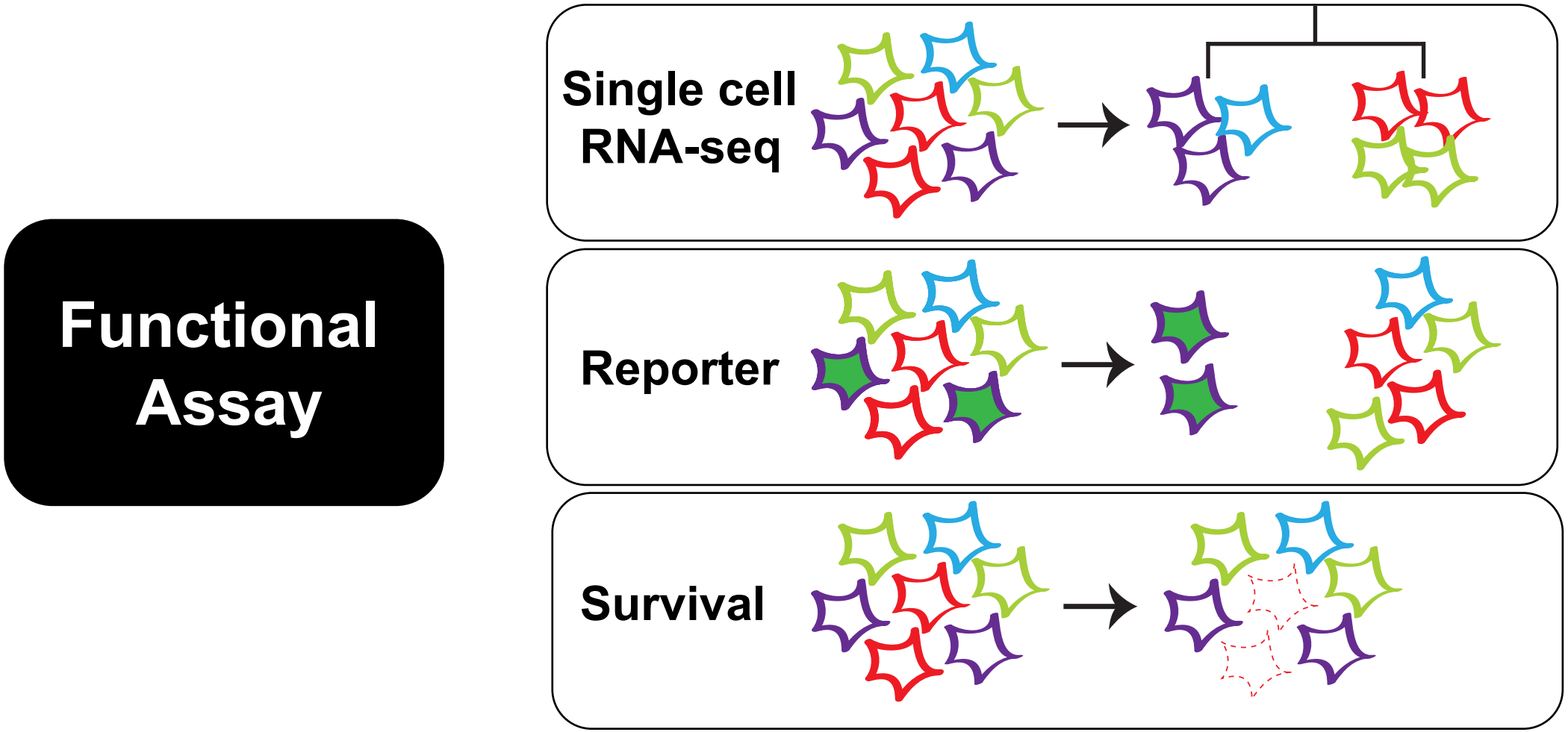
Gasperini, Starita, and Shendure (2016) Nature Protocols.

The variants are subjected to a functional assay

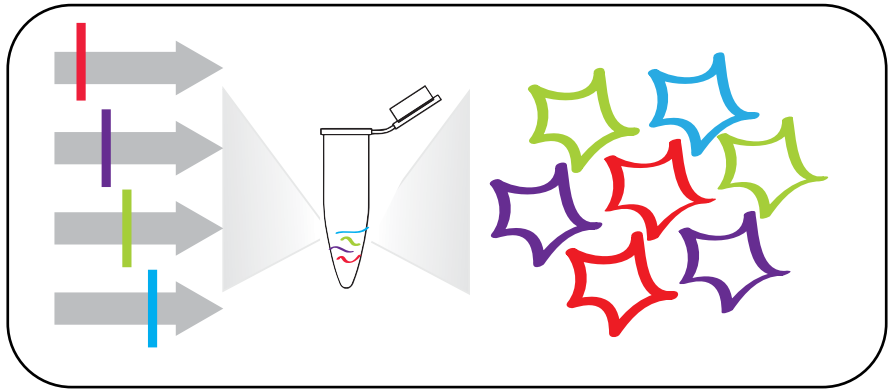


Functional Assay

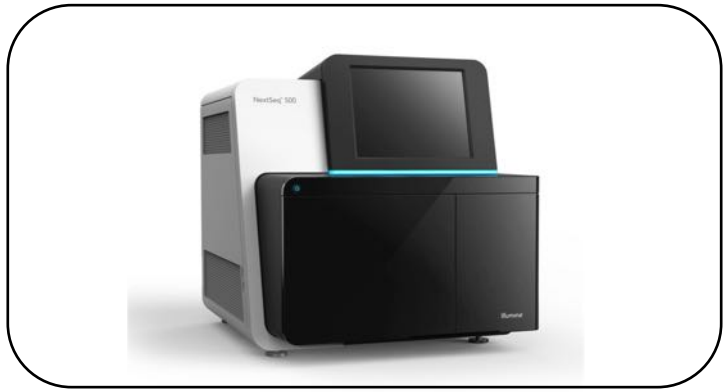
Examples of multiplexed functional assays



DNA sequencing is used to identify and count the variants



Functional Assay



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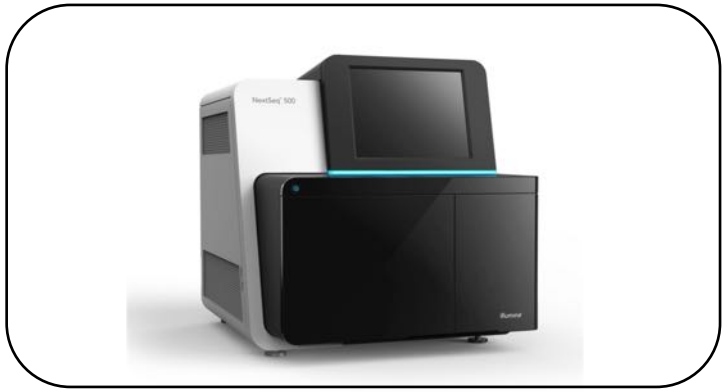
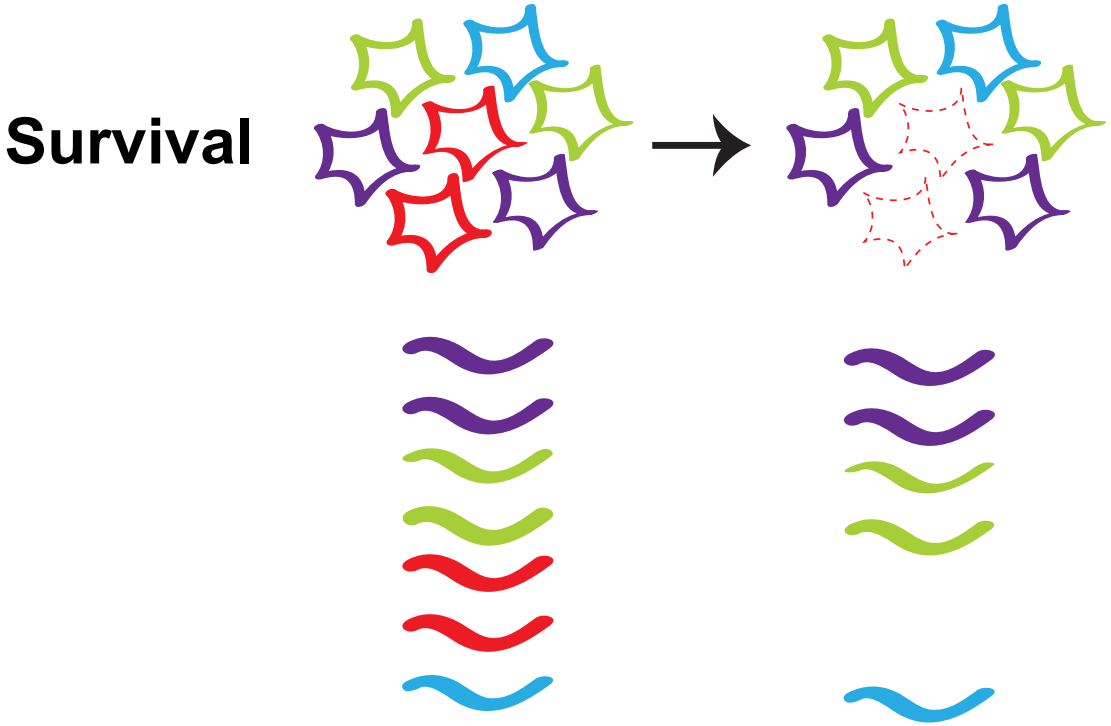
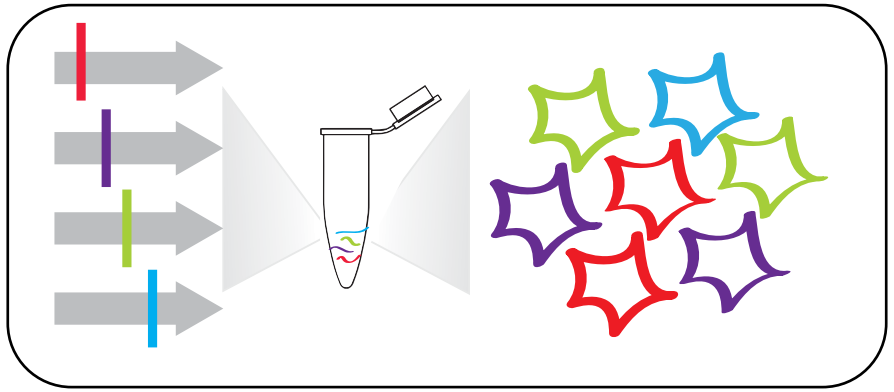
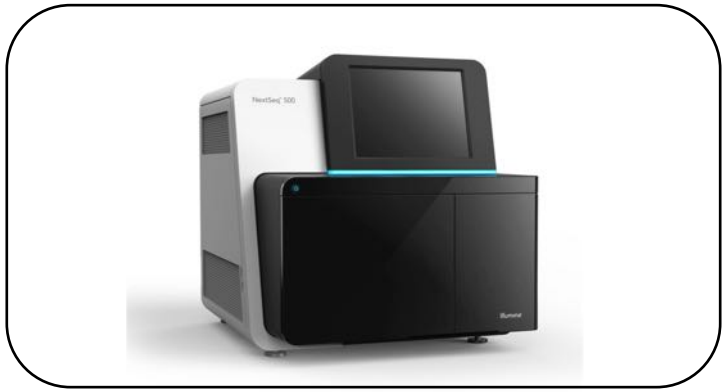




Functional scores are calculated for all variants

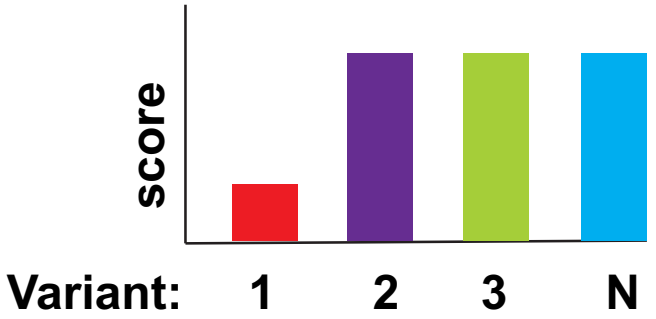


Functional Assay



Calculate scores for variants

After / Before



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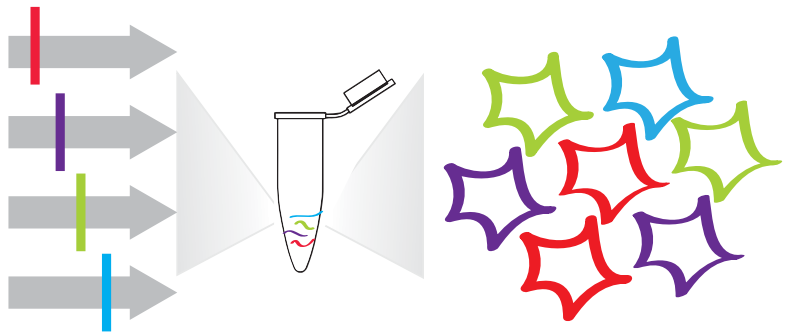
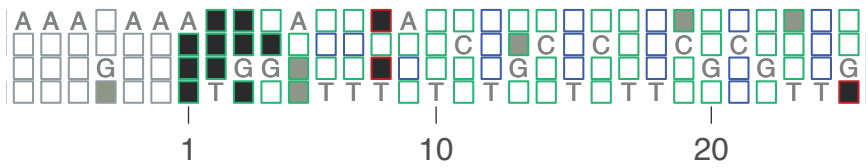


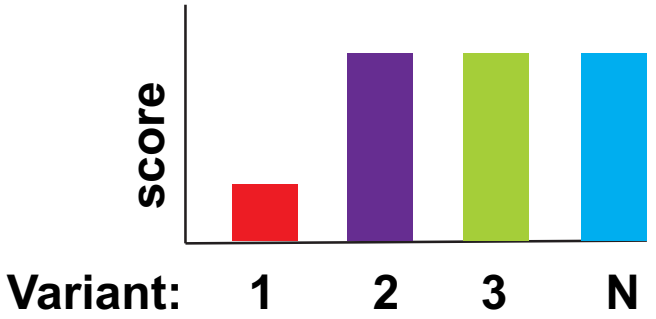
Functional Assay



Sequence–function map

Calculate scores for variants

After / Before

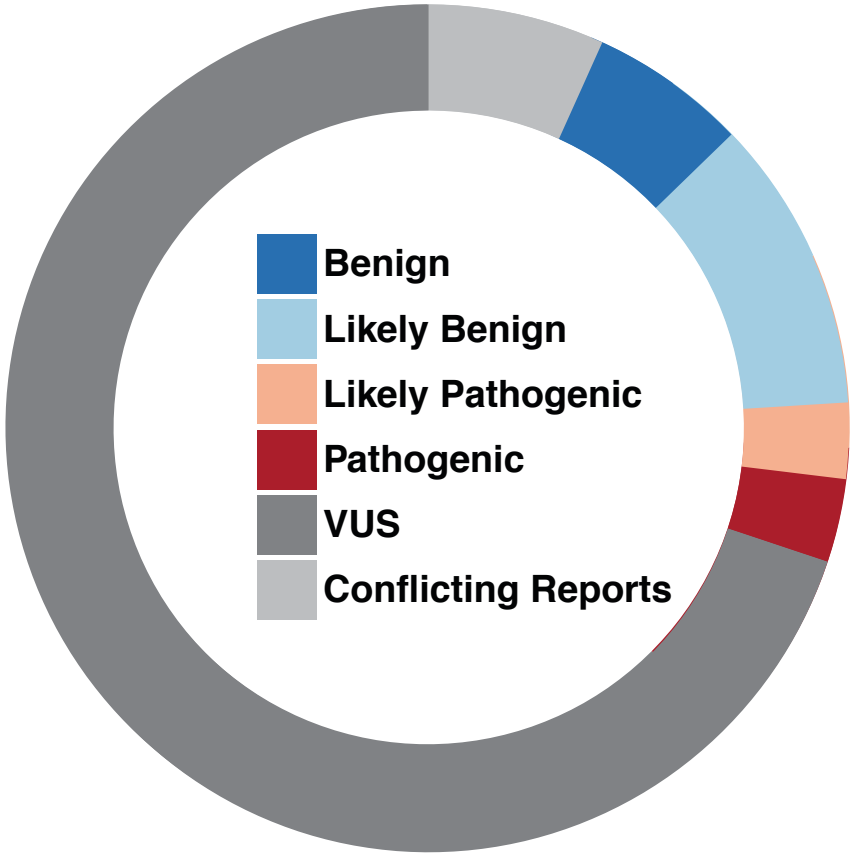


Outline

- What is a multiplexed functional assay?
- **Example of multiplexed functional assays for *BRCA1***
- Benefits and limitations for variant interpretation

There are a lot of VUS in *BRCA1*

All missense variants in *BRCA1*



N = 2,084

Greg Findlay

Riza Daza

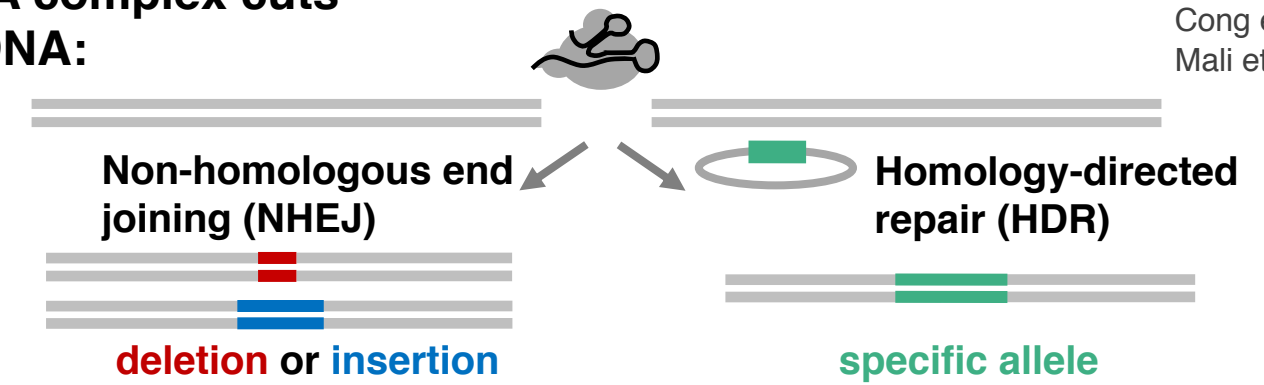
Beth Martin

Shendure lab

CRISPR for rapid, scalable, and efficient genome editing

Cas9:gRNA complex cuts genomic DNA:

Jinek (2012) *Science*
Cong et al. (2013) *Science*
Mali et al. (2013) *Science*



Efficiently mutate a single genomic site

CRISPR for rapid, scalable, and efficient genome editing

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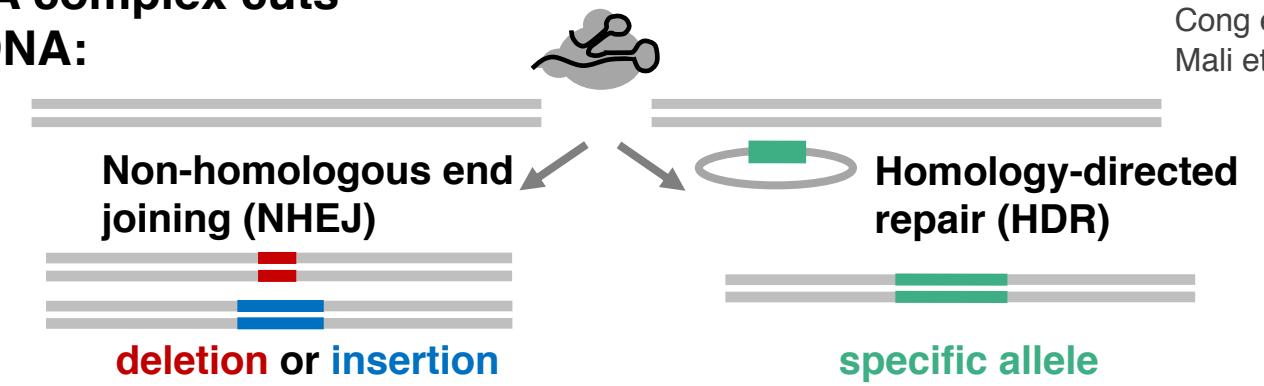
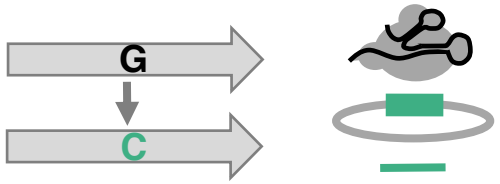
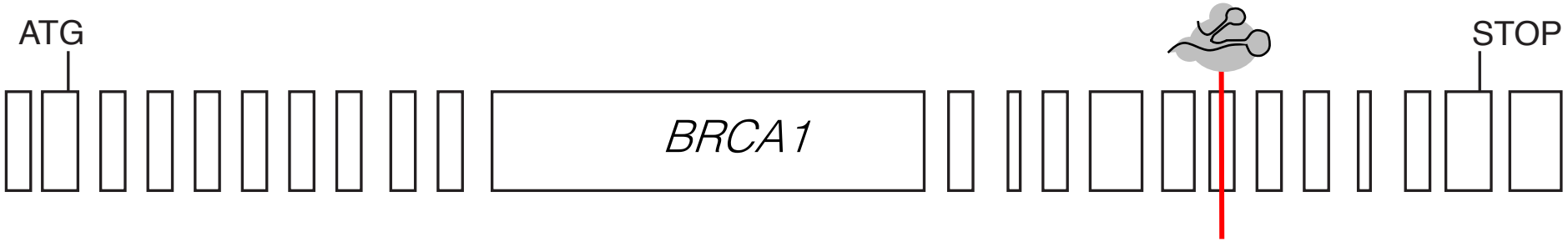


Efficiently mutate a single genomic site

Engineer a precise variant

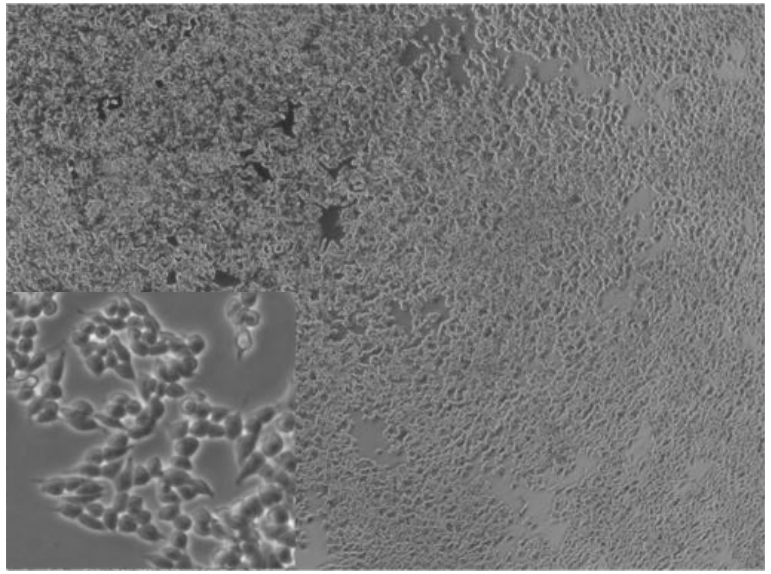


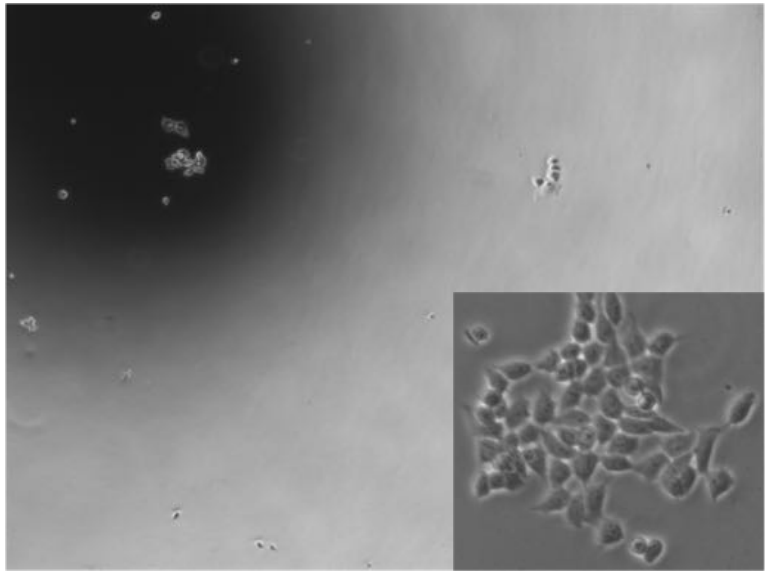
BRCA1 is essential in haploid cells



7 days after transfection:

Target: HPRT1 (control) BRCA1 exon 17





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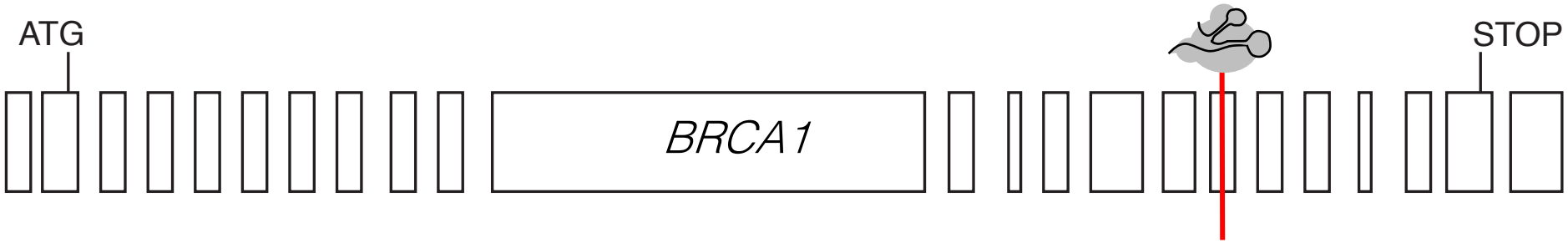
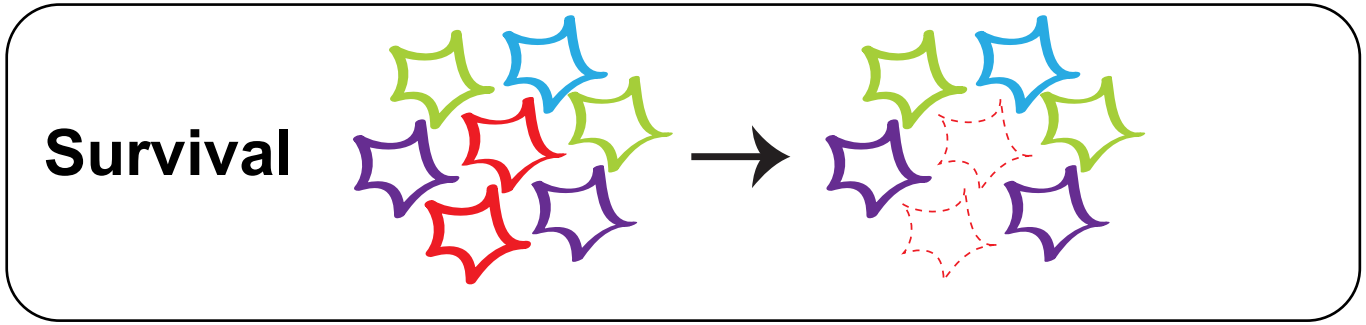
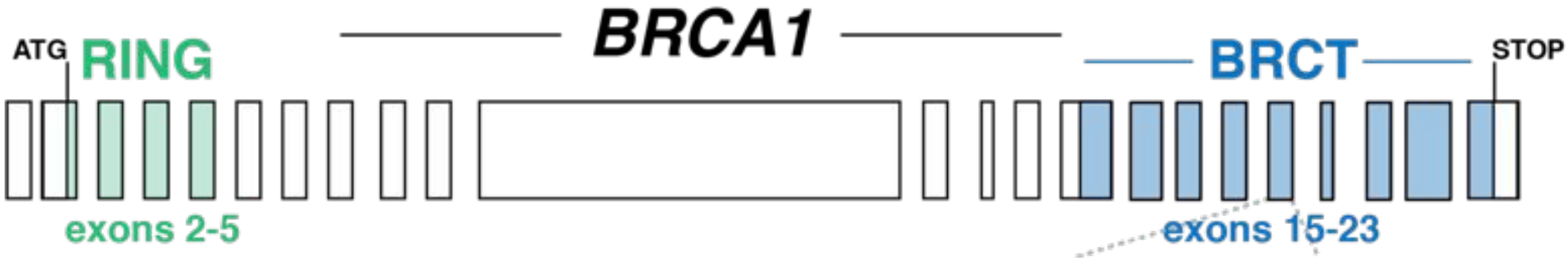
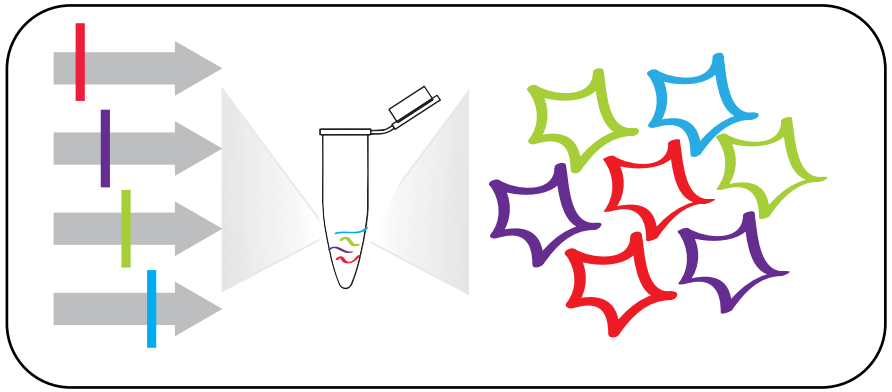


Functional Assay

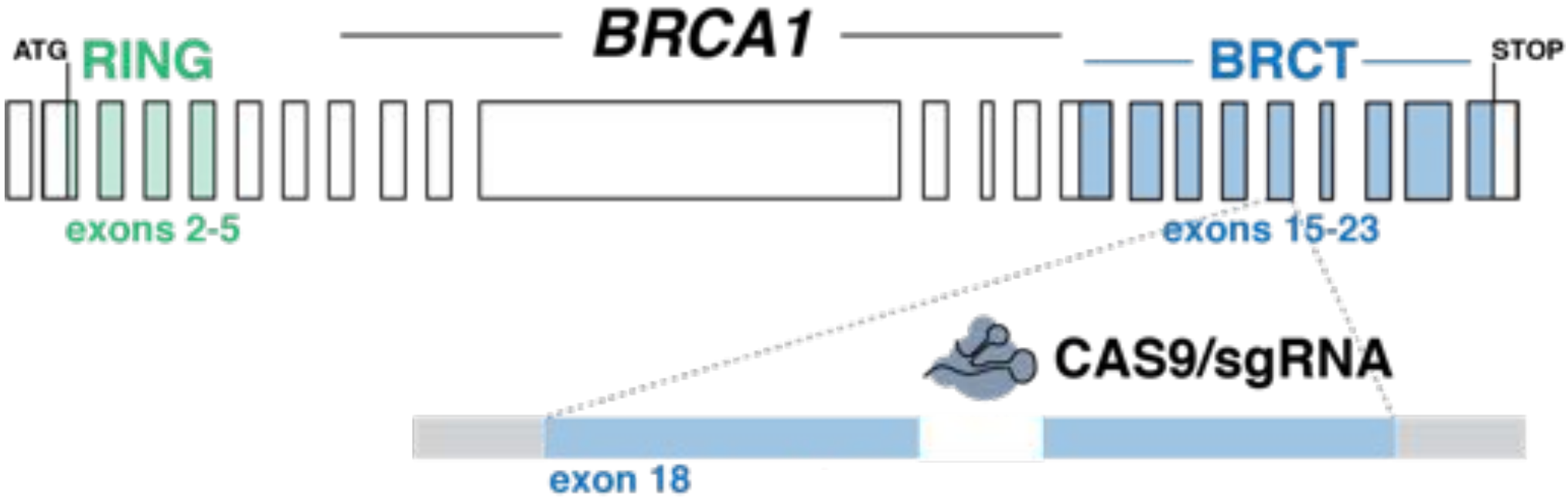


Saturation genome editing of BRCA1





Saturation genome editing of BRCA1



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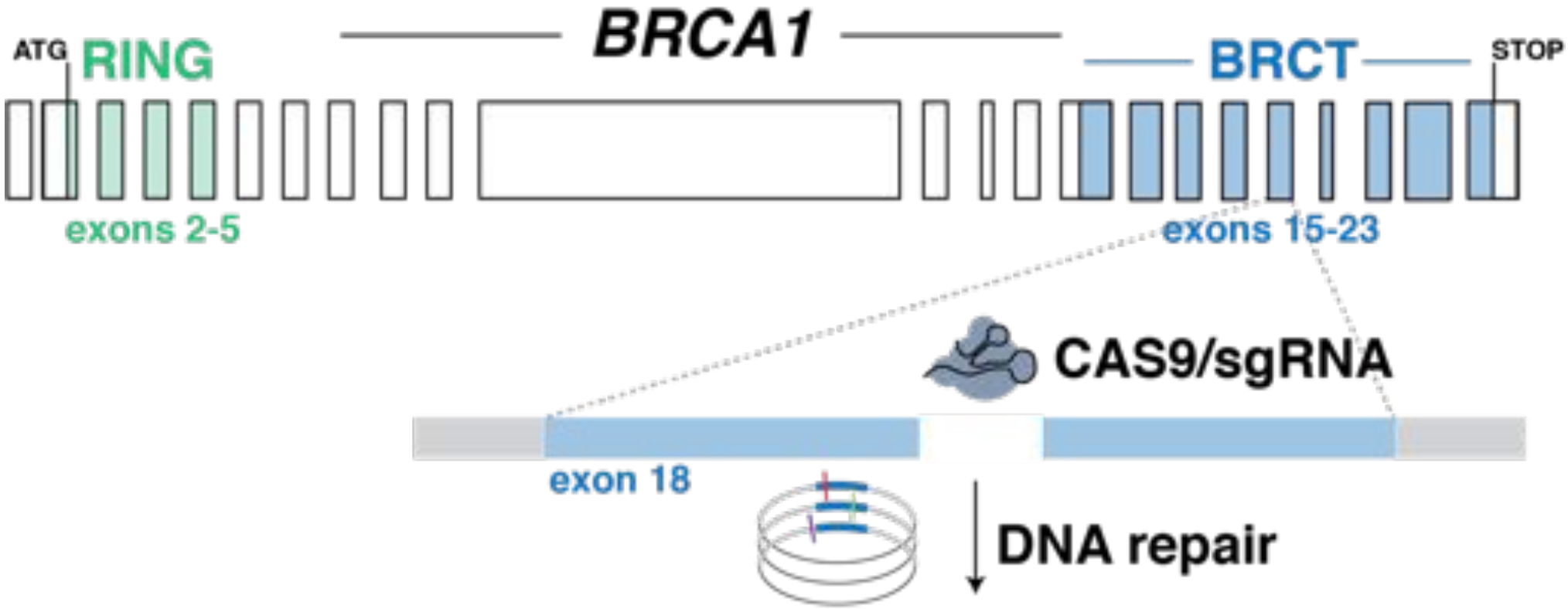
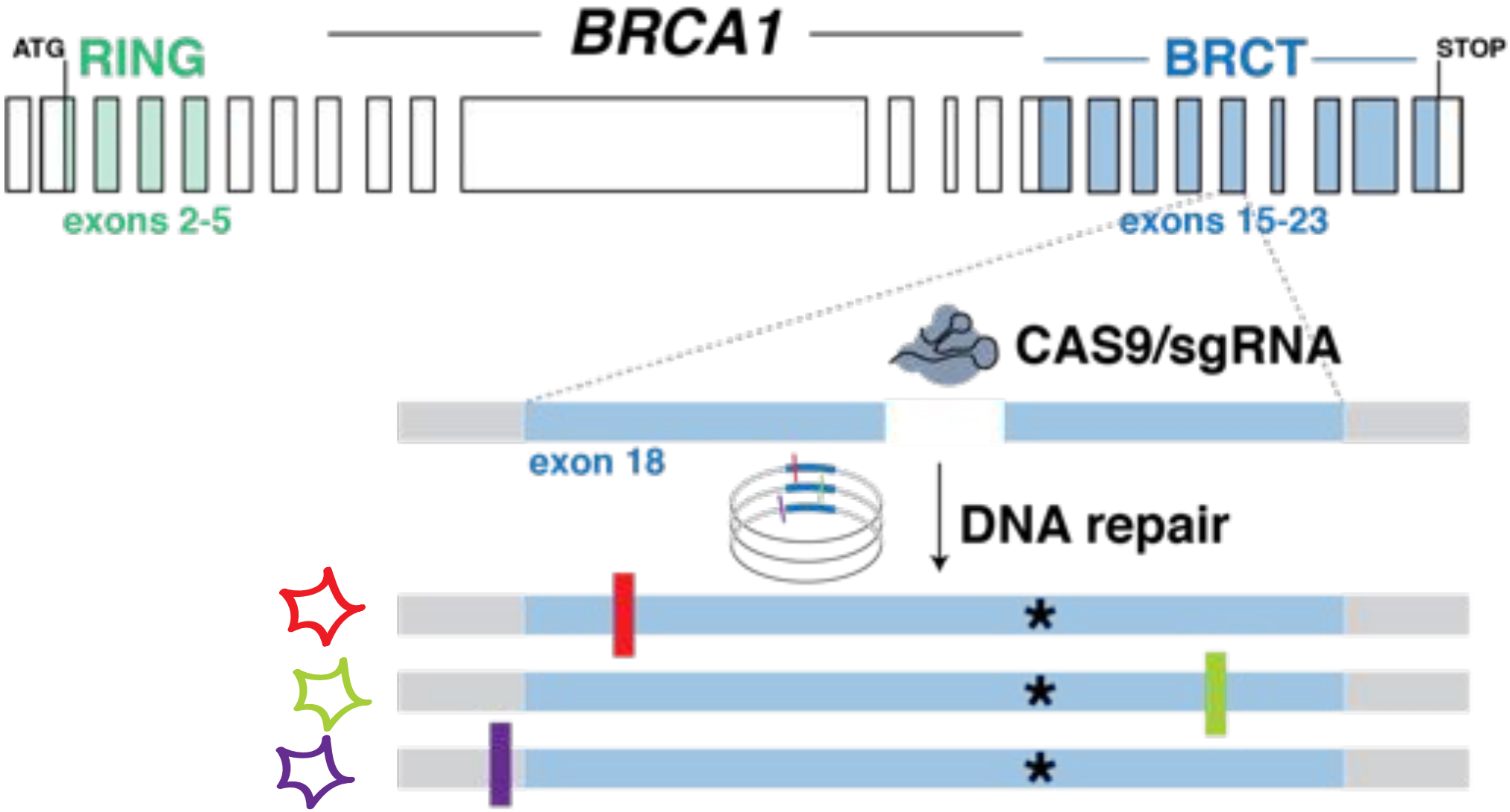
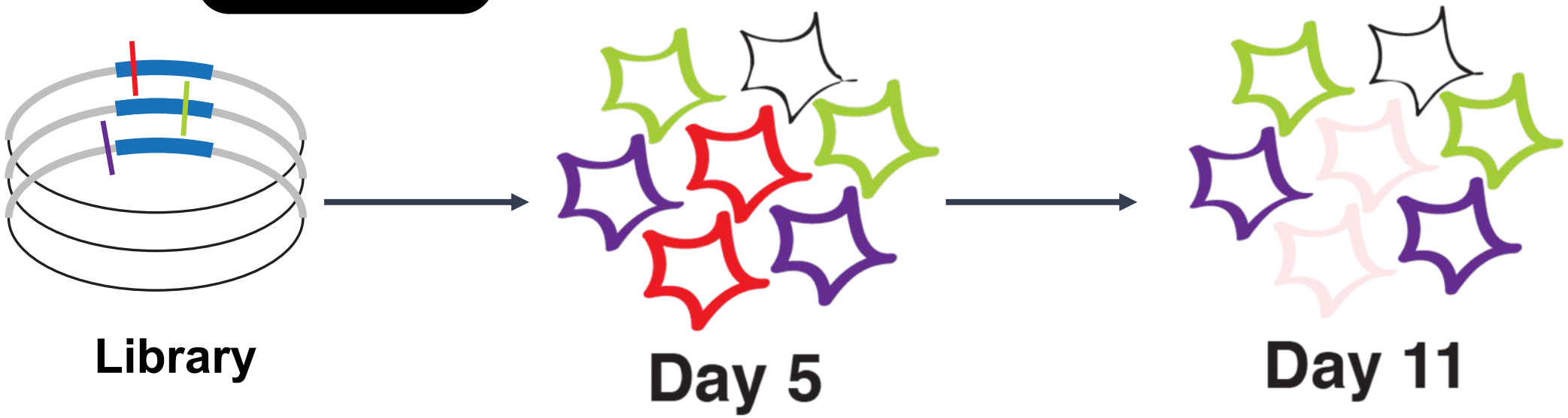


Saturation genome editing of BRCA1

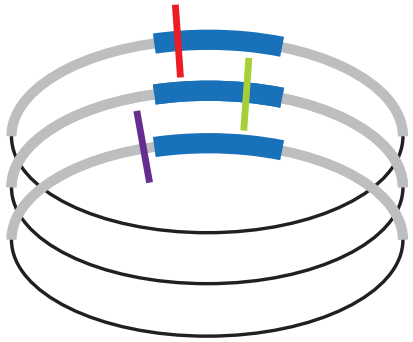


Functional Assay

Survival in haploid cells



Sequence and count variants from DNA and RNA



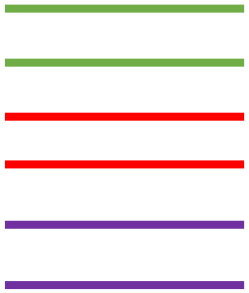
Library

Day 5

Day 11

Plasmid library

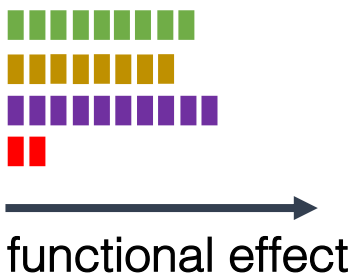
DNA



RNA

DNA

Calculate functional scores
RNA expression scores



Library

Plasmid library

Day 5

DNA

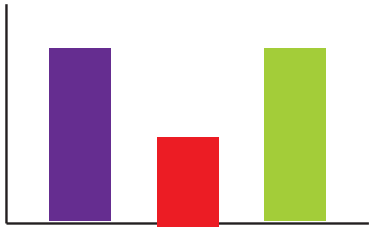
RNA

Day 11

DNA

Expression Score:

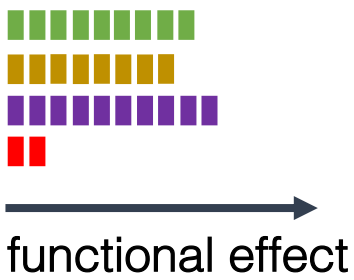
$$\frac{\text{Day 5 RNA}}{\text{Day 5 DNA}}$$



Functional Score:

$$\frac{\text{Day 11 DNA}}{\text{Day 5 DNA}}$$

Calculate functional scores
RNA expression scores



Library

Plasmid library

Day 5

DNA

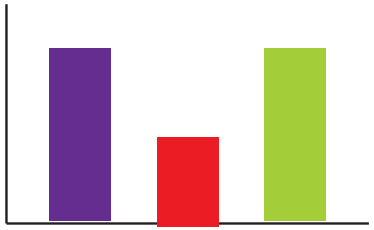
RNA

Day 11

DNA

Expression Score:

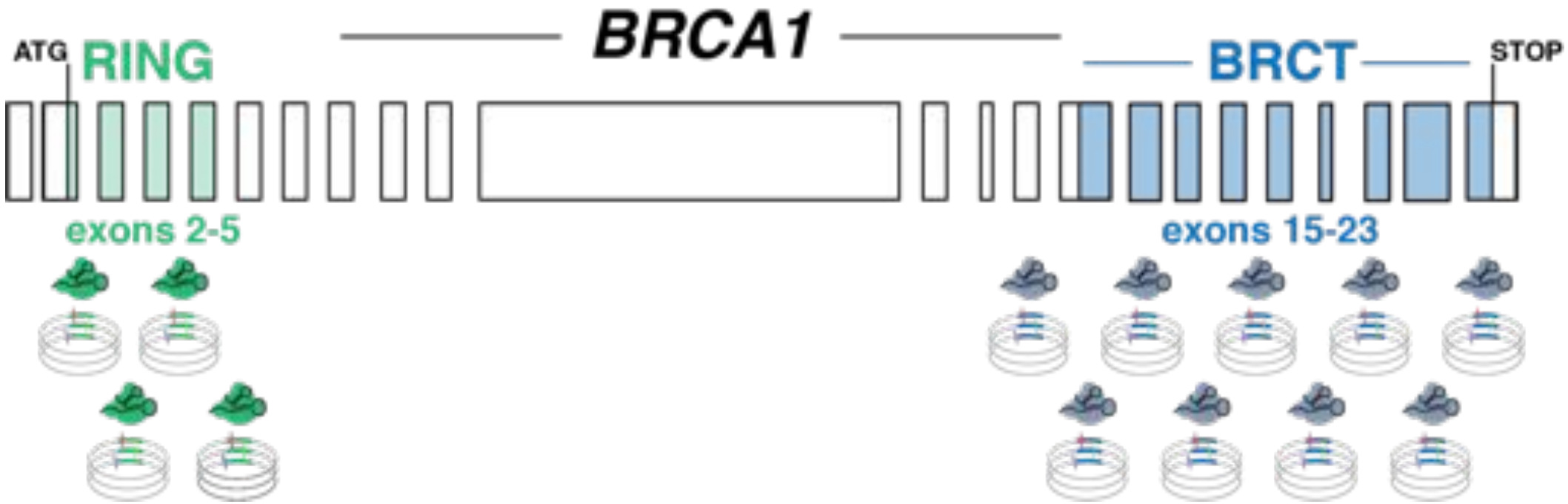
$$\frac{\text{Day 5 RNA}}{\text{Day 5 DNA}}$$



Functional Score:

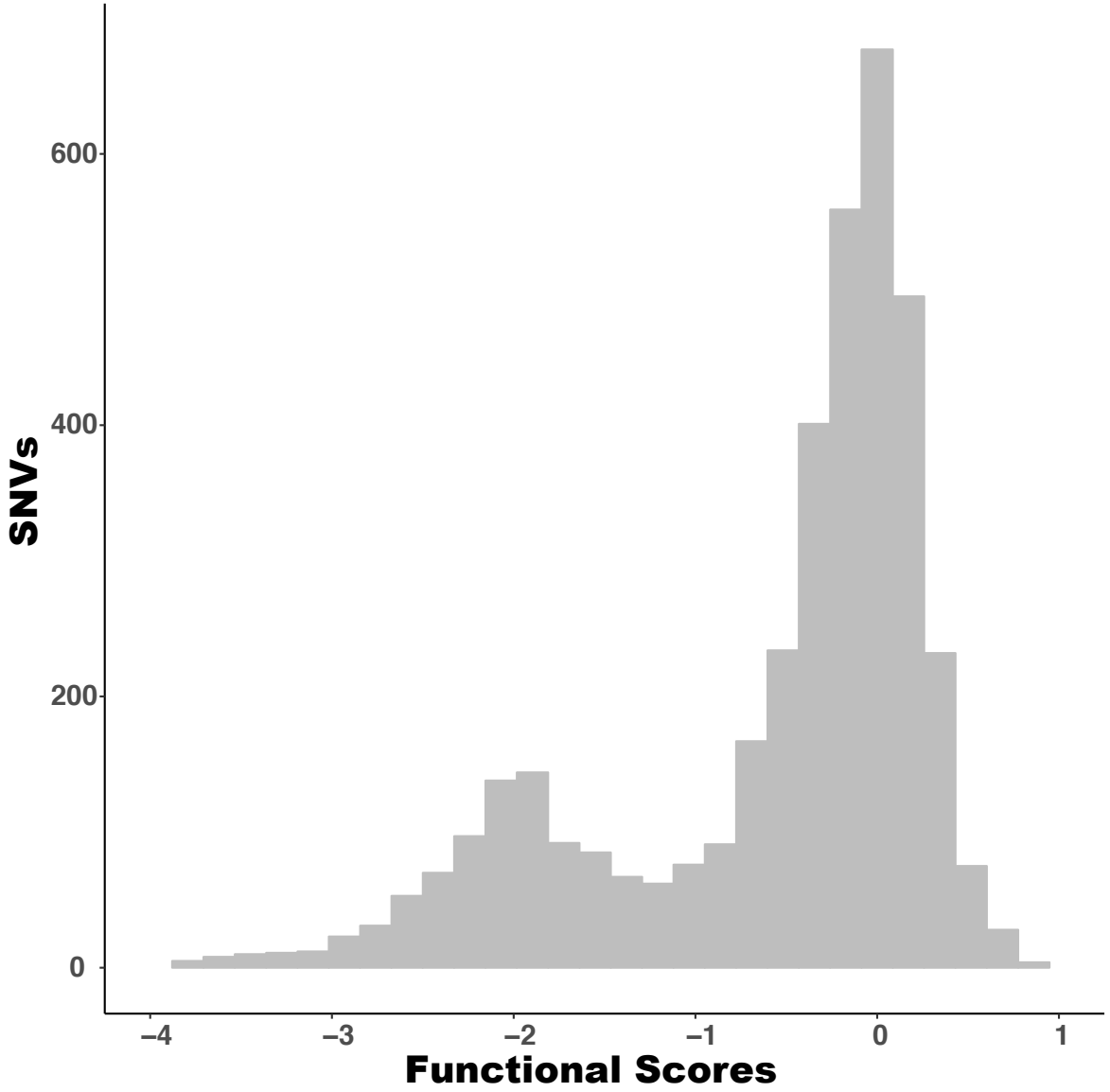
$$\frac{\text{Day 11 DNA}}{\text{Day 5 DNA}}$$

Saturation genome editing of 13 exons of BRCA1 encoding the RING and BRCT

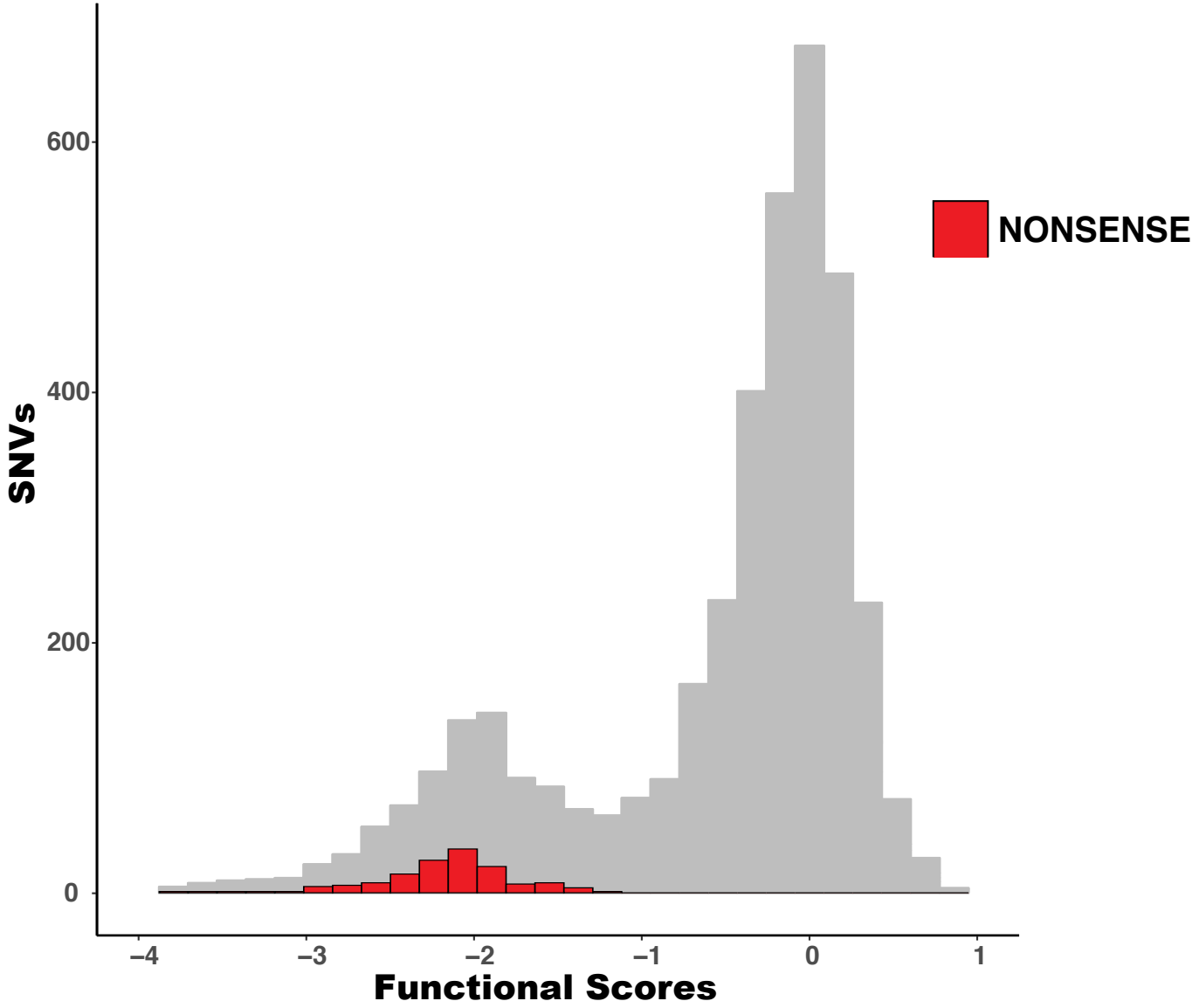


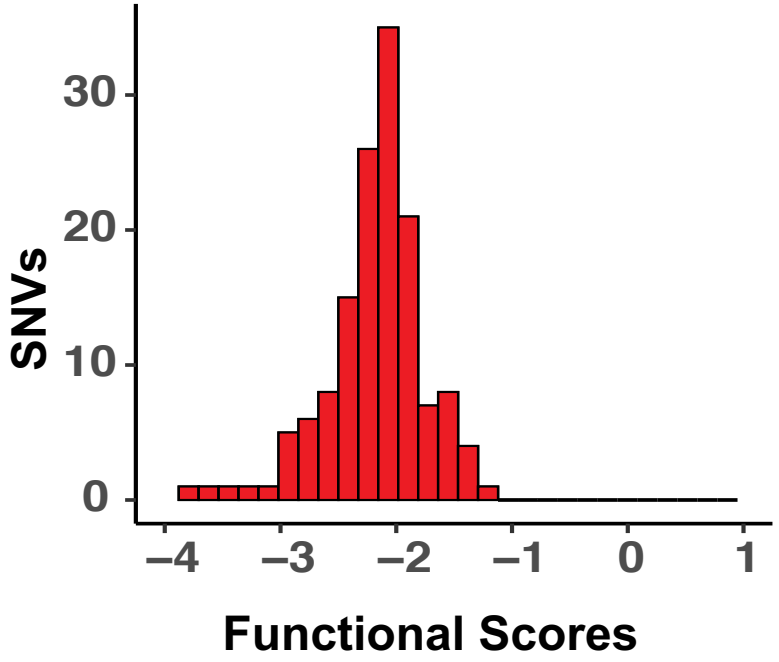
~4000 possible SNVs

Functional scores for 3958 SNVs

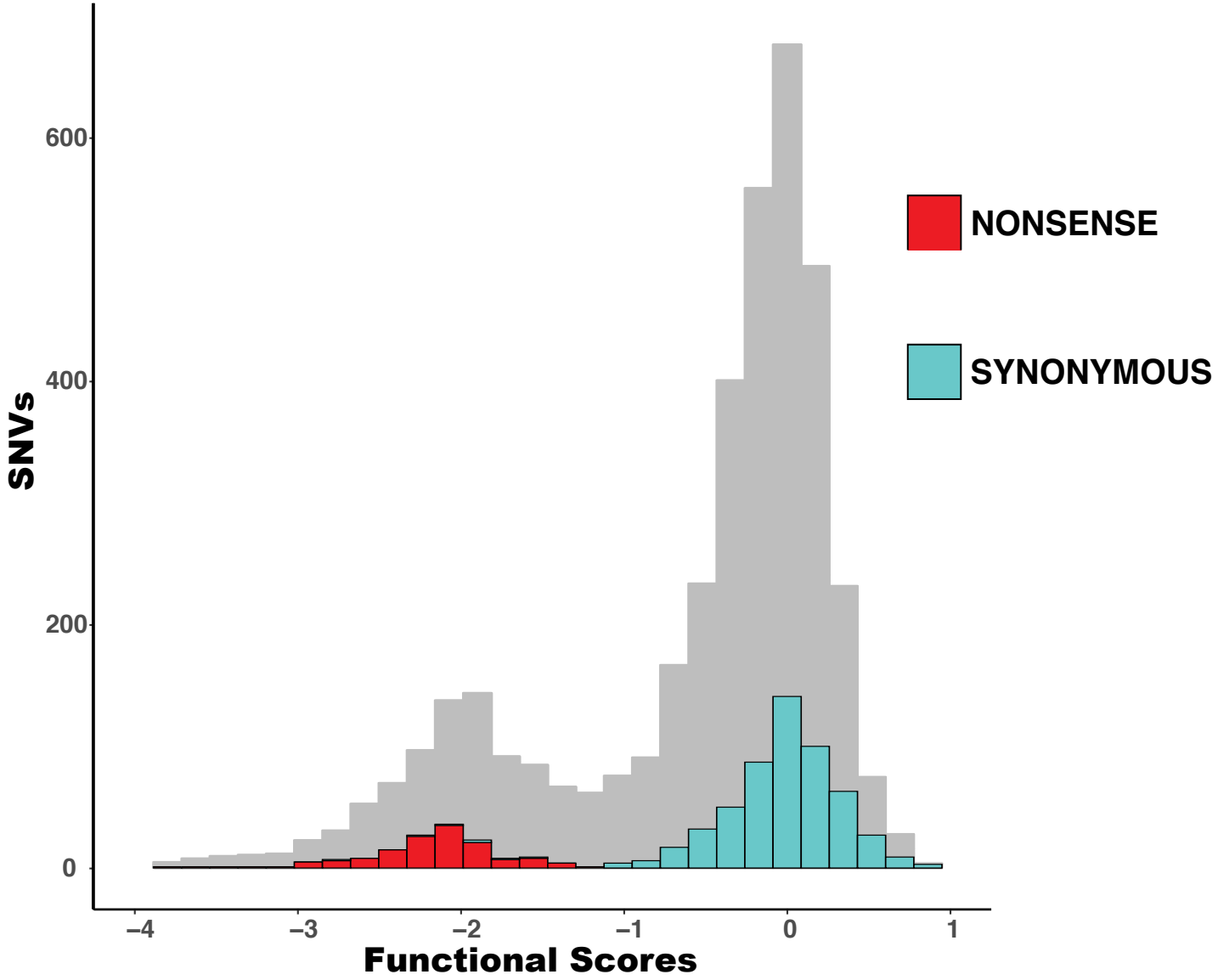


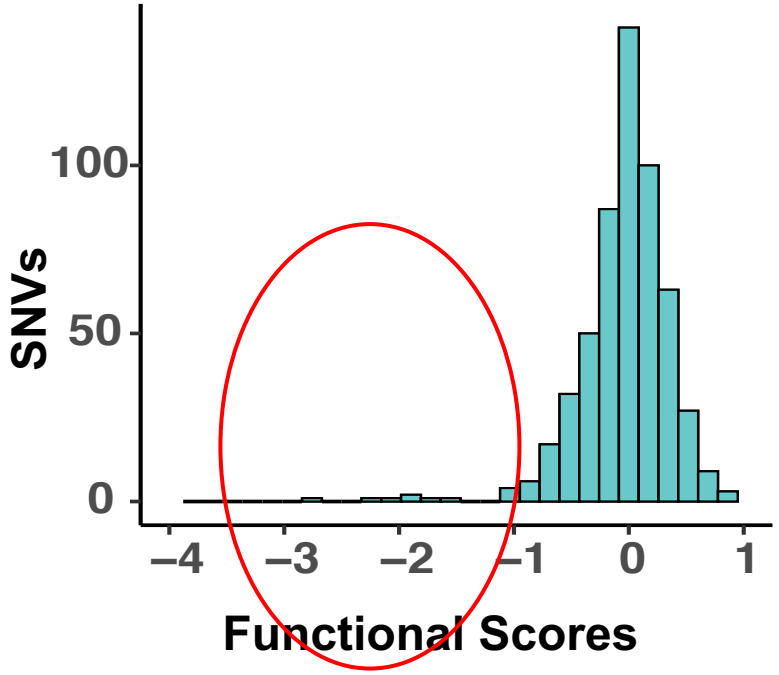
Nonsense codons are bad, whew!





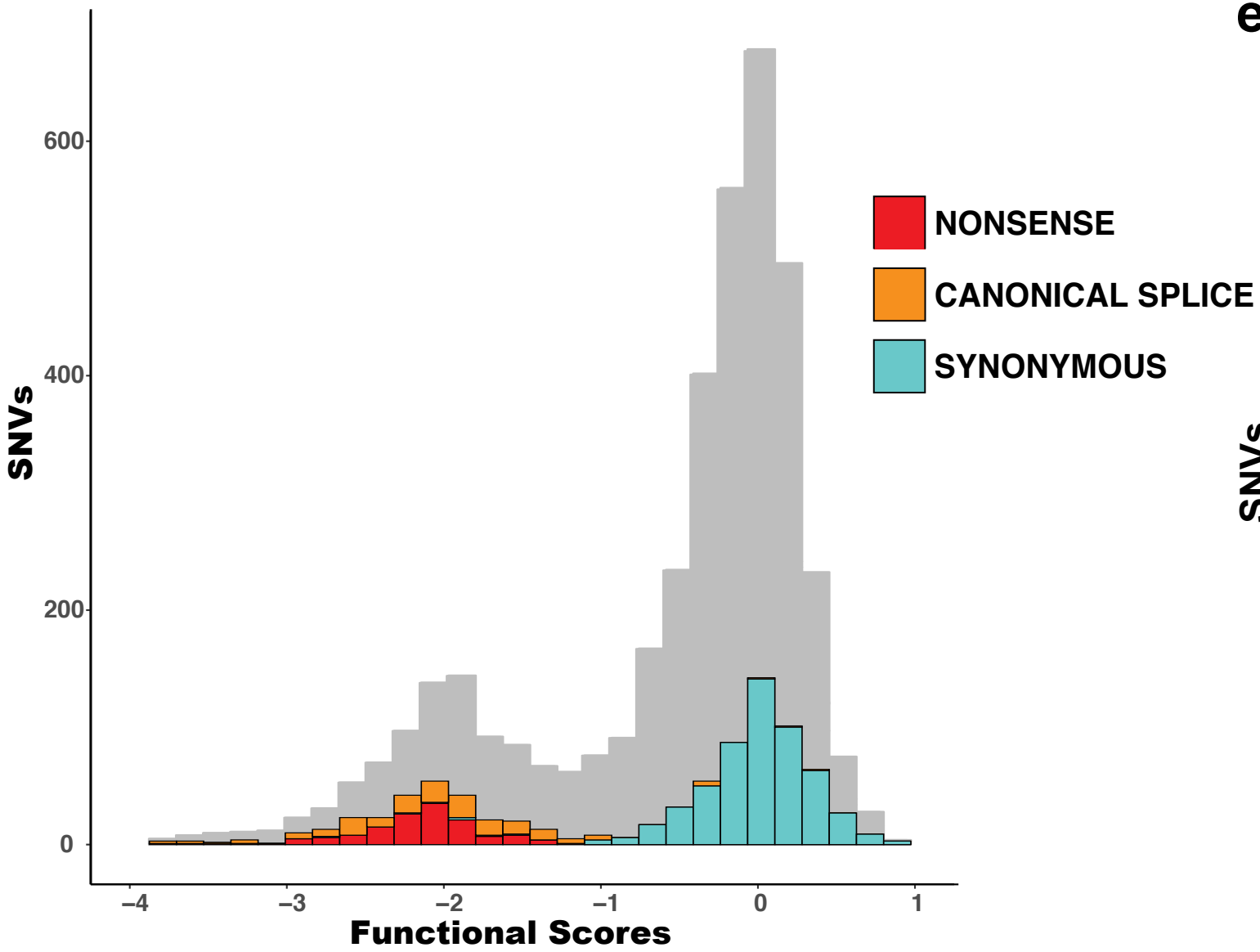
Synonymous variants are rarely damaging

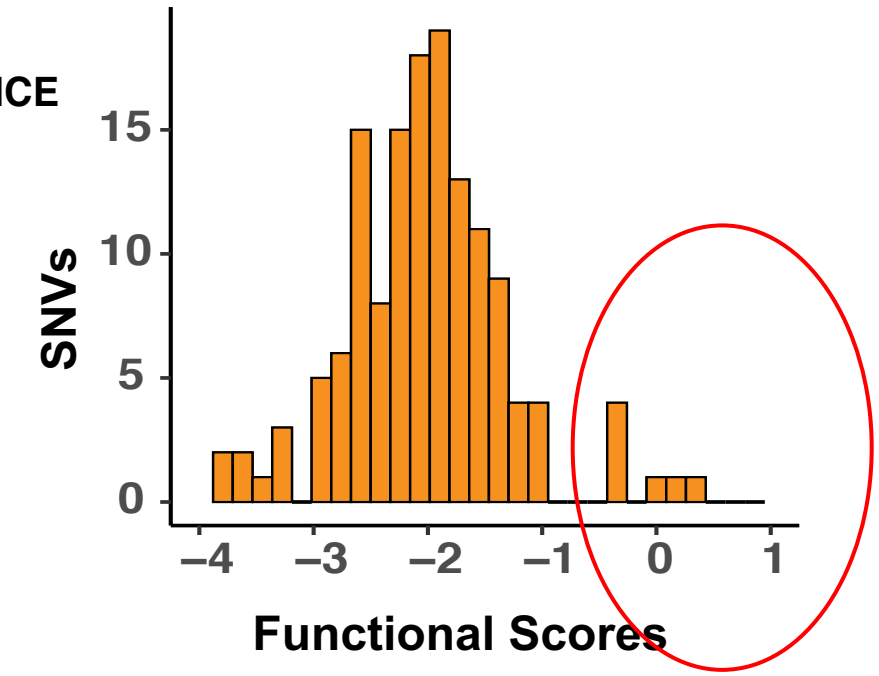




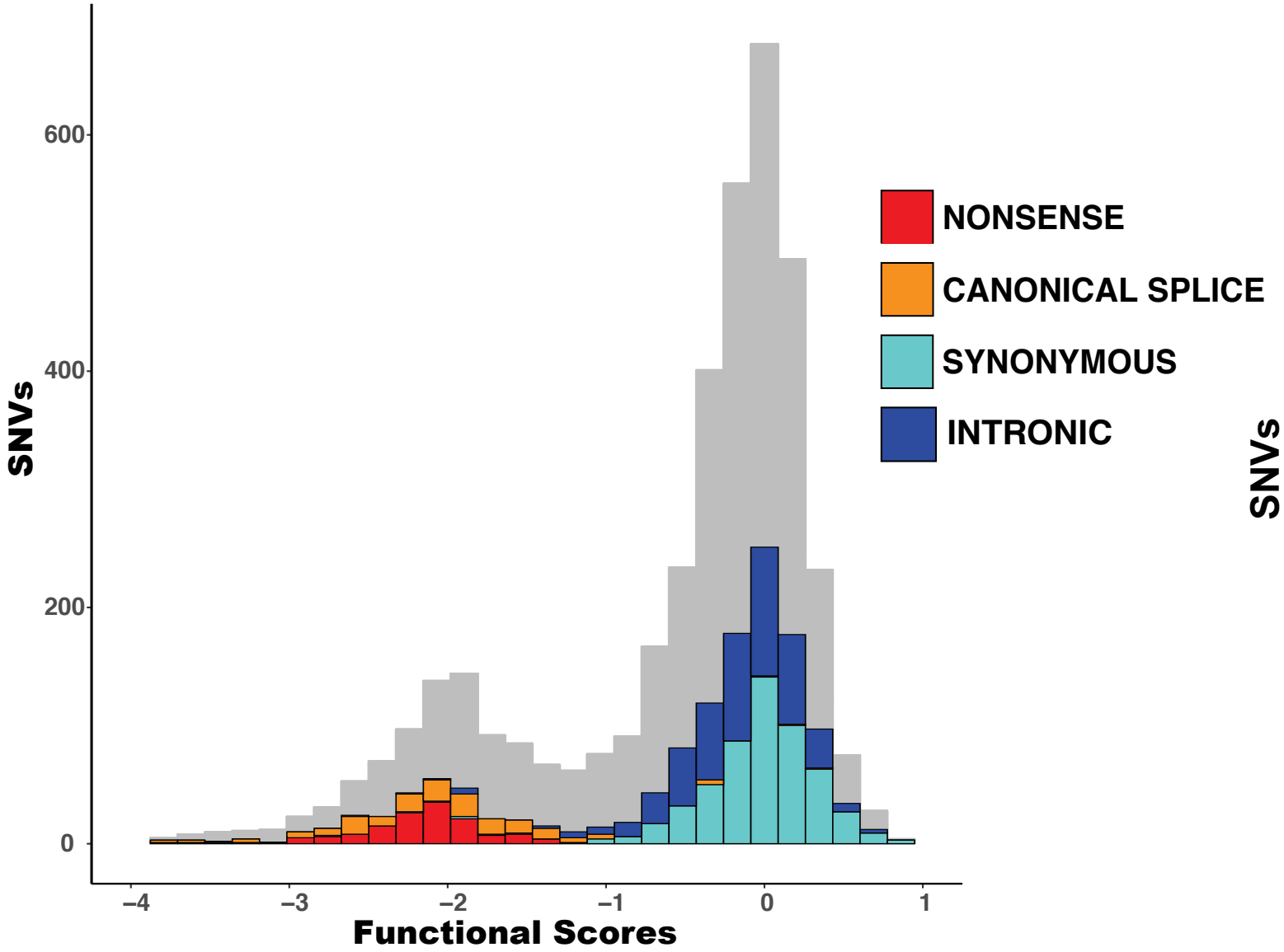
SNVs at canonical splice positions are damaging,

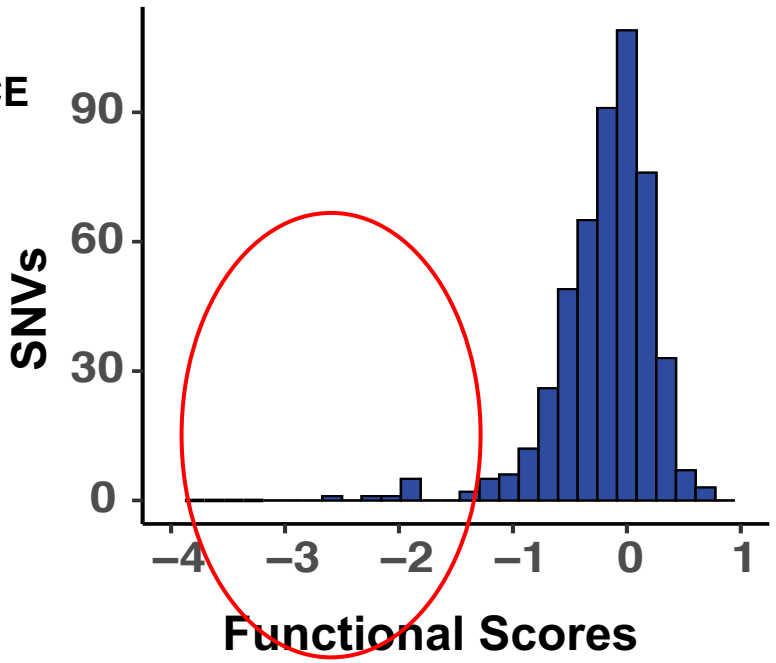
except when they aren't…



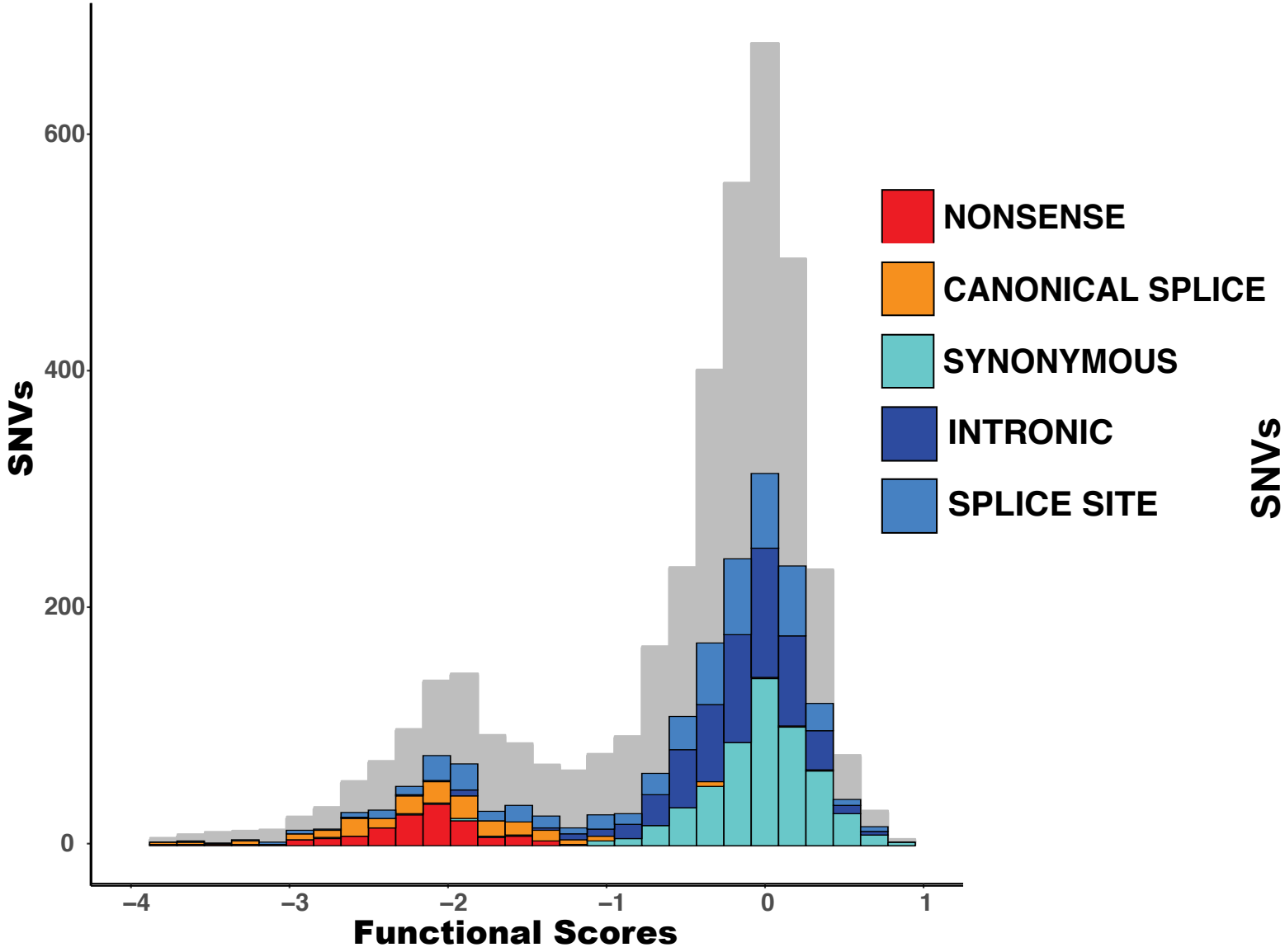


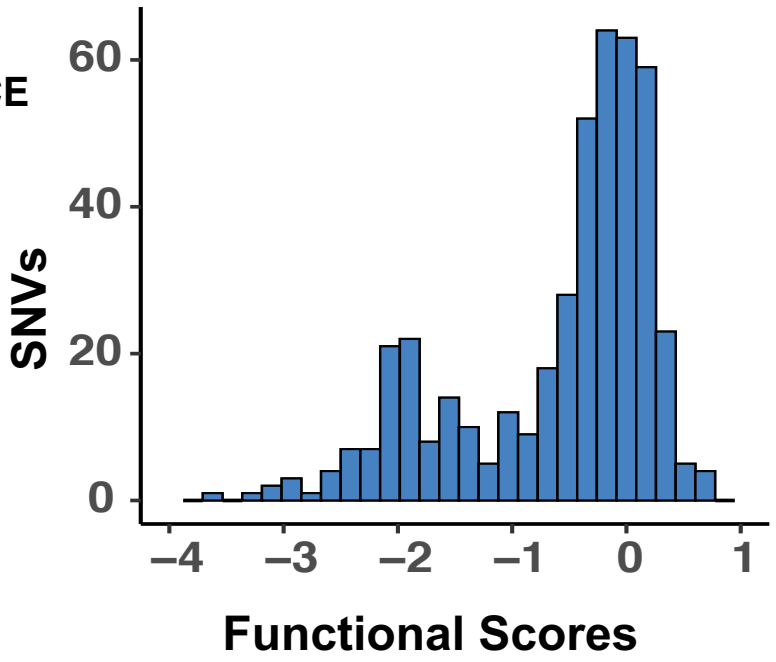
Deep intron variants are rarely damaging



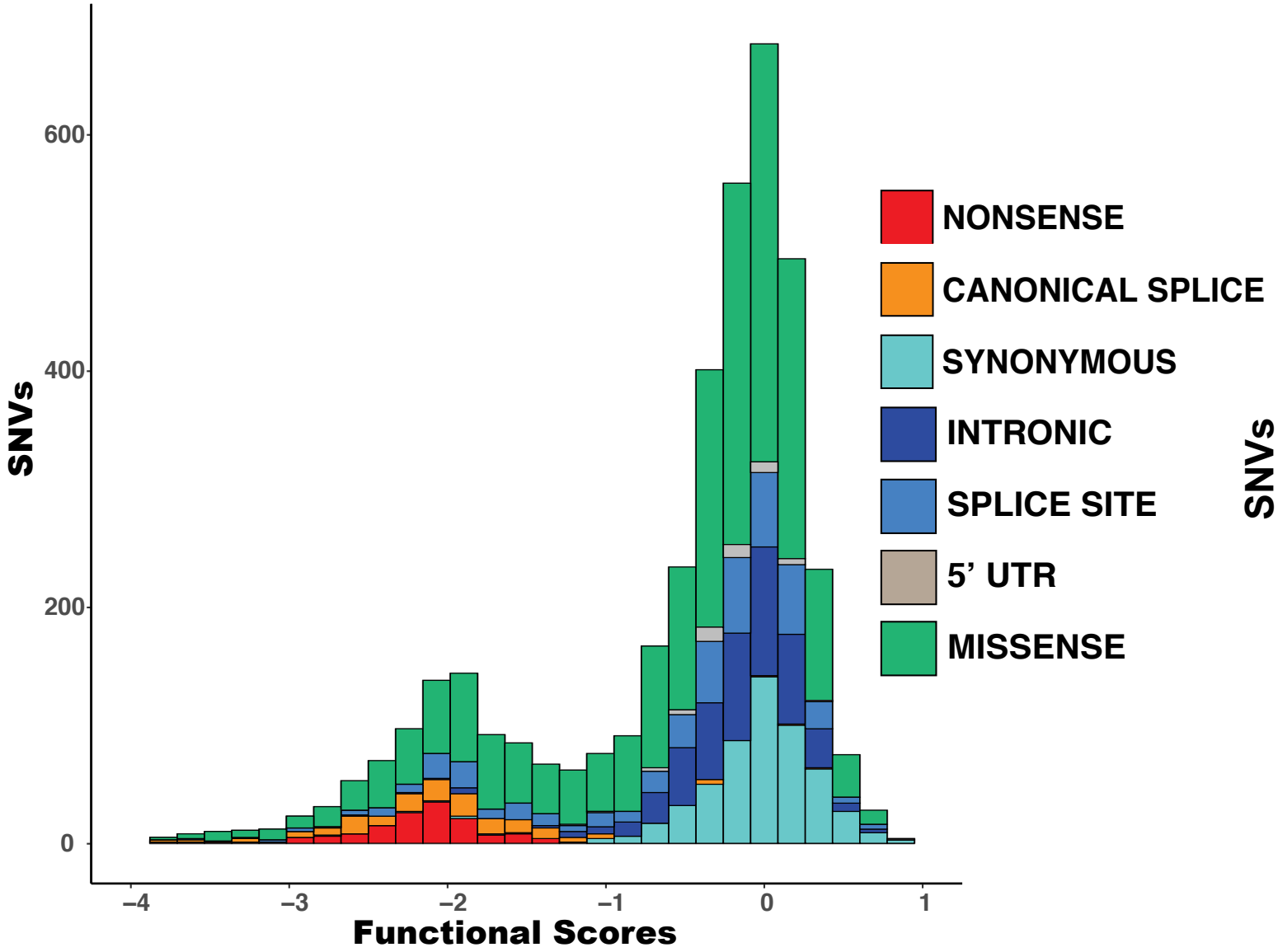


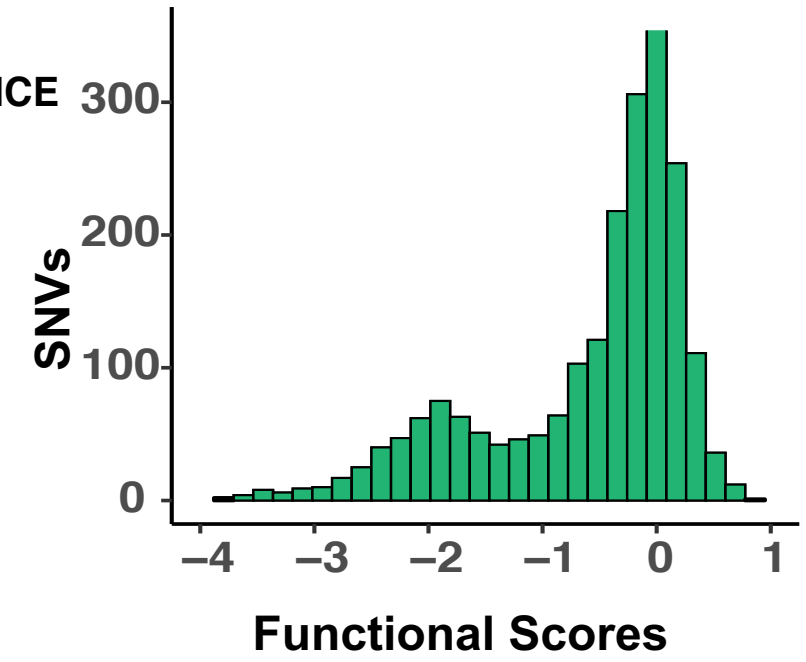
SNVs closer to the canonical splice positions are more likely to be damaging



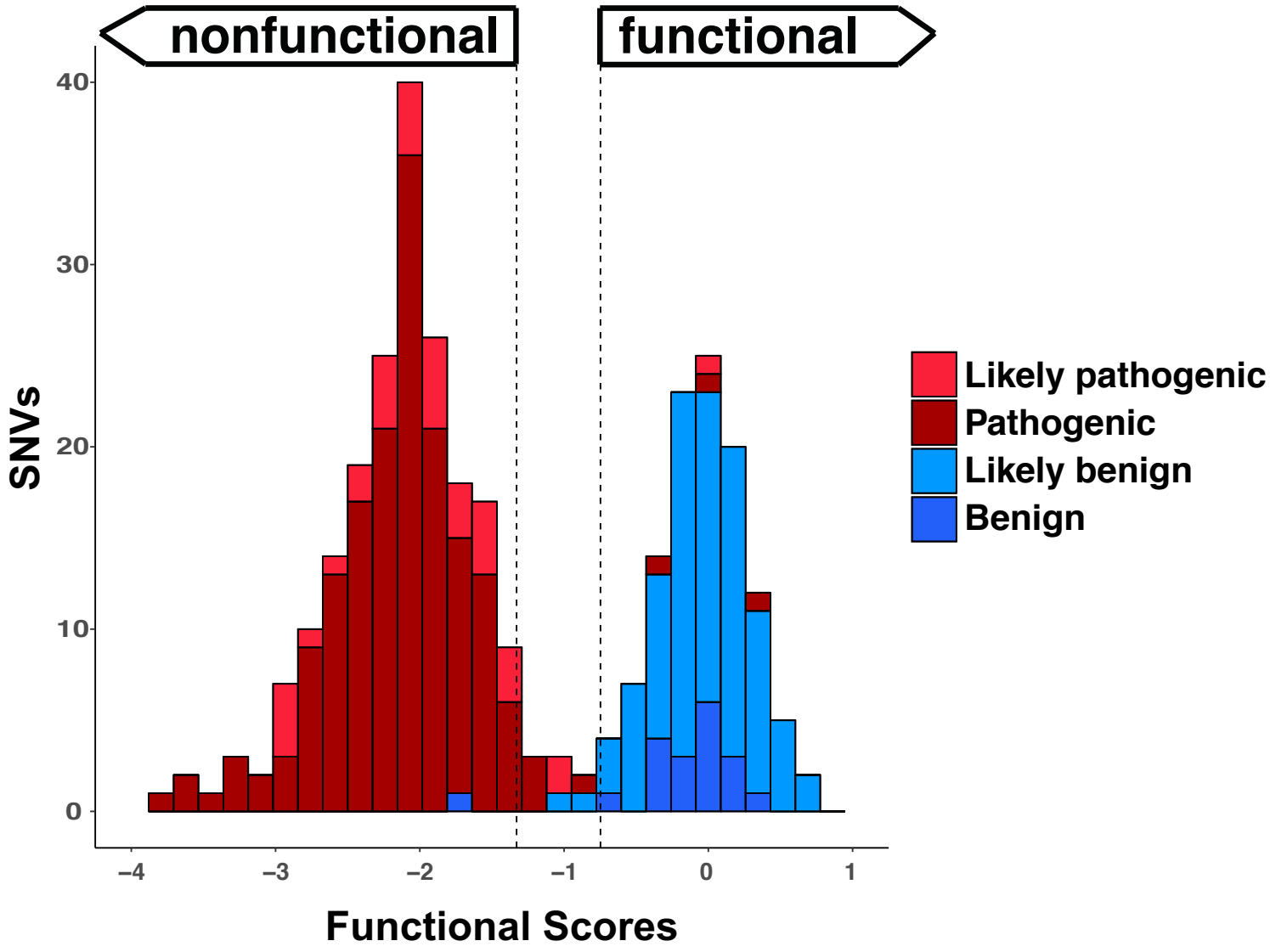


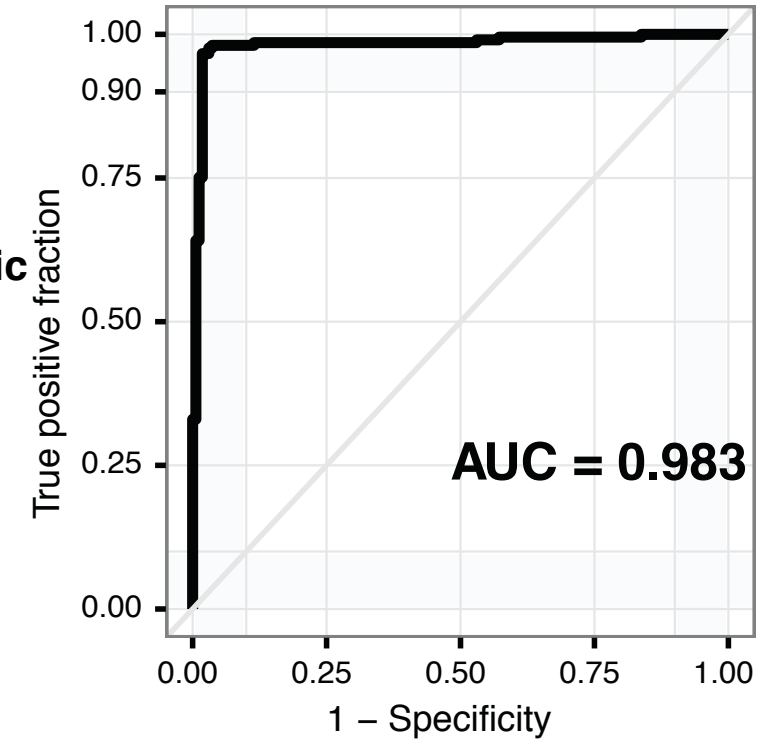
Missense variants are bimodal



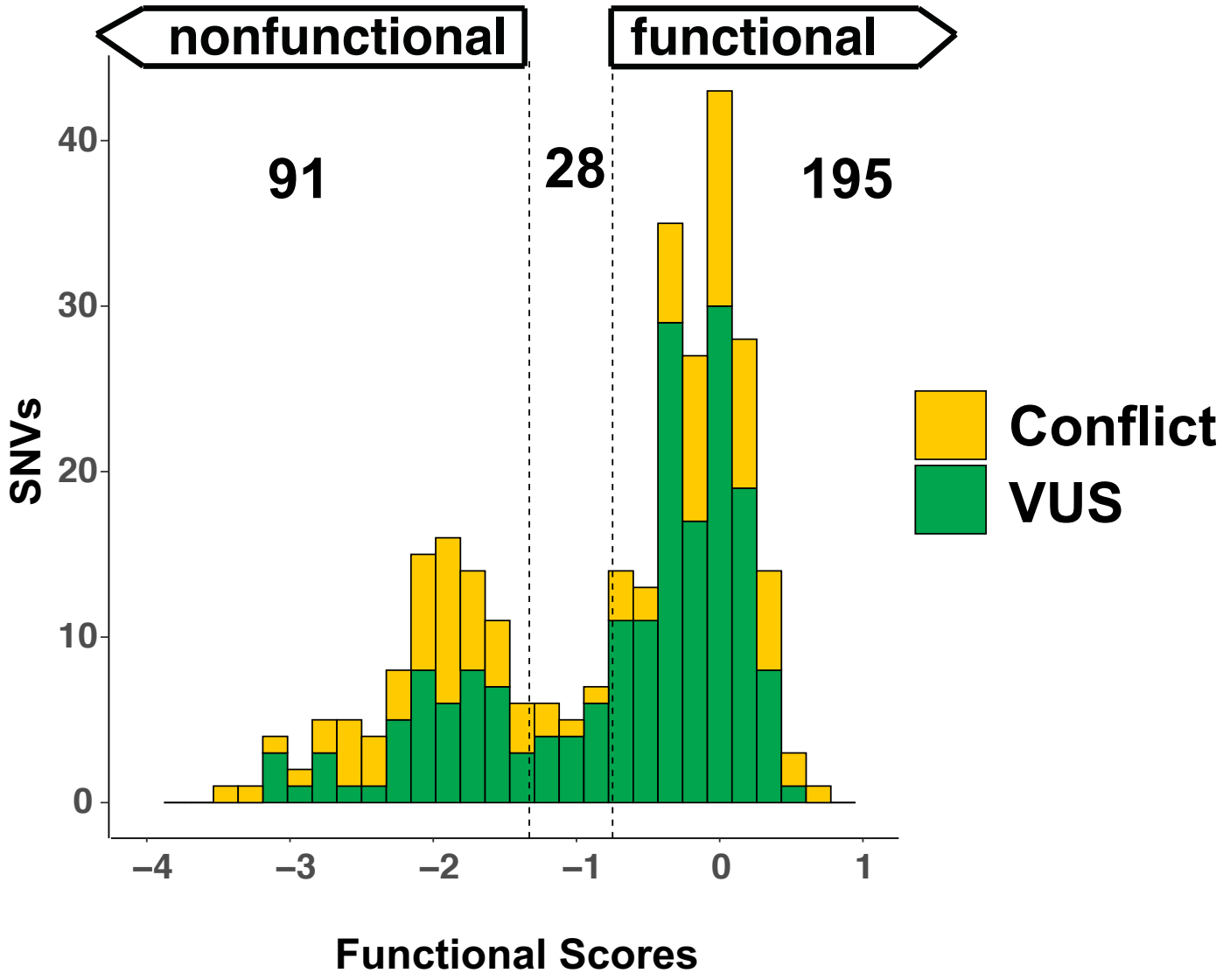


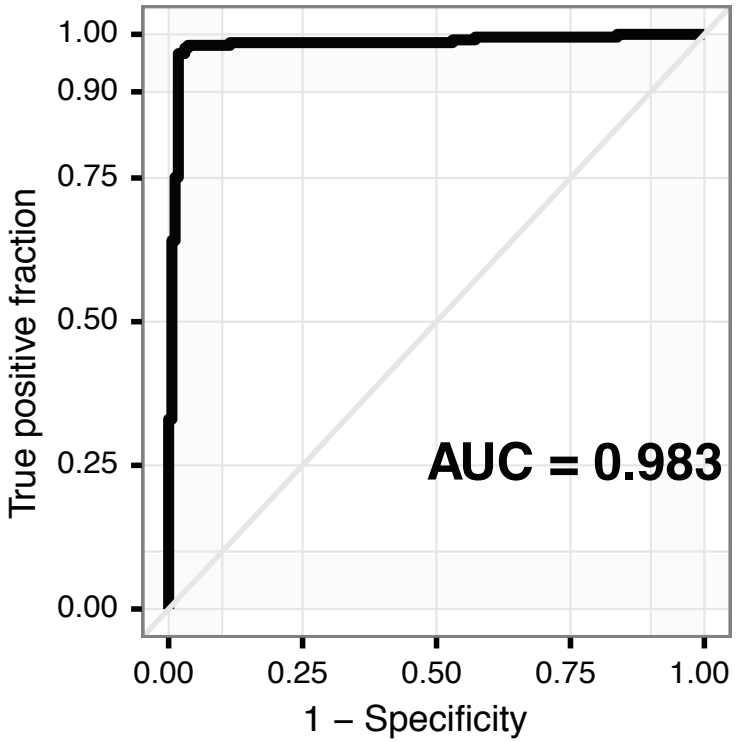
Functional scores separate known pathogenic mutations from benign variants





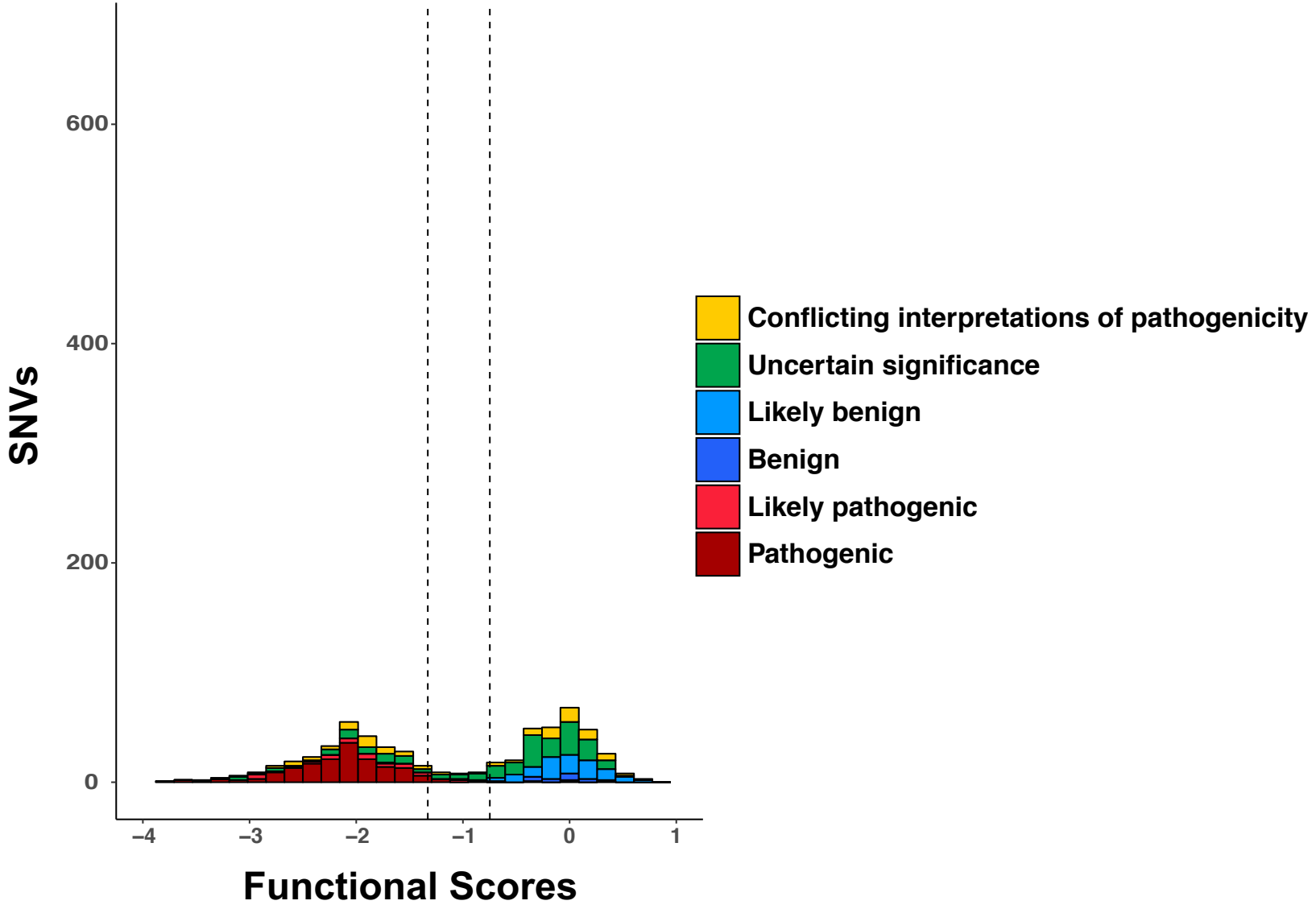
Functional scores separate 90% VUS into functional categories



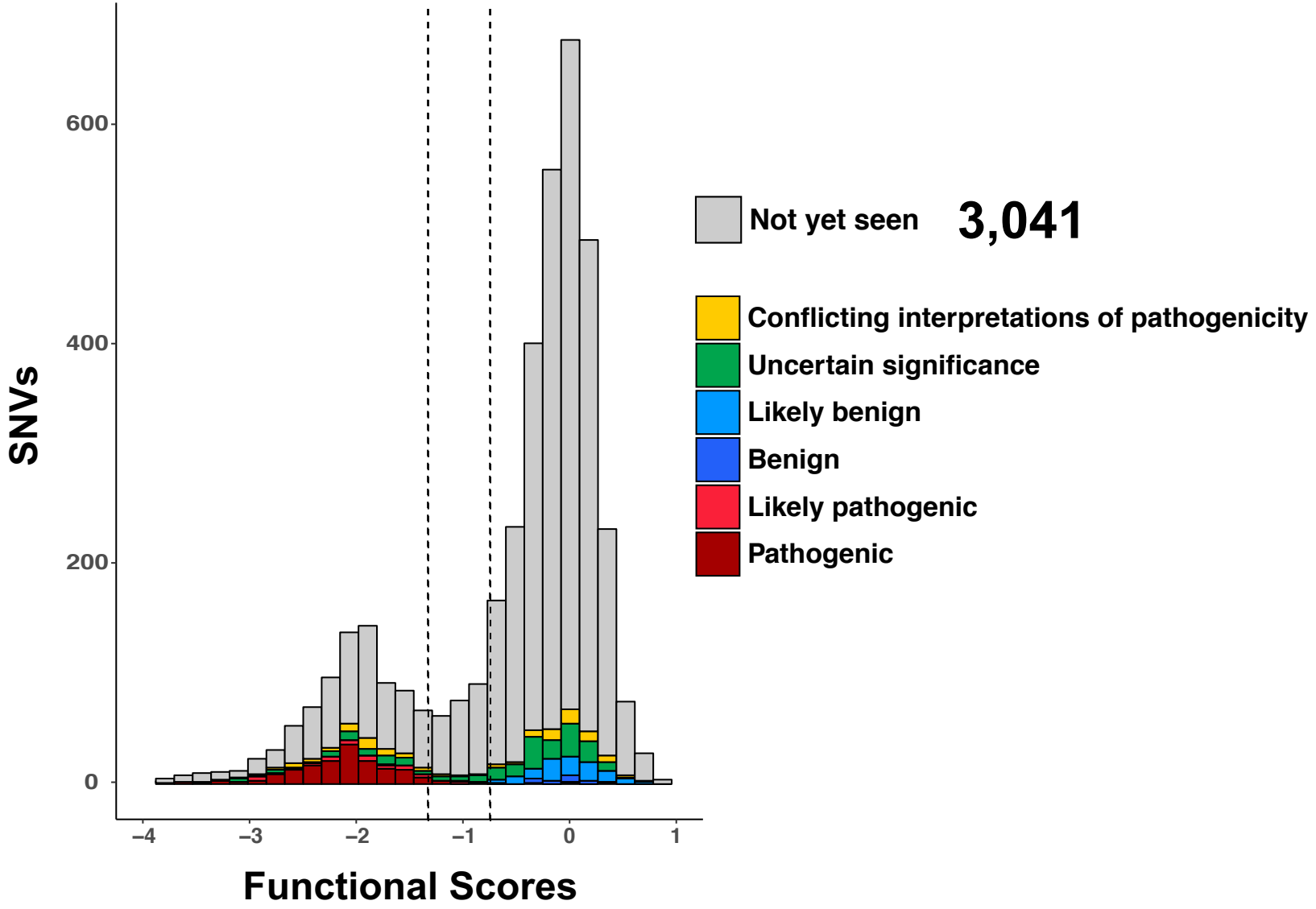


Findlay et al. Nature 2018

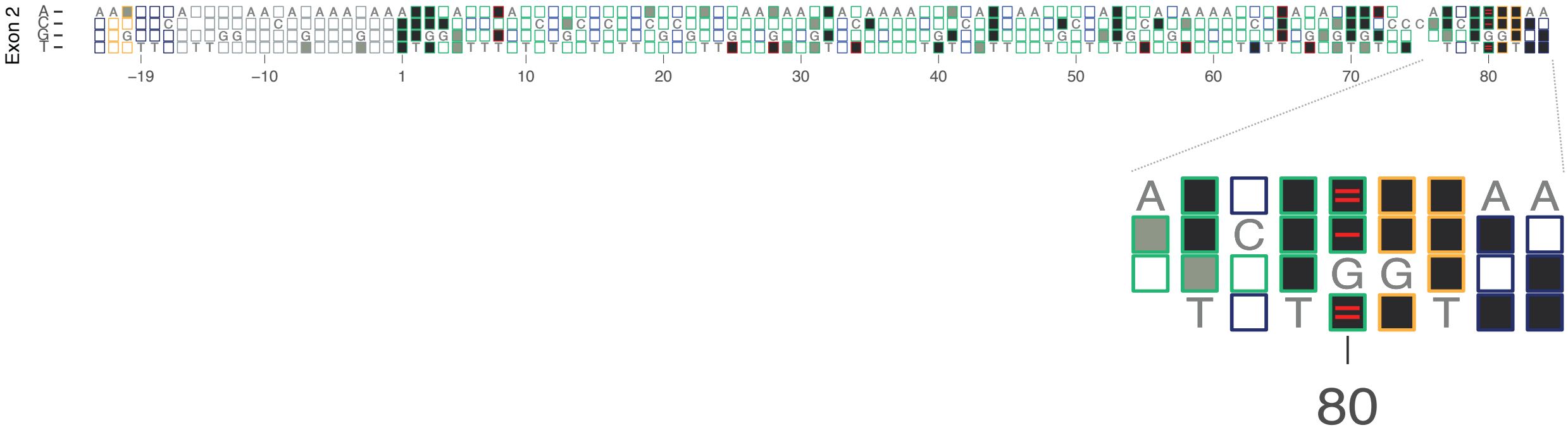
Functional scores separate known pathogenic mutations from benign variants



Functional scores separate known pathogenic mutations from benign variants



SNVs across 13 exons with mechanistic information of functional effects



SNV consequence

- 5' UTR
- Canonical splice
- Intronic
- Missense
- Splice region
- Nonsense
- Synonymous

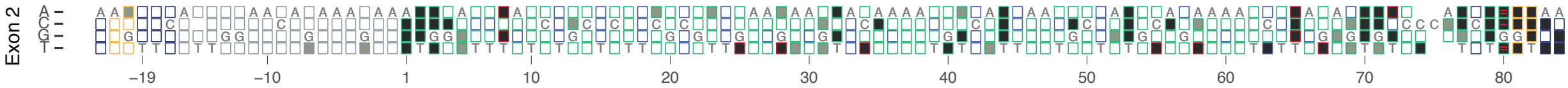
Function score

- non-functional
- intermediate
- functional

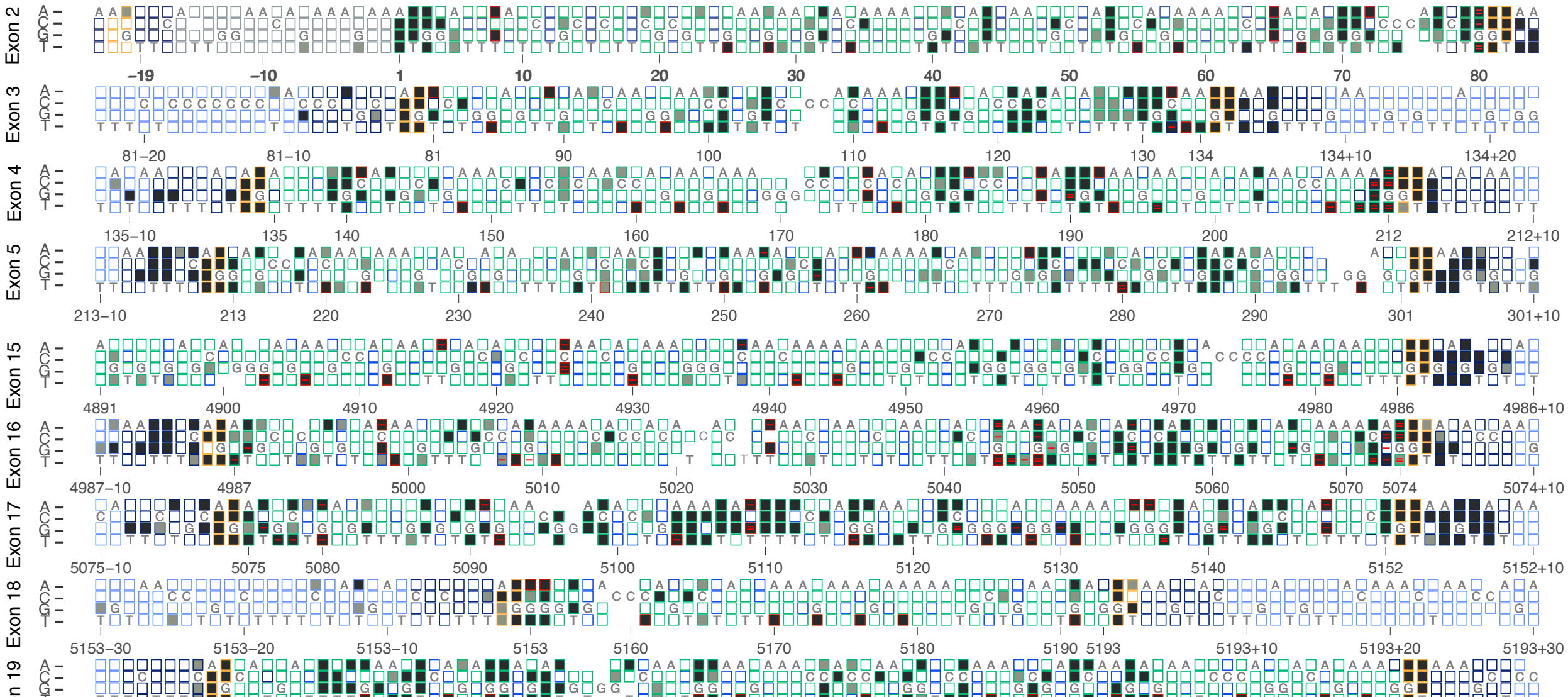
RNA score

- > -2
- -2 to -3
- < -3

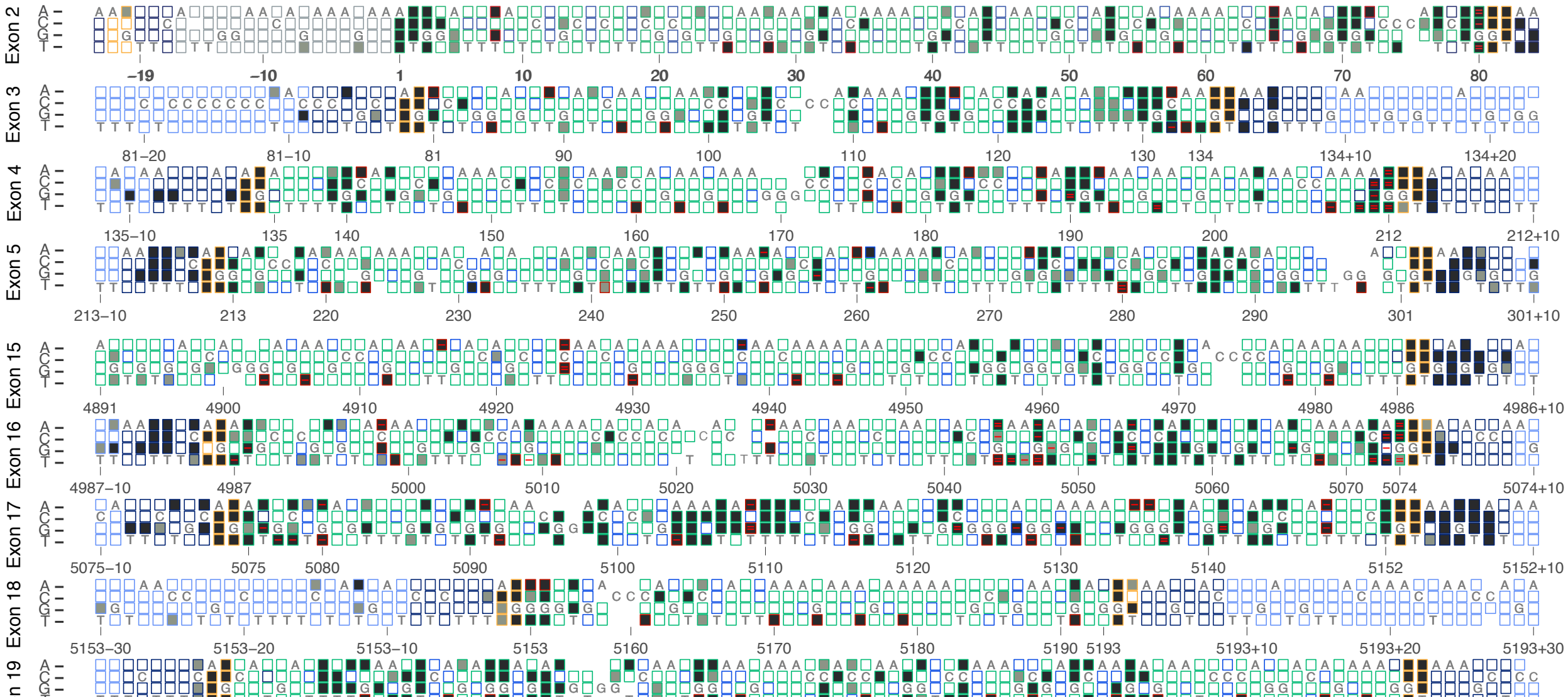
SNVs across 13 exons with mechanistic information of functional effects

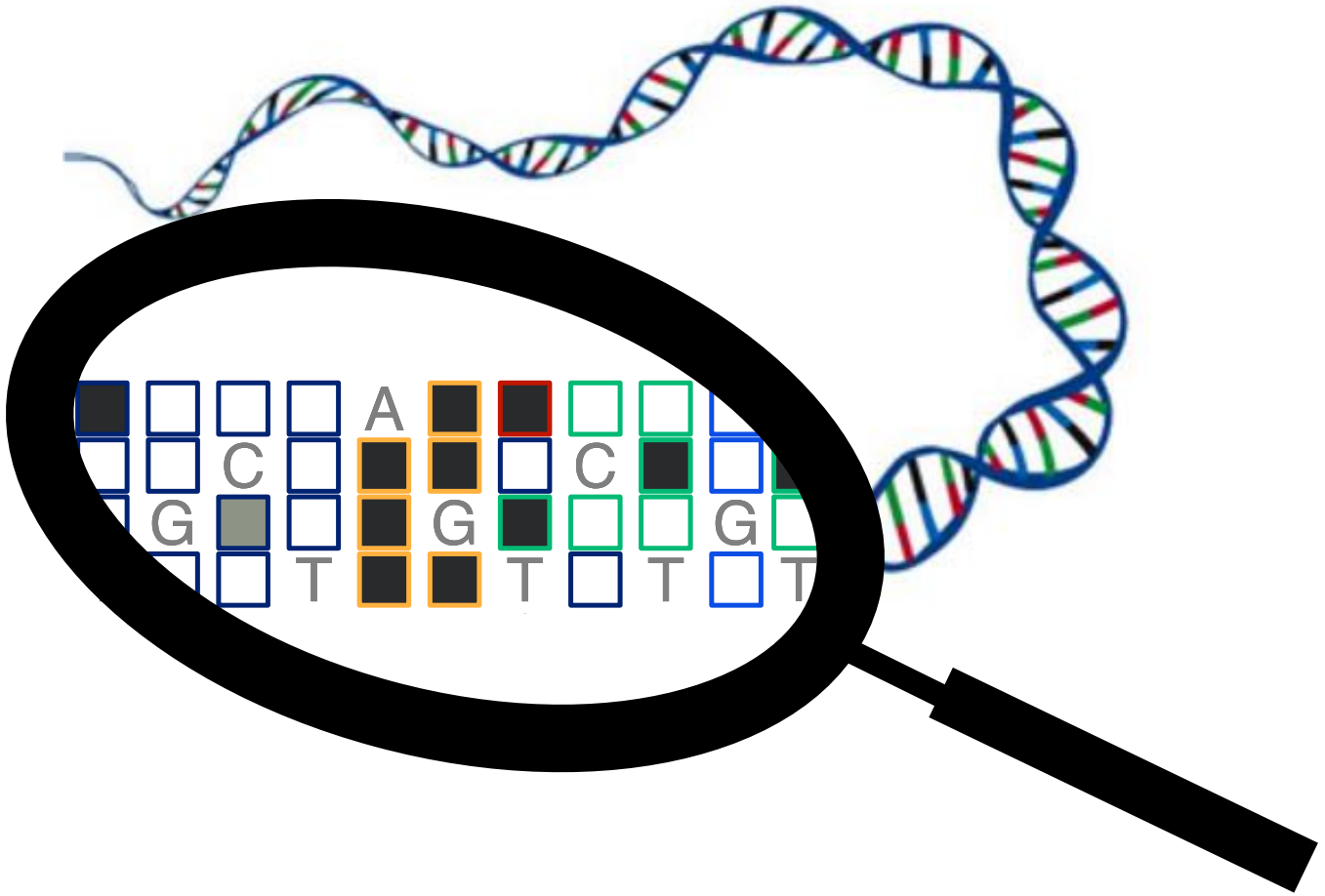


SNVs across 13 exons with functional categories *plus mechanistic information*



SNVs across 13 exons with functional categories *plus mechanistic information*



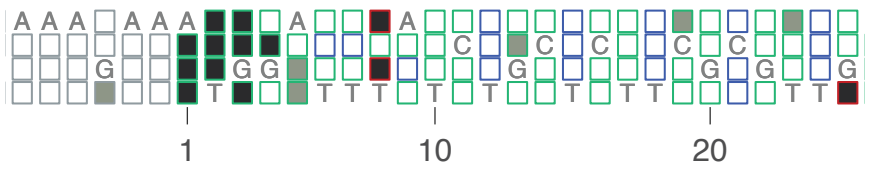


sge.gs.washington.edu/BRCA1/

Outline

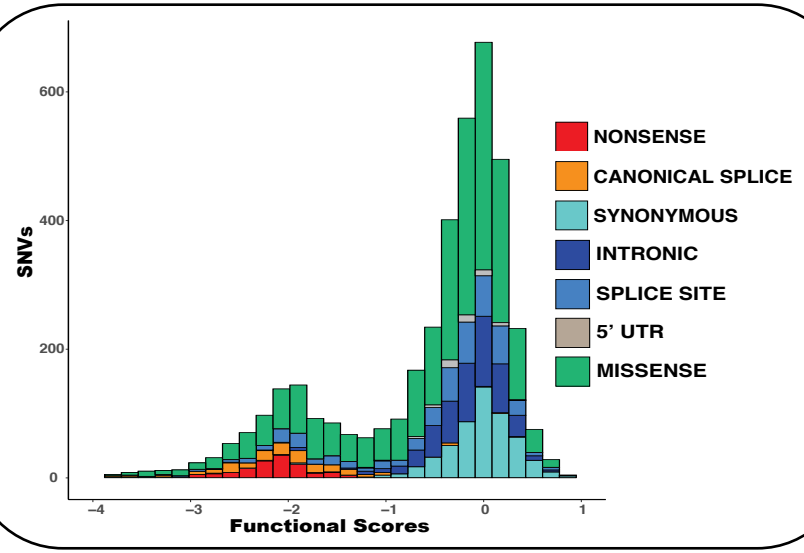
- What is a multiplexed functional assay?
- Example of multiplexed functional assays for *BRCA1*
- **Benefits and limitations for variant interpretation**

Benefits of multiplexed functional assays



Sequence–function map

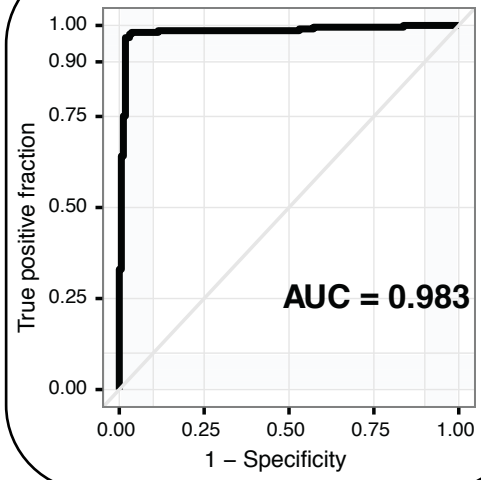
Data for variant is in the same place
Comprehensive coverage



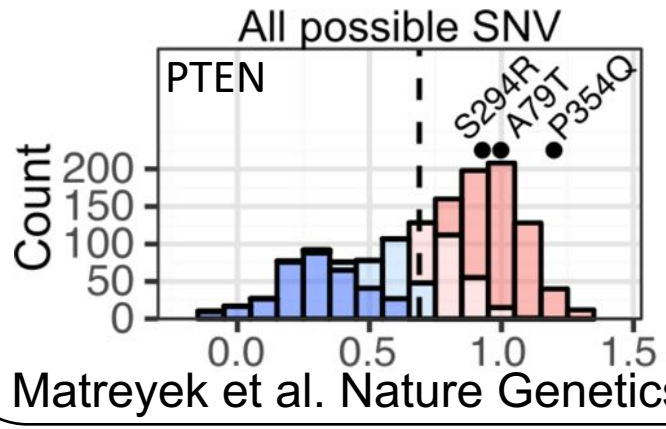
Internally controlled

Continuous, quantitative scores

specificity and sensitivity
can be calculated

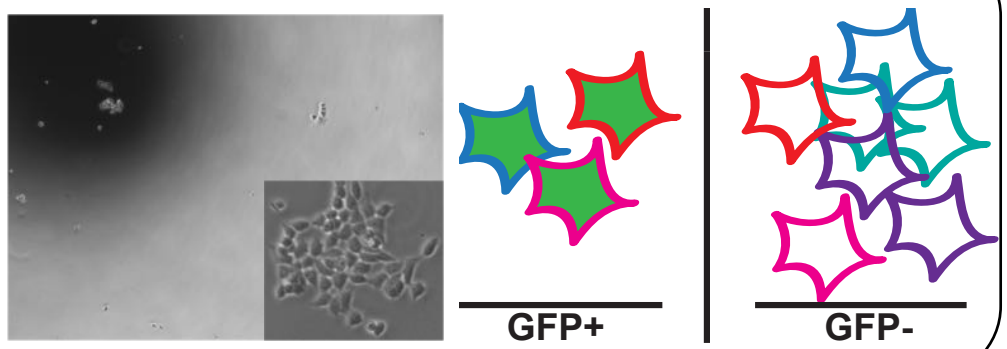


Limitations of multiplexed functional assays



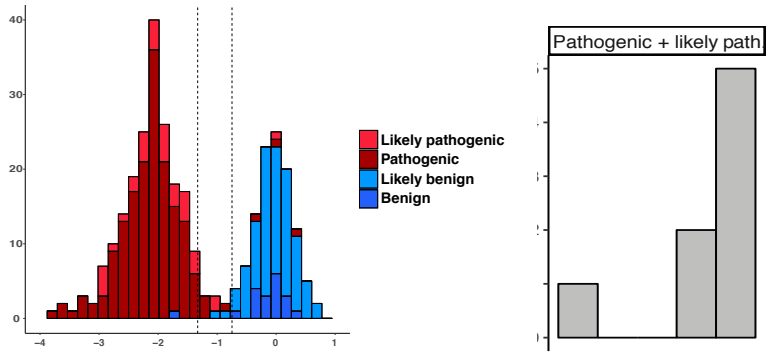
Matreyek et al. Nature Genetics 2018

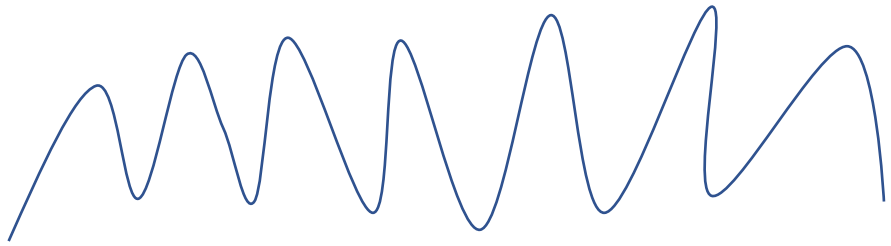
Lack of missense controls



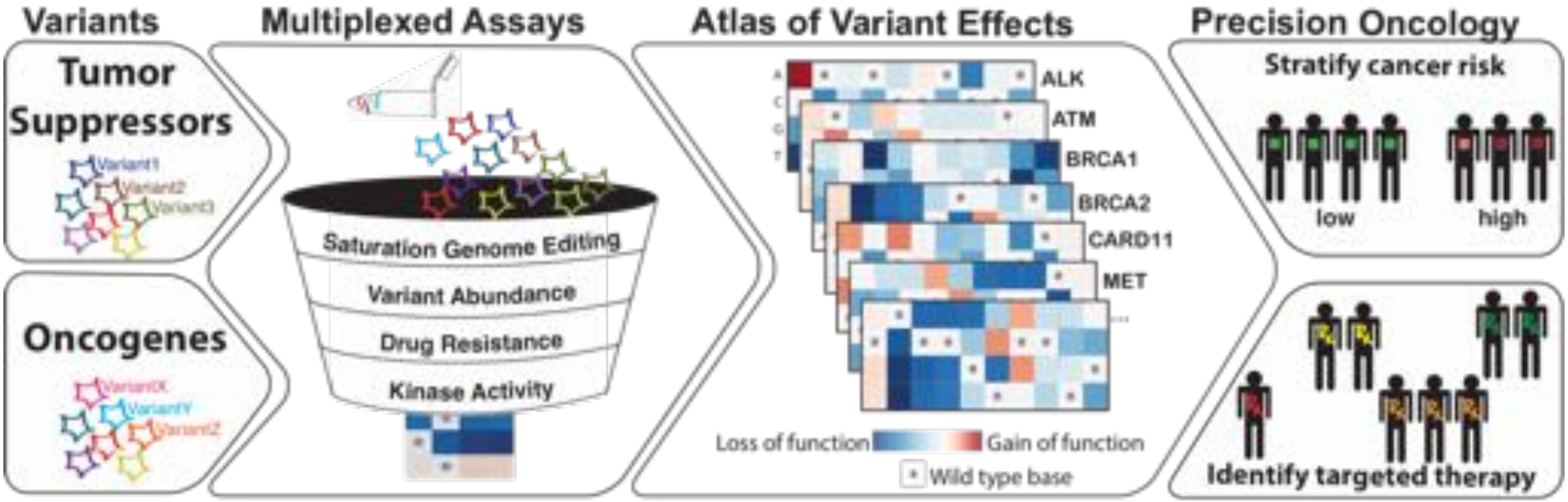
Inference distance from function queried and molecular function underlying disease risk

Differences in predictive value of assays





Data can be noisy

Functional assays to the rescue?

Starita et al. AJHG 2017