Prioritizing Variants in Personal Genomes:
Using functional impact & recurrence, with particular application to cancer

Mark Gerstein
Yale

Slides freely downloadable from Lectures.GersteinLab.org & “tweetable” (via @MarkGerstein).
No Conflicts for this Talk
See last slide for more info.
Personal Genomics as a Gateway into Biology

Personal genomes will soon become a commonplace part of medical research & eventually treatment (esp. for cancer). They will provide a primary connection for biological science to the general public.
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Keys to genome interpretation

Relating individuals' variants to DBs

**Scaling** DBs to the **population**

Identifying **key variants** - separating into rare, recurrent, common, &c
DB Growth: explosion of data scale & a diversity of uses

- The type of sequence data deposited has changed as well.
  - Protected data represents an increasing fraction of all submitted sequences.

[Muir et al. ('15) GenomeBiol.]
Growth of ICGC datasets

ICGC Data Portal Cumulative Donor Count for Member Projects

Release 22 (August 2016):
- 70 projects
- 19,290 donors total
- 16,236 donors w/ molecular data
In the early 2000's, improvements in Sanger sequencing produced a scaling pattern similar to Moore's law.

The advent of NGS was a shift to a new technology with dramatic decrease in cost.
Moore’s Law: Exponential Scaling of Computer Technology

- Exponential increase in the number of transistors per chip.
- Led to improvements in speed and miniaturization.
- Drove widespread adoption and novel applications of computer technology.

[Waldrop ('15) Nature]
Kryder’s Law and S-curves underlying exponential growth

- Moore’s & Kryder’s Laws
  - As important as the increase in computer speed has been, the ability to store large amounts of information on computers is even more crucial

- Exponential increase seen in Kryder’s law is a superposition of S-curves for different technologies

[Muir et al. ('15) GenomeBiol.]
The changing costs of a sequencing pipeline

From ‘00 to ~’20, cost of DNA sequencing expt. shifts from the actual seq. to sample collection & analysis

[Sboner et al. (‘11), Muir et al. (‘15) Genome Biology]
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Alignment algorithms scaling to keep pace with data generation

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[Sboner et al. (‘11), Muir et al. (‘15) Genome Biology]
Human Genetic Variation

A Cancer Genome

A Typical Genome

Population of 2,504 peoples

Origin of Variants

<table>
<thead>
<tr>
<th></th>
<th>Coding</th>
<th>Non-coding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germ-line</td>
<td>22K</td>
<td>4.1 – 5M</td>
</tr>
<tr>
<td>Somatic</td>
<td>~50</td>
<td>5K</td>
</tr>
</tbody>
</table>

Class of Variants

<table>
<thead>
<tr>
<th>Variant Type</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNP</td>
<td>3.5 – 4.3M</td>
</tr>
<tr>
<td>Indel</td>
<td>550 – 625K</td>
</tr>
<tr>
<td>SV</td>
<td>2.1 – 2.5K (20Mb)</td>
</tr>
<tr>
<td>Total</td>
<td>4.1 – 5M</td>
</tr>
</tbody>
</table>

Prevalence of Variants

<table>
<thead>
<tr>
<th>Variant Type</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNP</td>
<td>84.7M</td>
</tr>
<tr>
<td>Indel</td>
<td>3.6M</td>
</tr>
<tr>
<td>SV</td>
<td>60K</td>
</tr>
<tr>
<td>Total</td>
<td>88.3M</td>
</tr>
</tbody>
</table>

The 1000 Genomes Project Consortium, Nature. 2015. 526:68-74

* Variants with allele frequency < 0.5% are considered as rare variants in 1000 genomes project.
Finding Key Variants

Germline

• **Common variants**
  • Can be most readily associated with phenotype (i.e., disease) via GWAS
  • Usually their functional effect is weaker
  • Many are non-coding
  • Issue of LD in identifying the actual causal variant.

• **Rare variants**
  • Associations are usually underpowered due to low frequencies but often have larger functional impact
  • Can be collapsed in the same element to gain statistical power (burden tests).

Finding Key Variants

Somatic

- **Overall**
  - Often these can be thought of as *very rare variants*

- **Drivers**
  - Driver mutation is a mutation that directly or indirectly confers a selective growth advantage to the cell in which it occurs.
  - A typical tumor contains 2-8 drivers; the remaining mutations are passengers.

- **Passengers**
  - Conceptually, a passenger mutation has no direct or indirect effect on the selective growth advantage of the cell in which it occurred.
Prioritizing Variants in Personal Genomes: Using functional impact & recurrence, with particular application to cancer

- **Introduction**
  - An individual’s disease variants as the public's gateway into genomics & biology
  - **The exponential scaling** of data generation & processing
  - Mining big data to prioritize key variants as cancer drivers

- **Functional impact #1: Coding**
  - **ALoFT**: Annotation of Loss-of-Function Transcripts.
  - LoF annotation as a complex problem + finding deleterious LoFs
  - **Frustration** as a localized metric of SNV impact.
    Differential profiles for oncogenes v. TSGs

- **Functional impact #2: Non-coding**
  - **FunSeq** integrates evidence, with a “surprisal” based weighting scheme
  - Prioritizing rare variants with “sensitive sites” (human conserved)

- **Recurrence**: Statistics for driver identification
  - **Background mutation rate** significantly varies & is correlated with replication timing & TADs
  - Developed a variety of parametric & non-parametric methods taking this into account
  - **LARVA** uses parametric beta-binomial model, explicitly modeling covariates
  - **MOAT** does a variety of non-param. shuffles (annotation, variants, &c). Useful when explicit covariates not available. Slower but speeded up w/ GPUs

- **Recurrence #2**:
  (Low-power) application to pRCC
  - WGS finds additional facts on the canonical driver, MET. Other suggestive non-coding hotspots.
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**Variant Annotation Tool (VAT), developed for 1000G FIG**

VCF Input

Output:
- Annotated VCFs
- Graphical representations of functional impact on transcripts

Access:
- Webserver
- AWS cloud instance
- Source freely available

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vat.gersteinlab.org
Complexities in LOF annotation

Transcript isoforms, distance to stop, functional domains, protein folding, etc.

Balasubramanian S. et al., *Genes Dev.*, ’11
Balasubramanian S.*, Fu Y.* et al., *NComms.*, ’17
Annotation of Loss-of-Function Transcripts (ALoFT)

Runs on top of VAT

Output:

- Impact score: benign or deleterious.
- Decorated VCF.

Balasubramanian S.*, Fu Y.* et al., NComms., ’17
LoF distribution varies as expected by mutation set (from healthy people v from disease)

Balasubramanian S.*, Fu Y.* et al., NComms., '17
ALoFT identifies deleterious somatic LoF variants

**Cancer genes:**
- COSMIC consensus.
- *Enriched in deleterious LoFs.*

**LoF tolerant genes:**
- LoF in the 1KG cohort.
- *Depleted in deleterious LoFs.*

Balasubramanian S.*, Fu Y.* et al., *NComms.*, ’17
ALoFT refines cancer mutation characterization

Vogelstein et al. '13: if >20% of mutations in gene inactivating → tumor suppressor gene (TSG).
ALoFT further refines 20/20 rule predictions.

Balasubramanian S.*, Fu Y.* et al., NComms., '17
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What is localized frustration?

[Ferreiro et al., PNAS ('07)]
Workflow for evaluating localized frustration changes (ΔF)

\[ F_{\text{TYR}} - F_{\text{TRP}} = \Delta F < 0 \]

Model of mutated structure

\[ \langle E \rangle' - E_{\text{TYR}}' = F_{\text{TYR}}' < 0 \]

\[ \langle E \rangle - E_{\text{TYR}} \quad \sigma_E = F_{\text{TYR}} < 0 \]

\[ \langle E \rangle' - E_{\text{TRP}}' \quad \sigma_E = F_{\text{TRP}} > 0 \]
Complexity of the second order frustration calculation

<table>
<thead>
<tr>
<th>Time</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>First order frustration calculation (F)</td>
<td>E_{WT}</td>
</tr>
<tr>
<td>Second order frustration calculation (ΔF)</td>
<td>E_{MUT}</td>
</tr>
<tr>
<td>MD-assisted free energy calculation (ΔG)</td>
<td>Energy</td>
</tr>
</tbody>
</table>

Landscape
Comparing ΔF values across different SNV categories: disease v normal

Normal mutations (1000G) tend to unfavorably frustrate (less frustrated) surface more than core, but for disease mutations (HGMD) no trend & greater changes

[Kumar et al, NAR (2016)]
Comparison between \( \Delta F \) distributions: TSGs v. oncogenes

SNVs in TSGs change frustration more in core than the surface, whereas those associated with oncogenes manifest the opposite pattern. This is consistent with differences in LOF v GOF mechanisms.

[Kumar et al., NAR (2016)]
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Funseq: a flexible framework to determine functional impact & use this to prioritize variants

Annotation (tf binding sites open chromatin, ncRNAs) & Chromatin Dynamics

Conservation (GERP, allele freq.)

Mutational impact (motif breaking, Lof)

Network (centrality position)
Finding "Conserved" Sites in the Human Population:
Negative selection in non-coding elements based on Production ENCODE & 1000G Phase 1

Broad categories of regulatory regions under negative selection
Related to:

Ward & Kellis, Science, 2012
Mu et al, NAR, 2011
Differential selective constraints among specific sub-categories

Sub-categorization possible because of better statistics from 1000G phase 1 v pilot

[Khurana et al., Science (‘13)]
Sub-categorization possible because of better statistics from 1000G phase 1 v pilot

Defining Sensitive non-coding Regions

Start 677 high-resolution non-coding categories; Rank & find those under strongest selection

[Khurana et al., Science ('13)]
SNPs which break TF motifs are under stronger selection

[Khurana et al., Science ('13)]
FunSeq.gersteinlab.org

- Info. theory based method (ie annotation “surprisal”) for weighting consistently many genomic features

- Practical web server

- Submission of variants & pre-computed large data context from uniformly processing large-scale datasets

\[ w_d = 1 + p_d \log_2 p_d + (1 - p_d) \log_2 (1 - p_d) \]

[Fu et al., GenomeBiology (’14)]
Germline pathogenic variants show higher core scores than controls

3 controls with natural polymorphisms (allele frequency >= 1%

1. Matched region: 1kb around HGMD variants
2. Matched TSS: matched for distance to TSS
3. Unmatched: randomly selected

Ritchie et al., Nature Methods, 2014

[Fu et al., GenomeBiology ('14, in revision)]
Flowchart for 1 Prostate Cancer Genome (from Berger et al. '11)

[Khurana et al., Science ('13)]
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Mutation recurrence

Cancer Type 1

Cancer Type 2

Cancer Type 3
Mutation recurrence

Cancer Type 1

Cancer Type 2

Cancer Type 3

Early replicated regions → Late replicated regions
Cancer Type 1

Cancer Type 2

Cancer Type 3

Early replicated regions

Late replicated regions

Noncoding annotations
Noncoding annotations

Cancer Type 1

Cancer Type 2

Cancer Type 3

Early replicated regions

Late replicated regions
Cancer Somatic Mutational Heterogeneity, across cancer types, samples & regions

[Lochovsky et al. NAR (’15)]
Variation in somatic mutations is closely associated with chromatin structure (TADs) & replication timing.
mrTADFinder: Identifying TADs at multiple resolutions by maximizing modularity vs appropriate null

Choose a particular resolution $\gamma$
Optimize $Q$ over all possible partitions

$$Q = \frac{1}{2N} \sum_{i,j} (W_{ij} - \gamma E_{ij}) \delta_{i,j}$$

$\gamma$: resolution parameter

Multiple runs to define boundary scores for all pairs of adjacent bins

Consensus boundaries based on the boundary scores

Consensus TADs

Output

[Yan et al., PLOS Comp. Bio. (‘17)]
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Cancer Somatic Mutation Modeling

**PARAMETRIC MODELS**

**Model 1: Constant Background Mutation Rate (Model from Previous Work)**

\[ x_i \sim Binomial(n_i, p) \]

**Model 2a: Varying Mutation Rate with Single Covariate Correction**

\[ x_i \sim Binomial(n_i, p_i) \]
\[ p_i \sim Beta(\mu|R_i, \sigma|R_i) \]
\[ \mu|R_i, \sigma|R_i : \text{constant within the same covariate rank} \]

**Model 2b: Varying Mutation Rate with Multiple Covariate Correction**

\[ x_i \sim Binomial(n_i, p_i) \]
\[ p_i \sim Beta(\mu|R_i, \sigma|R_i) \]
\[ \mu|R_i, \sigma|R_i : \text{constant within the same covariate rank} \]

**Non-parametric model is useful when covariate data is missing for the studied annotations**

- Also sidesteps issue of properly identifying and modeling every relevant covariate (possibly hundreds)

**NON-PARAMETRIC MODELS**

**Model 3a: Random Permutation of Input Annotations**

Shuffle annotations within local region to assess background mutation rate.

**Model 3b: Random Permutation of Input Variants**

Shuffle variants within local region to assess background mutation rate.

[Lochovsky et al. *Bioinformatics* in press]
MOAT-a: Annotation-based permutation

[Lochovsky et al. Bioinformatics in press]
MOAT-v: Variant-based Permutation

Can preserve tri-nt context in shuffle

\[ \text{bin width } W \approx 2^{d_{\text{max}}} \]

[Lochovsky et al. Bioinformatics in press]
MOAT-s: a variant on MOAT-v

- A somatic variant simulator
  - Given a set of input variants, shuffle to new locations, taking genome structure into account

[Lochovsky et al. Bioinformatics in press]
LARVA Model Comparison

- Comparison of mutation count frequency implied by the binomial model (model 1) and the beta-binomial model (model 2) relative to the empirical distribution
- The beta-binomial distribution is significantly better, especially for accurately modeling the over-dispersion of the empirical distribution

[Lochovsky et al. NAR ('15)]
LARVA Results

TSS LARVA results

Noncoding annotation p-values in sorted order

These have literature-verified cancer associations

[Lochovsky et al. NAR ('15)]
MOAT: recapitulates LARVA with GPU-driven runtime scalability

Computational efficiency of MOAT’s NVIDIA™ CUDA™ version, with respect to the number of permutations, is dramatically enhanced compared to CPU version.

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Documented role with cancer</th>
<th>Pubmed ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLC3A1</td>
<td>Cysteine transporter SLC3A1 promotes breast cancer tumorigenesis</td>
<td>28382174</td>
</tr>
<tr>
<td>ADRA2B</td>
<td>reduce cancer cell proliferation, invasion, and migration</td>
<td>25026350</td>
</tr>
<tr>
<td>SIL1</td>
<td>subtype-specific proteins in breast cancer</td>
<td>23386393</td>
</tr>
<tr>
<td>TCF24</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>AGAP5</td>
<td>significant mutation hotspots in cancer</td>
<td>25261935</td>
</tr>
<tr>
<td>TMPRSS13</td>
<td>Type II transmembrane serine proteases in cancer and viral infections</td>
<td>19581128</td>
</tr>
<tr>
<td>ERO1L</td>
<td>Overexpression of ERO1L is Associated with Poor Prognosis of Gastric Cancer</td>
<td>26987398</td>
</tr>
</tbody>
</table>

MOAT’s high mutation burden elements recapitulate LARVA’s results & published noncoding cancer-associated elements.

<table>
<thead>
<tr>
<th>Number of permutations</th>
<th>Fold speedup of CUDA version</th>
</tr>
</thead>
<tbody>
<tr>
<td>1k</td>
<td>14x</td>
</tr>
<tr>
<td>10k</td>
<td>100x</td>
</tr>
<tr>
<td>100k</td>
<td>256x</td>
</tr>
</tbody>
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[Lochovsky et al. Bioinformatics in press]
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Power, as an issue in driver discovery

Better annotation or large number of samples could help.

[Kumar & Gerstein, Nature (‘17)]
An (underpowered) case study: pRCC

- Kidney cancer lifetime risk of 1.6% & the papillary type (pRCC) counts for ~10% of all cases
- TCGA project sequenced 161 pRCC exomes & classified them into subtypes
  - Yet, cannot pin down the cause for a significant portion of cases....
- 35 WGS of TN pairs, perhaps useful? But not that definitive from a recurrence perspective

• MET is long known pRCC driver
• In MET, TCGA found somatic SNVs, duplications & an alt. splicing event as drivers (43/161).
• In addition, from 35 WGS we found
  – A noncoding hotspot associated with MET
  – Lack of SVs & breakpoints disrupting MET
  – Germline SNP (rs11762213) predicts survival in type 2 patients

[A. Gentile, L. Trusolino and PM. Comoglio, Cancer and Metastasis Reviews ('08); S. Li, B. Shuch and M. Gerstein PLOS Genetics ('17)]
Beyond **MET**: 2 non-coding hotspots in NEAT & ERRFI1, supported by expr. changes & survival analysis

[Li et al. PLOS Genetics (17)]
Tumor Evolution: Highlight the Ordering of Key Mutations

Yates et al, NRG (2012)
Construct evolutionary trees in pRCC

- Infer mutation order and tree structure based on mutation abundance (PhyloWGS, Deshwar et al., 2015)
- Some of the key mutations occur in all the clones while others are just in some parts of the tree

[S. Li, B. Shuch and M. Gerstein PLOS Genetics ('17)]
Mutation distance
Germline

0.5
Populations (%)

[S. Li, B. Shuch and M. Gerstein PLOS Genetics ('17)]
Mutation distance

Germline

Populations (%)

0.5

[S. Li, B. Shuch and M. Gerstein PLOS Genetics ('17)]
Tree topology correlates with molecular subtypes

[Li et al., PLOS Genetics ('17)]

<table>
<thead>
<tr>
<th>Histological type/Patient ID</th>
<th>Type 1</th>
<th>Type 2</th>
<th>Unclassified</th>
</tr>
</thead>
<tbody>
<tr>
<td>COCA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copy number gain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Somatic mutation</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Splicing event</td>
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<td>Germline mutation</td>
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<td>BAP1/PBRM1/SETD2 mut.</td>
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<tr>
<td>CDKN2A copy number loss</td>
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<td>SDHB deletion</td>
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<td>Metastasis</td>
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<td>Promoter mutation</td>
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<td>1-2 intronic mutation</td>
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<td>NEAT1 somatic mutation</td>
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<td>ERRFI1 promoter mutation</td>
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<tr>
<td>Mutation Processes</td>
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<tr>
<td>Whole genome mutation rate</td>
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<tr>
<td>DHS mutation percentage</td>
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<td>SV number</td>
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<tr>
<td>Evolution tree topology</td>
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**Mutation rate/percentage/SV number**

- **High**
- **Medium**
- **Low**

**affected**

[NA, NA]
Mutational processes carry context-specific signatures

$$M = S \times W + \varepsilon$$

CpGs drive inter-patient variation in pRCC mutational spectra

- The loadings on PC1 are mostly \([C>\text{T}]G\)
- Confirmed by higher \(C>\text{T}\)% in CpGs in the hypermethylated group (cluster1)

[S. Li, B. Shuch and M. Gerstein PLOS Genetics ('17)]
Chromatin remodeling defect ("mut") leads to more mutations in open chromatin (raw number & fraction) in those pRCC cases with the mutation. Key mutation affects mutational landscape which, in turn, affects overall burden in pRCC.

[S. Li, B. Shuch and M. Gerstein PLOS Genetics ('17)]
Prioritizing Variants in Personal Genomes: Using functional impact & recurrence, with particular application to cancer

• Introduction
  • An individual’s disease variants as the public’s gateway into genomics & biology
  • The exponential scaling of data generation & processing
  • Mining big data to prioritize key variants as cancer drivers

• Functional impact #1: Coding
  • ALoFT: Annotation of Loss-of-Function Transcripts.
  • LoF annotation as a complex problem + finding deleterious LoFs
  • Frustration as a localized metric of SNV impact.
  • Differential profiles for oncogenes v. TSGs

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  • Prioritizing rare variants with “sensitive sites” (human conserved)

• Recurrence: Statistics for driver identification
  • Background mutation rate significantly varies & is correlated with replication timing & TADs
  • Developed a variety of parametric & non-parametric methods taking this into account
  • LARVA uses parametric beta-binomial model, explicitly modeling covariates
  • MOAT does a variety of non-parm. shuffles (annotation, variants, &c). Useful when explicit covariates not available. Slower but speeded up w/ GPUs

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  • WGS finds additional facts on the canonical driver, MET. Other suggestive non-coding hotspots.
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Acknowledgments!

Also, Hiring: See Jobs.gersteinlab.org

github.com/gersteinlab/Frustration
S Kumar, D Clarke

github.com/gersteinlab/MrTADfinder
KK Yan, S Lou

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L Habegger, S Balasubramanian, DZ Chen, E Khurana, A Sboner, A Harmanci, J Rozowsky, D Clarke, M Snyder

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Y Fu, E Khurana, Z Liu, S Lou, J Bedford, XJ Mu, KY Yip

pRCC - S Li, B Shuch

CostSeq2 - P Muir, S Li, S Lou, D Wang, DJ Spakowicz, L Salichos, J Zhang, GM Weinstock, F Isaacs, J Rozowsky

LARVA.gersteinlab.org
L Lochofsky, J Zhang, Y Fu, E Khurana

MOAT.gersteinlab.org - L Lochofsky, J Zhang
Info about this talk

No Conflicts

Unless explicitly listed here. There are no conflicts of interest relevant to the material in this talk.

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