Mapping Toxicity Traits using Diversity Outbred Mice

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HESI Genomics Genetically Diverse Mouse Models
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The Diversity Outbred (DO) mice are a mapping population

Ideal for mapping complex traits because:
  – High genetic diversity,
  – Small recombination blocks,
  – Balanced allele frequencies.

Outbred nature increases variance for other experiments.

Unique genetic makeup of each mouse means that a specific mouse is not reproducible.

However, the overall results of a mapping experiment are reproducible.
DO mice are derived from 8 inbred founders

<table>
<thead>
<tr>
<th>Strain</th>
<th>Letter</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/J</td>
<td>A</td>
<td>Yellow</td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>B</td>
<td>Gray</td>
</tr>
<tr>
<td>129S1/SvImJ</td>
<td>C</td>
<td>Pink</td>
</tr>
<tr>
<td>NOD/ShiLtJ</td>
<td>D</td>
<td>Blue</td>
</tr>
<tr>
<td>NZO/LtJ</td>
<td>E</td>
<td>Green</td>
</tr>
<tr>
<td>CAST/EiJ</td>
<td>F</td>
<td>Red</td>
</tr>
<tr>
<td>PWK/PhJ</td>
<td>G</td>
<td>Orange</td>
</tr>
<tr>
<td>WSB/EiJ</td>
<td>H</td>
<td>Purple</td>
</tr>
</tbody>
</table>
The founder strains contribute high genetic diversity

<table>
<thead>
<tr>
<th>Number of variants</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>SNPs</td>
<td>38,261,117</td>
<td></td>
</tr>
<tr>
<td>Indels</td>
<td>5,376,127</td>
<td></td>
</tr>
<tr>
<td>Struct. Var.</td>
<td>228,286</td>
<td></td>
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</tbody>
</table>

% of protein coding genes with variants in exons or UTRs.

<table>
<thead>
<tr>
<th></th>
<th>SNPs</th>
<th>Indels</th>
<th>Struct. Var.</th>
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</thead>
<tbody>
<tr>
<td>SNPs</td>
<td>94%</td>
<td>66%</td>
<td>12%</td>
</tr>
</tbody>
</table>
DO mice were derived from Collaborative Cross funnels

144 pairs of mice from different inbreeding funnels (between G2:F4 & G2:F12)
DO Mice are maintained in a random mating scheme

175 Breeding Pairs

Generations are bred 4 times per year.

Breeding is expanded to meet demand in each generation.
DO genomes are heterozygous mixture of the 8 founders
Number of recombinations per mouse increases with generation

Currently at Generation 12
Founder allele proportions are (mostly) balanced

A/J
C57BL/6J
129S1/SvImJ
NOD/ShiLtJ
NZO/HILtJ
CAST/EiJ
PWK/PhJ
WSB/EiJ
Steps in a QTL mapping study with DO mice

1. Obtain ~200 – 300 DO mice

2. Genotype mice
   – MEGA MUGA array run by GeneSeek (Lincoln, NE)
   – ~70,000 SNPs ($100/mouse)

3. Phenotype mice
   – Ideally phenotype pre- and post-dose.
   – Post-dose phenotype has worked well.

4. QTL Mapping: DOQTL package in R
Case Study: Benzene Inhalation

National Institute of Environmental Health Sciences (NIEHS)

Study benzene genotoxicity in reticulocytes.

Search for new genes involved in susceptibility or resistance.

*Cyp2e1? Epoxide hydrolase? Phase II enzymes?*
**Study Design**

<table>
<thead>
<tr>
<th>Day 0</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ppm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 ppm</td>
<td>75</td>
<td>75</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>10 ppm</td>
<td>75</td>
<td>75</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>100 ppm</td>
<td>75</td>
<td>75</td>
<td>75</td>
<td>75</td>
</tr>
</tbody>
</table>

- **6 hrs per day**
- **5 days per week**

*Note: The chart indicates the exposure schedule for different concentrations of a substance.*
Benzene Inhalation Endpoints

Pre- and post-exposure **blood**. Bone marrow at necropsy.

MN-RET: micronucleated reticulocytes indicator of DNA damage.
Micronucleated Reticulocytes in Blood

Peripheral Blood, MN-RET/1000

DNA Damage
Micronucleated Reticulocytes in Bone Marrow

Bone Marrow, MN-RET/1000

DNA Damage
QTL mapping model estimates founder effects

Map with 150 samples from 100 ppm dose.

\[ y_{ij} = \sum_{j=1}^{8} \beta_j g_{ij} + \lambda + \epsilon \]

- \( y_{ij} \) = phenotype for sample \( i \) at SNP \( j \).
- \( \beta_j \) = founder allele effect at SNP \( j \).
- \( g_{ij} \) = genotype for sample \( i \) at SNP \( j \).
- \( \lambda \) = correction for population structure.

R packages: DOQTL and QTLRel
Blood MN-RET has a QTL on Chr 10

Post-Exposure Blood, MN-RET/1000

Ephx1  Ephx4  Cyp2e1  Ephx2  Ephx3

LOD

0.05
Bone Marrow MN-RET has a QTL on Chr 10

Bone Marrow, MN-RET/1000

LOD

0.05
CAST/EiJ allele is resistant
CAST/EiJ allele is dominant
Candidate genes in the QTL interval

SNPs from: [http://www.sanger.ac.uk/cgi-bin/modelorgs/mousegenomes/snps.pl](http://www.sanger.ac.uk/cgi-bin/modelorgs/mousegenomes/snps.pl)


Sult3a1 has high expression in liver

Sult3a1 Expression, Liver, Males only

Log2(expression)

129S1/SvImJ  A/J  C57BL/6J  CAST/EiJ  NOD/ShiLtJ  NZO/HILtJ  PWK/PhJ  WSB/EiJ
Sult3a1 expression in liver has a co-located QTL on Chr 10
Sult3a1 founder effect pattern is the same as MN-RET
CAST/EiJ haplotype blocks can be used to narrow the QTL

Sult3a1
Future work

No direct ortholog of *Sult3a1* in humans, but its active catalytic site accepts phenol, napthylamine, and paranitrophenol.

Investigation of the homology of the *Sult3a1* binding site and substrate affinity with phenol human SULTs in progress.

Sulfate conjugates of benzene are observed in urine.

Differential mRNA expression liver and bone marrow in progress.

Collaborative Cross mice and targeted knockouts will aid reproduction of phenotype and results.
Summary

Diversity Outbred mice are derived from 8 inbred founders.

DO mice have high genetic diversity, fine recombination block structure and balanced allele frequencies, making them ideal for mapping.

DO mice can be used to find genes underlying toxicity.

Data from the founders can help inform results.

CC mice may be useful for validation and follow up.
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