Measuring the molecular functional consequences of very large numbers of human genetic variants

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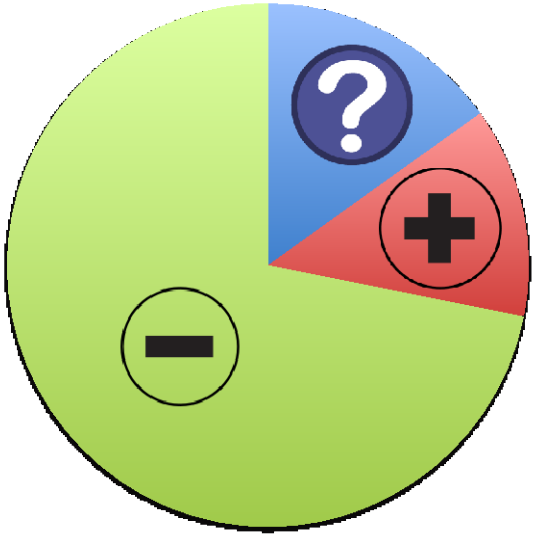
The interpretation of genetic variation is a NHGRI mission-critical challenge

Variants of uncertain significance

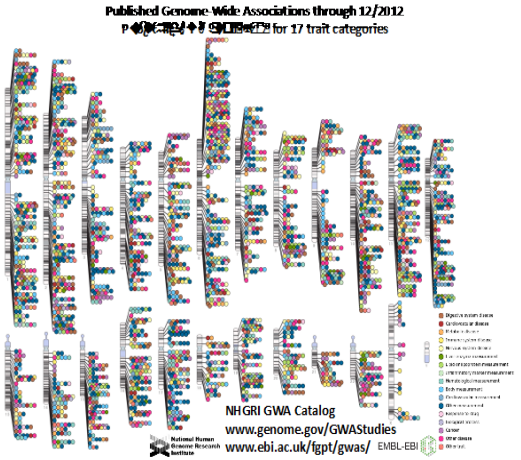
BRCA1&2 → 1M patients

Problem not going away



>1000 genome-wide associations

Small minority for which functional variant(s) known



Computational Prediction?

An Atlas of the Molecular Functional Consequences of Genetic Variation

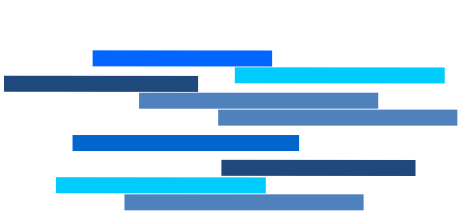
Can we **experimentally** measure the functional consequences of **10 million** human genetic variants?

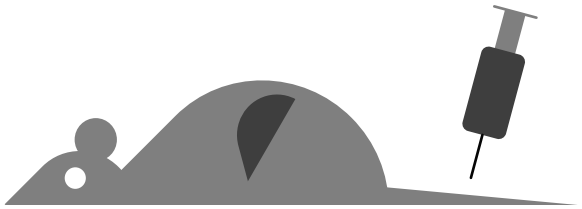
(0.1% of all possible SNVs)

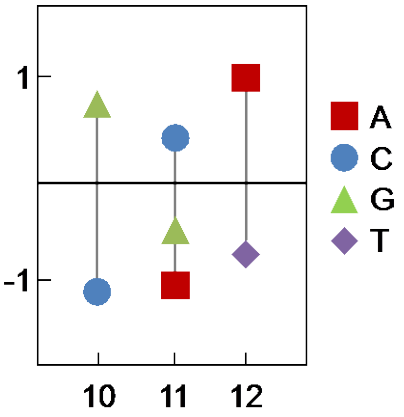
What would we gain?

- **A massive training dataset** for improving algorithms for predicting variant effects
- **Sequence-structure-function maps** for diverse elements (regulatory, protein)
- **The measurements themselves**, for better interpreting Variants of Uncertain Significance and fine-mapping associations

All possible SNVs of an **enhancer**







Doped synthesis & cloning of allelic series of enhancer

↓

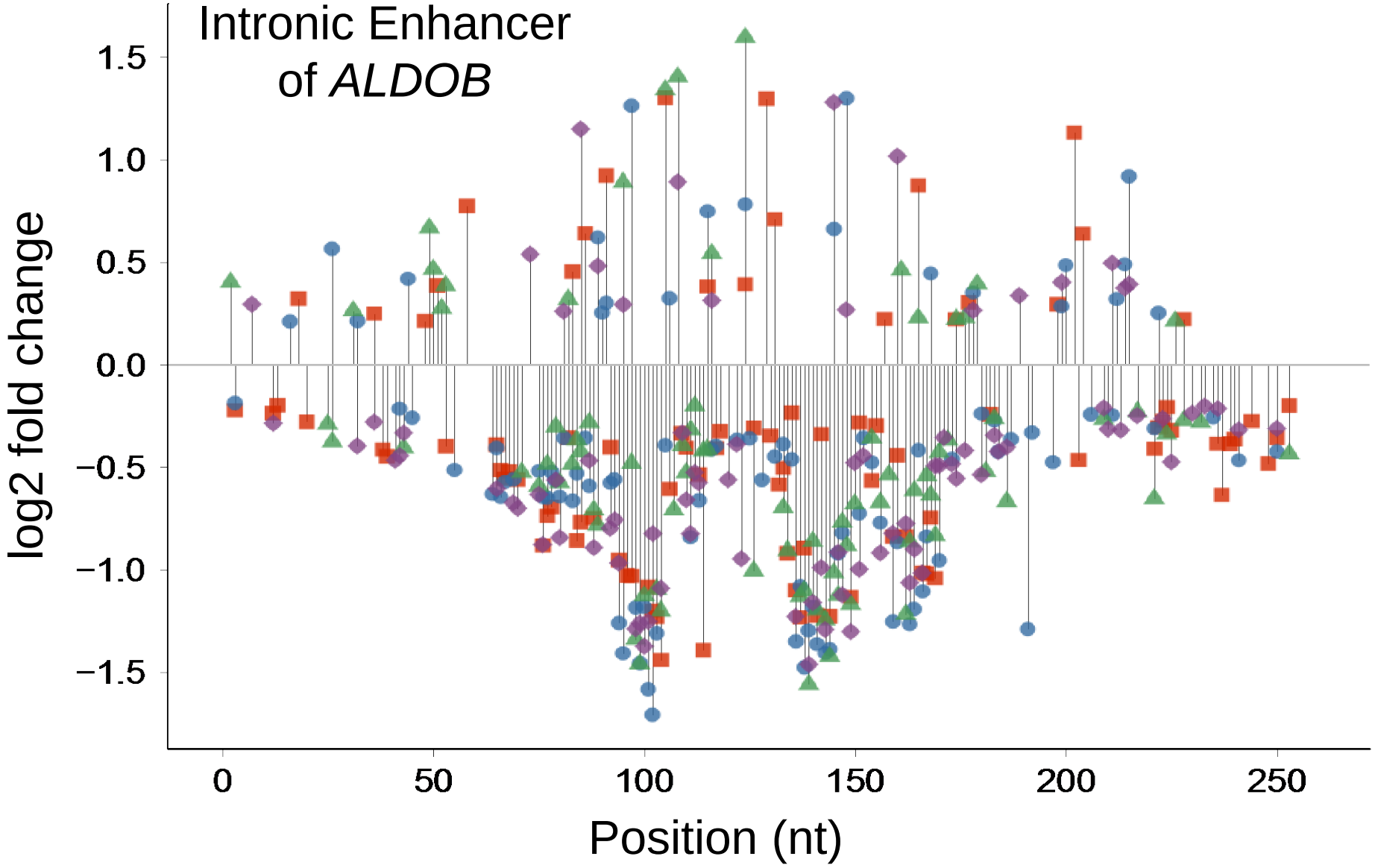
Massively parallel functional assay

↓

Sequence transcribed barcodes & estimate SNV or indel effect sizes

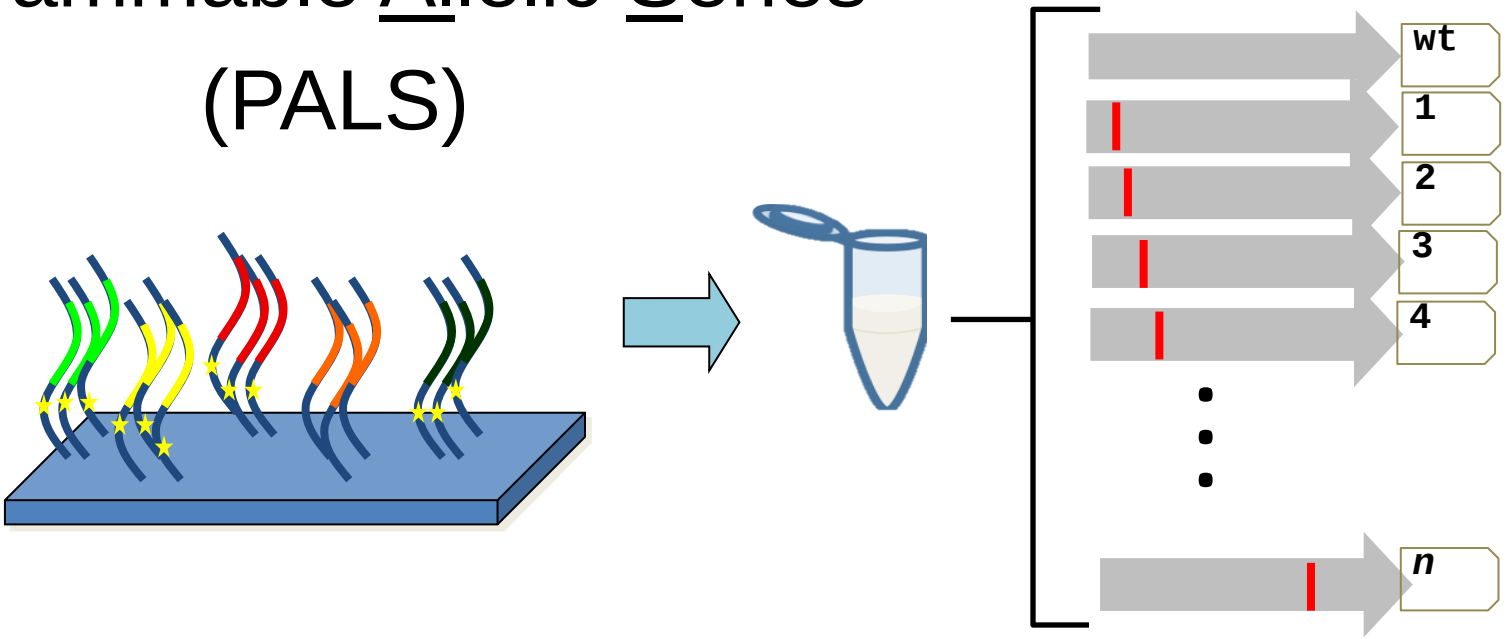
Patwardhan, Hiatt *et al.* Nature Biotechnology (2012)

All possible SNVs of an **enhancer**



All possible codon swaps of a **protein**

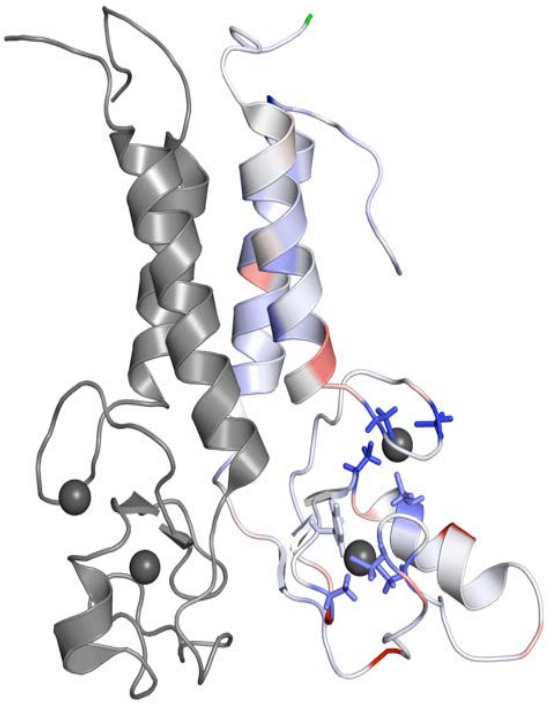
Programmable Allelic Series (PALS)

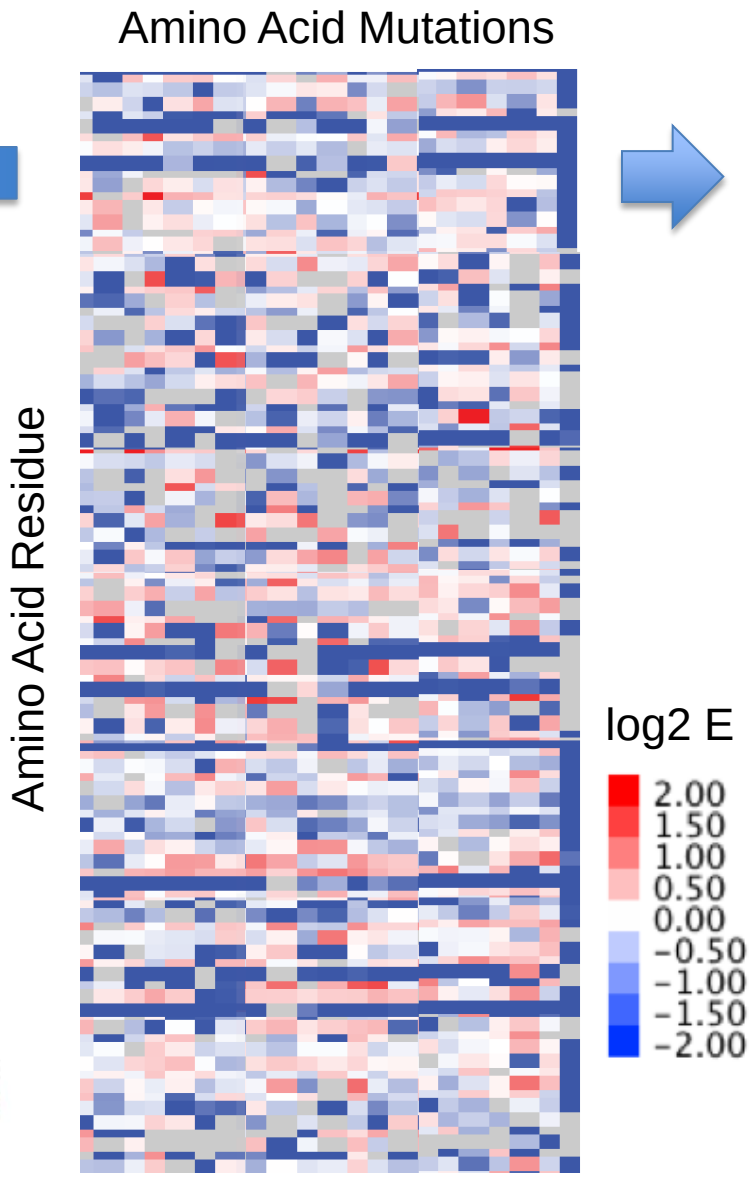


All possible codon substitutions of an ORF in a single, multiplex reaction

Jacob Kitzman

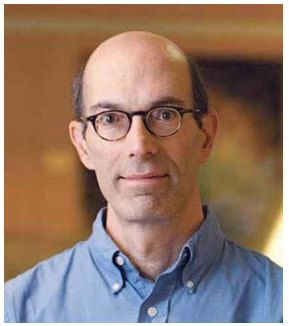
BRCA1 structure-function-pathogenicity





Classification of clinical variants

Lea Starita



Stan Fields

An Atlas of the Molecular Functional Consequences of Genetic Variation

Technologies are rapidly maturing

Rate-limiting factors:

- Allelic Series Production
- Multiplex Functional Assays

Sequencing is the least of the challenges

Which variants?

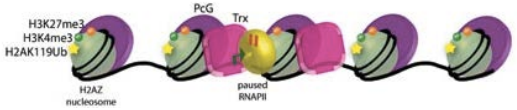
Dense: 2,500 x (1 Kb x 4 mutations) = 1e7

- 500 clinically relevant genes
- 2,000 *cis*-regulatory elements

Sparse: GWAS, eQTL candidates in LD blocks

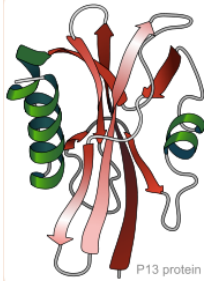
- Better methods are needed
- Much more expensive per variant

Recycle functional assays & allelic series

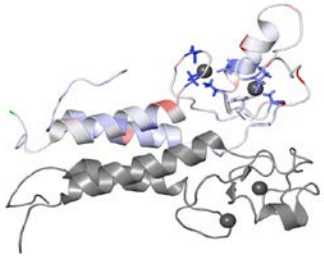


4D x Chromatin

Folding & Stability

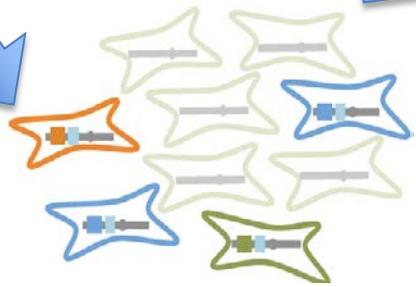


Two Hybrids

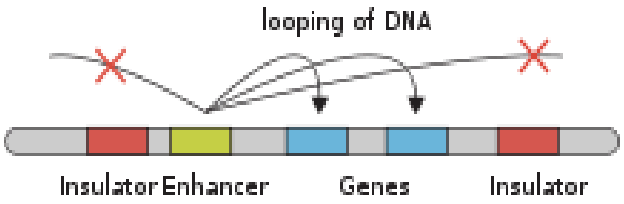


Enhancer Allelic Series

Protein Allelic Series



Cell Type 1
Cell Type 2
Cell Type 3



Transcriptional Activation

Doug Fowler

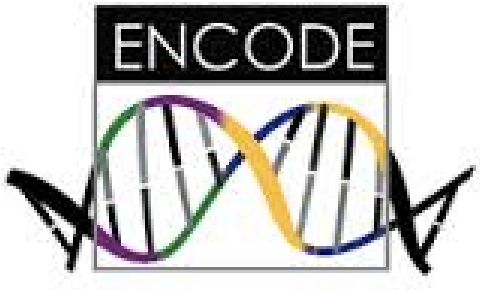
Limitations of Functional Assays

"Context problem" (Botstein & Shortle, Science 1985)

Functional assays ≠ humans

Molecular functionality ≠ pathogenicity

Choice of sequences & contexts informed by:



How would this resource be used?

Rich database of experimental measurements

- Training data for better algorithms
- “Pre-computed” VUS interpretations?

Understanding biology at single-base resolution

- Protein domains, *cis*-regulatory elements
- Learn rules and extrapolate to variants in other members of a functional class

What will success depend on?

- ($15M x 5 yrs) / 10M variants = $30K per 1 Kbp
- Doped oligonucleotide synthesis relatively cheap
- Scaling construction of allelic series
- Multiplex, scalable, recyclable functional assays

- NHGRI: funding, coordination, scaling
- Phase I (100K) → Phase II (1M) → Phase III (10M)
- Technology & assay development throughout