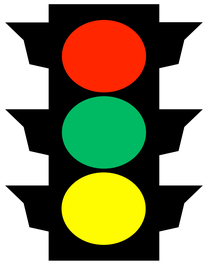

# Stop, go, slow: pathogenic, nonpathogenic, still don't know
# A Traffic Light Reporter Assay for Clinical Interpretation of Variants

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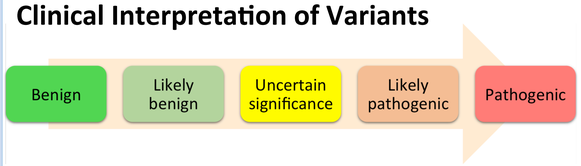
Curriculum in Genetics and Molecular Biology

## Clinical Interpretation of Variants



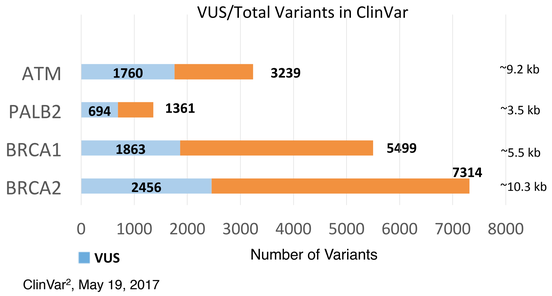

ACMG Variant Interpretation Guidelines[1] consider:

- Population data
- Computational and predictive data
- **Functional data**
- Segregation data
- De novo inheritance
- Allelic data

## The Problem

Our ability to generate data and identify genetic variants on a genome-wide scale has outpaced our ability to accurately interpret these findings, given limited data connecting them to disease. Even within genes that are known to play a role in disease, variants of uncertain significance (VUS) are numerous and complicate patient counseling.

### VUS Burden in Identified Risk Genes




ClinVar[2], May 19, 2017

## Functional Studies and Breast Cancer

Normally, tumor suppressor genes may function in cell cycle regulation, apoptosis, or DNA damage repair. Loss-of-function mutations in these genes contribute to cancer susceptibility.

Approximately 12.4% of women will be diagnosed with breast cancer in their lifetime.[3] About 5-10% of these cases are hereditary. Variants in genes such as *ATM*, *BRCA1*, *BRCA2*, and *PALB2* have been linked to hereditary breast cancer, with 1-3% of women with breast cancer carrying a pathogenic *PALB2* mutation.[4] These genes are all involved in the DNA double strand break repair pathways, providing an opportunity to assay groups of genes by repair pathway.[5]

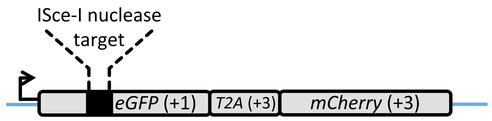
I aim to assess the utility of the fluorescent Traffic Light Reporter (TLR) system for clinical interpretation of genetic variants. I will begin by examining a validation panel of previously studied *BRCA2* variants before examining variants in *PALB2*.

## Hypothesis

The readout of HDR/NHEJ is a physiologically relevant indicator of functionality of these genetic variants.

## Traffic Light Reporter (TLR) Assay

**a** Used lentivirus to transduce TLR construct at low M.O.I. in HEK293T17 cells for a stable 293T/TLR cell line that is non-fluorescent at baseline




**b**

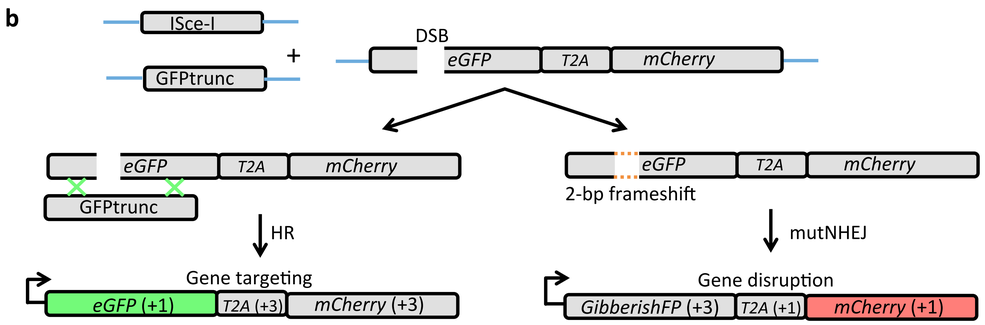
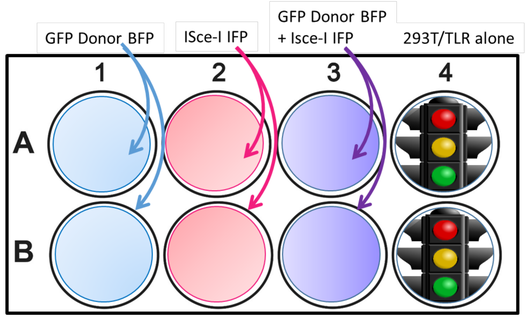


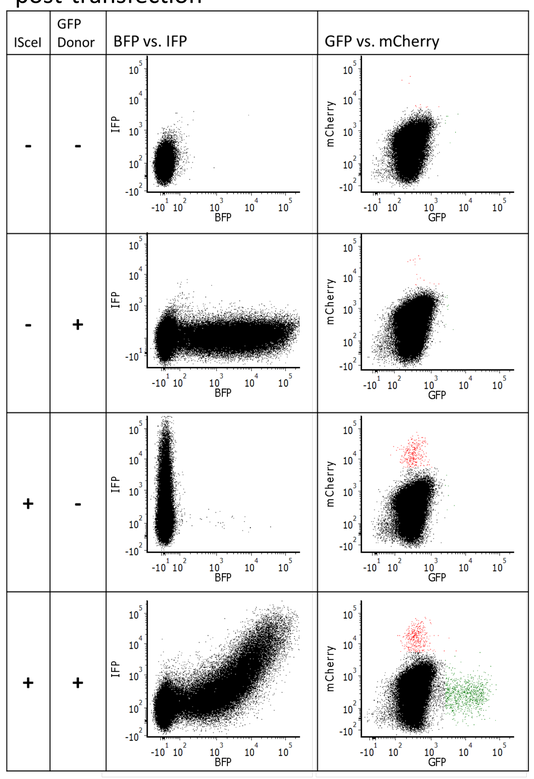

Figure adapted from Certo, et al., Nature Methods (2011)

## TLR Assay Validation and Pilot Experiment

### 1. Transient transfection of stable 293T/TLR cells




### 2. Flow cytometry analysis 72 hours post-transfection




### 3. Endogenous wild-type gene suppression & transient variant expression

I used site-directed mutagenesis PCR to introduce variants in plasmid DNA. These siRNA-resistant plasmids will be transiently transfected into 293T/TLR cells along with siRNA targeting the endogenous wild-type copy of the gene of interest and analyzed by flow cytometry.

| I-SceI | GFP donor | siRNA | Variant | GFP/ mCherry |
|---|---|---|---|---|
| + | + | Non-targeting control | --- | No change |
| + | + | *RAD51* | --- | Decrease |
| + | + | *BRCA2* | WT or non-pathogenic control | No change |
| + | + | *BRCA2* | Pathogenic control | Decrease |

## Anticipated Results

Known pathogenic and nonpathogenic controls will calibrate the assay's dynamic range and permit functional interpretation of unclassified variants.

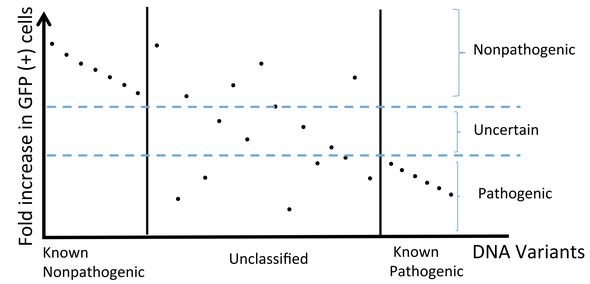



Figure adapted from Guidugli, et al., Cancer Res (2013)

## Validation Variant Panel

The Evidence-based Network for the Interpretation of Germline Mutant Alleles (ENIGMA) has published a panel of *BRCA2* variants for use in validation of functional assays of *BRCA2*.[6]

Batches of these variants will be assessed, with each batch including at least one known pathogenic and one known benign as controls to be run alongside variants of uncertain significance.

## *PALB2* Variant Selection

51% of clinically identified *PALB2* variants are classified as VUS, and of these, 89% are missense.[2] Most truncating variants in *PALB2* are pathogenic and there is new functional evidence of pathogenic missense mutations in *PALB2*.[7]

| Gene | Protein Change | DNA Change | Assay Category | ClinVar Assertion | Source |
|---|---|---|---|---|---|
| PALB2 | L939W | c. 2816 T>G | VUS | Conflicting Interpretations | Park, et al. 2014 |
| PALB2 | T1030I | c. 3089 C>T | VUS | VUS | Park, et al. 2014 |
| PALB2 | L1143P | c. 3428 T>C | VUS | VUS | Park, et al. 2014 |
| PALB2 | L21P | c. 61 T>C, c.62 T>C | VUS | | Zhang, et al. 2009 |
| PALB2 | L24P | c. 70 T>C, c.71 T>C | VUS | | Zhang, et al. 2009 |
| PALB2 | F404L | c.1212 T>A | VUS | VUS | 2 NCGenes |
| PALB2 | A712V | c. 2135 C>T | Benign/LB | Benign/Likely Benign | 2 NCGenes |
| PALB2 | N497Lfs*64 | c. 1490_1490delA | VUS | | 1 NCGenes |
| PALB2 | C77Vfs*100 | c. 229delT | Pathogenic | Pathogenic | Pauty, et al. 2014 |
| PALB2 | L531Cfs*30 | c. 1592delT | Pathogenic | Pathogenic, risk factor | Pauty, et al. 2014 |
| PALB2 | L35P | c. 104 T>C | Pathogenic | | Foo, et al. 2017 |

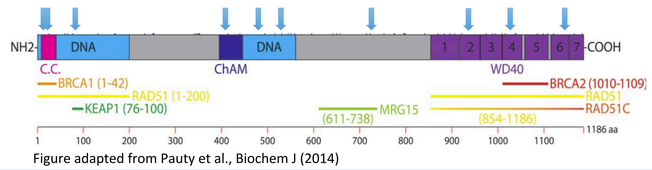



Figure adapted from Pauty et al., Biochem J (2014)

## Acknowledgements and References

We would like to acknowledge funding from the UNC School of Medicine Yang Family Biomedical Scholars Award and NCGenes. Sarah was also supported in part by the UNC MSTP Training Grant 2T32GM008719-6, and a grant from the National Institute of General Medical Sciences under award 5T32 GM007092.

[1] Richards et al. Genetics in Medicine (2015)
[2] https://www.ncbi.nlm.nih.gov/clinvar/
[3] https://seer.cancer.gov/statfacts/html/breast.html
[4] Daly et al., NCCN (2017)
[5] Prakash R. et al. Cold Spring Harbor Perspect Biol (2015)
[6] Guidugli et al., Human Mutation (2013)
[7] Foo et al., Oncogene (2017)