Phenotype Sequencing

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Collaborators

- Statistical analysis, simulations: Chris Lee (UCLA Bioinformatics, Genomics and Proteomics, Computer Science)
- Sequencing: Stan Nelson, Zugen Chen (UCLA Sequencing Center)
- E. coli mutants, screening: James Liao, Luisa Gronenberg (UCLA Chemical and Biomolecular Engineering)

The Basic Biological Problem

Relating Genotype and Phenotype

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Experiment Design

More generally, how can we design experiments to efficiently and confidently determine such genes given a set of (independently generated) individuals with a particular phenotype?

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- Can take advantage of known gene and mutation databases

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- Open source simulation and computation pipeline
- Easy to extend and combine experimental results

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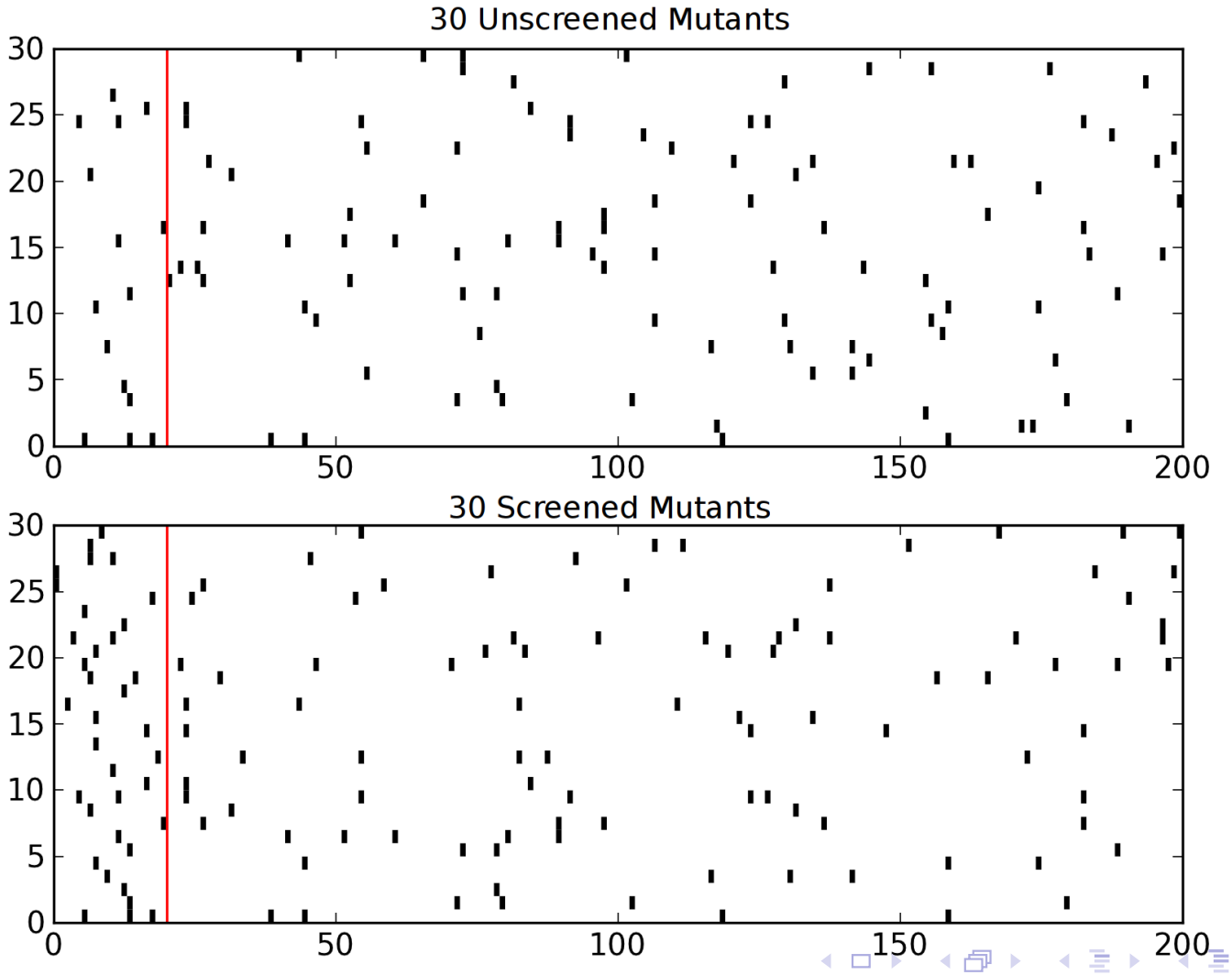
- Lower mean sequencing error $\rightarrow$ more pooling, typically 3-5 genomes into up to 12 tags (depending on genome size)

Effects of Screening

Screening boosts the mutation count signal in target genes.

Simulation: 20 targets in 5000 genes, 30 unscreened genomes and 30 screened genomes.



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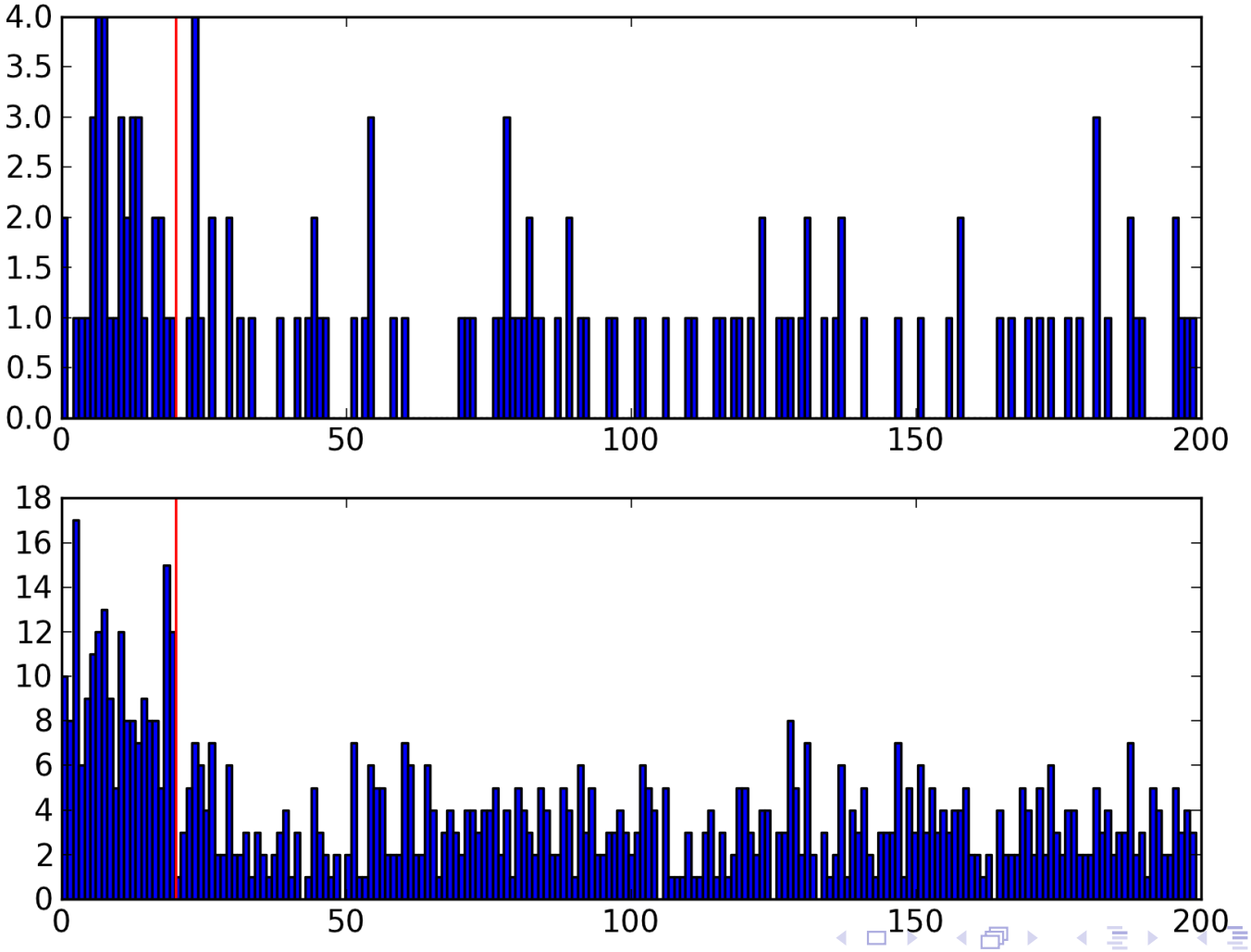

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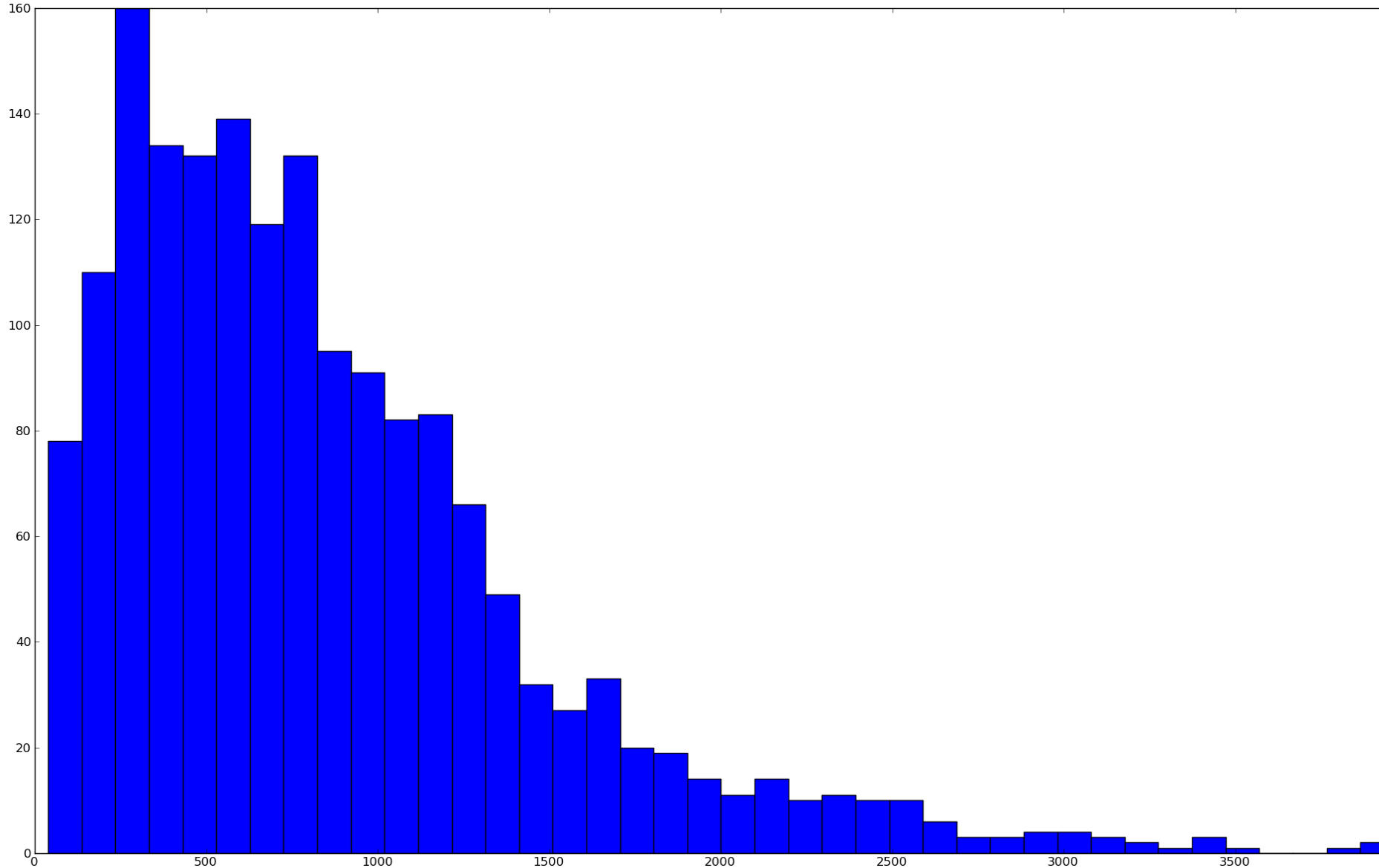
- Filter out synonymous, non-functional mutations (if possible)
- Correct for multiple hypothesis testings

E. coli Gene Length Distribution



Mutagenesis Bias

Mutation Spectra: Comparison

Organism	**Mutagenesis**	**AT → GC**	**GC → AT**	**AT → TA**	**GC → TA**	**AT → CG**	**GC → CG**
E. coli	NTG	2.17%	96.6%	0.07%	0.07%	0.46%	0.61%
T. reesei	UV then NTG	30%	26%	15%	13%	10%	6%
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Scoring

P-values

P-values are computed from a Poisson model for the target size λ and observed mutations k_{obs}, for the null hypothesis that the gene is not a target:

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Multiple Hypothesis Testing: Bonferroni Correction

Finally we apply a Bonferroni correction to the p-values to reduce false positives due to chance in multiple hypothesis tests. In this case that means multiplying the resultant p-values by the total number of genes or pathways being tested.

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Each experiment cost approx \$2400 (\$1200 for sequencer lane + \$1200 in reagents and labor for pooling)

Results – 24 E. coli mutants

Top hits

Gene	p-value
iclR	1.39×10^{-25}
aceK	8.43×10^{-14}
malT	4.81×10^{-4}
malE	0.045
yjbH	0.088

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- EcoCyc pathways and functional groups allow the concentrating of the signal
- Finds many more genes than single-gene level analysis

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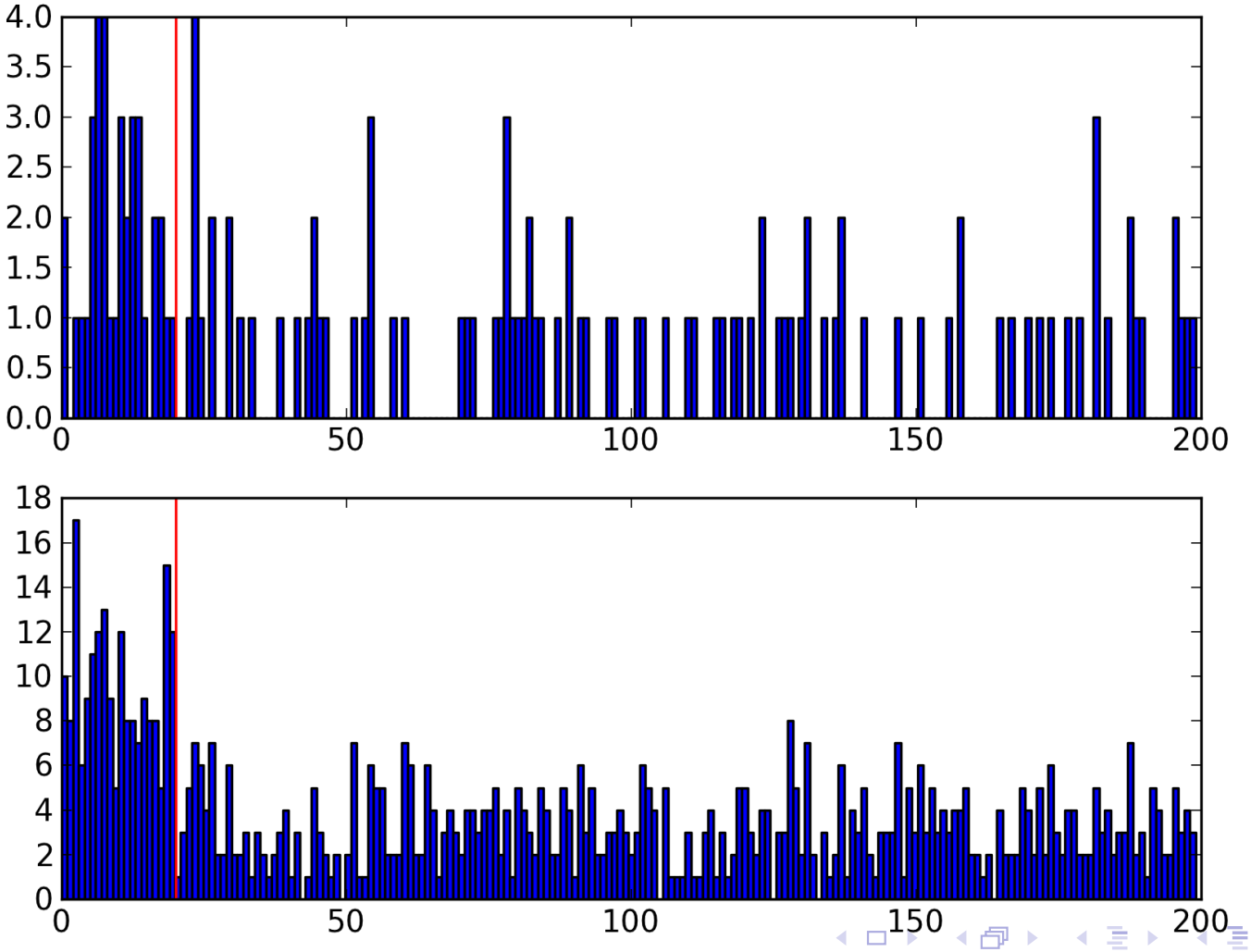
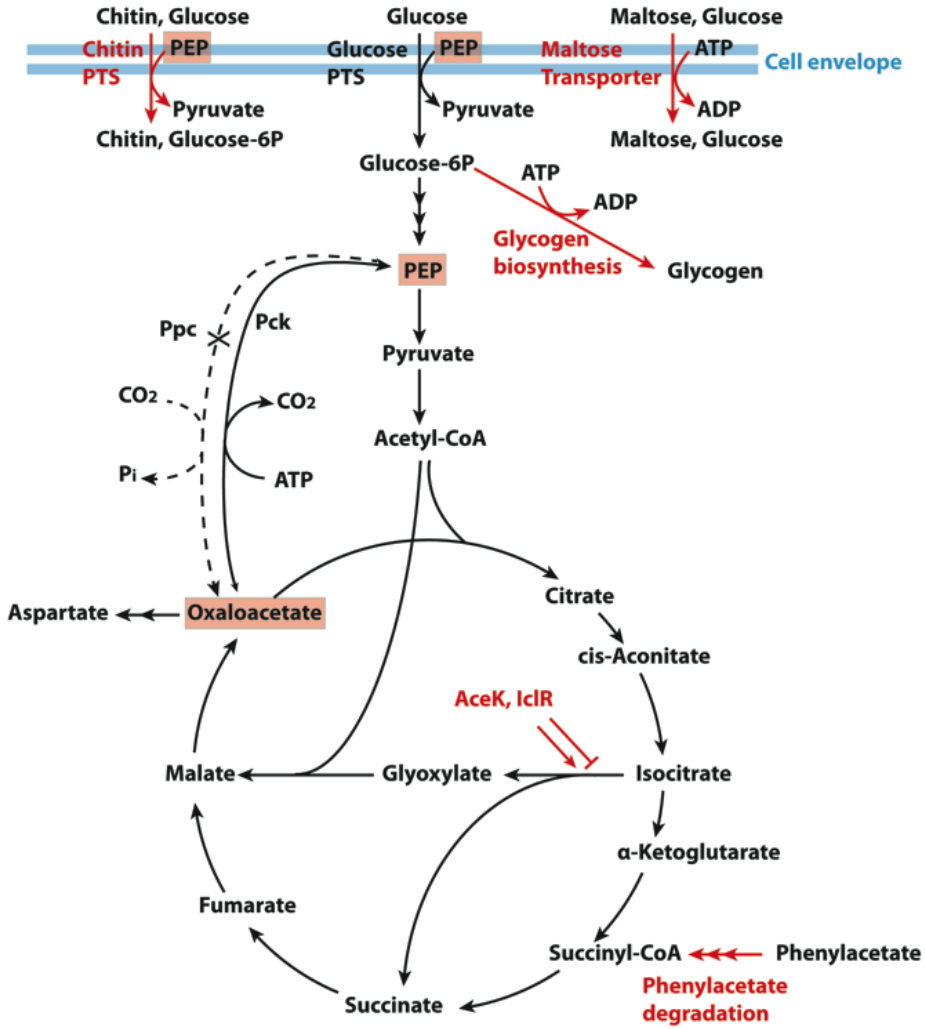


Metabolic Pathways



Results

Table: Top 10 gene groups ranked by pathway-phenoseq p-value (Bonferroni corrected for 536 tests)

Group	Genes	p-value (phenoseq)
PD04099	*aceK iclR*	2.01×10^{-39}
CPLX0-2101	*malE malF malG malK lamB*	2.84×10^{-9}
ABC-16-CPLX	*malF malE malG malK*	7.17×10^{-8}
PD00237	*malS malT*	4.29×10^{-4}
GLYCOGENSYNTH-PWY	*glgA glgB glgC*	4.25×10^{-3}
CPLX-155	*chbA chbB chbC ptsH ptsI*	0.145
PWY0-321	*paaZ paaA paaB paaC paaD paaE paaF paaG paaH paaJ paaK*	0.146
RNAP54-CPLX	*rpoA rpoB rpoC rpoN*	0.53
APORNAP-CPLX	*rpoA rpoB rpoC*	0.62
APORNAP-CPLX	*rpoA rpoB rpoC rpoD*	0.71

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Looking for collaborators for two larger-scale projects.

References

(1) Phenotype Sequencing, PLoS ONE, Feb 2011. Marc Harper, Zugen Chen, Traci Toy, Iara M. P. Machado, Stanley F. Nelson, James C. Liao, Chris Lee (http://www.plosone.org/article/info:doi/10.1371/journal.pone.0016517)
(2) ArXiv: "Comprehensive Discovery of Genes Causing a Phenotype using Phenotype Sequencing and Pathway Analysis", Marc Harper, Luisa Gronenberg, James Liao, Chris Lee

Software

Open source package *phenoseq* available at github: https://github.com/cjlee112/phenoseq

Contact

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