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Mouse Biology Program

UC Davis Haploessentiality Pilot

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Background

- 3,000 human genes cannot tolerate the loss of one of the two alleles.
- These genes are over-represented in variants identified by developmental diseases.
- It is hypothesized that these failed lines are orthologues of human dominant LOF genes.
- To address this question a pilot project was awarded to the major KOMP centers (BasH, Jax, DTCC(including UCD).
- The goal of this pilot project (UCD) is to identify a subset of these failed lines for further phenotypic characterization.
- **We evaluated three endpoints: 1. Blastocyst culture 2. In vivo development 3. Strain background**

Methods

- We identified thirty candidate haploessential genes.
- Genes were edited in C57/BL6N by Cas9 RNP at the zygotic stage by electroporation.
- preimplantation lethality of these candidate genes was assessed *in vitro* by culturing to blastocyst.
  - Each embryo culture batch also had a sham control group that was also electroporated with Cas9 absent of a gRNA.
  - Zygotes were then culture to e4.5 and genotyped.
- post-implantation: A subset (n= 15) of genes with viable blastocysts were evaluated for viability and morphology by collecting embryos at day 9.5 of development following engineering as described above.
  - Yolk sacs were harvested for genotyping.
- inbred genetic backgrounds: To screen for modifiers of lethality, 8 genes (of 15) were re-electroporated onto embryos from FVB/NJ, 129S1/SvImJ, and DBA/2J and collected at e9.5.

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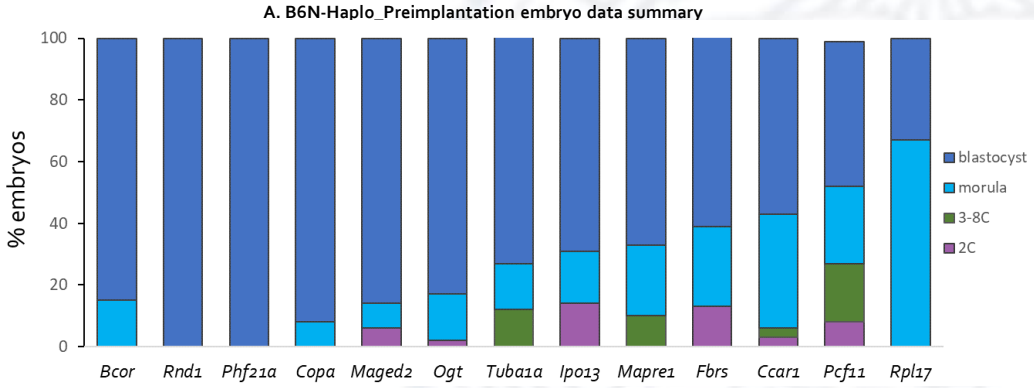
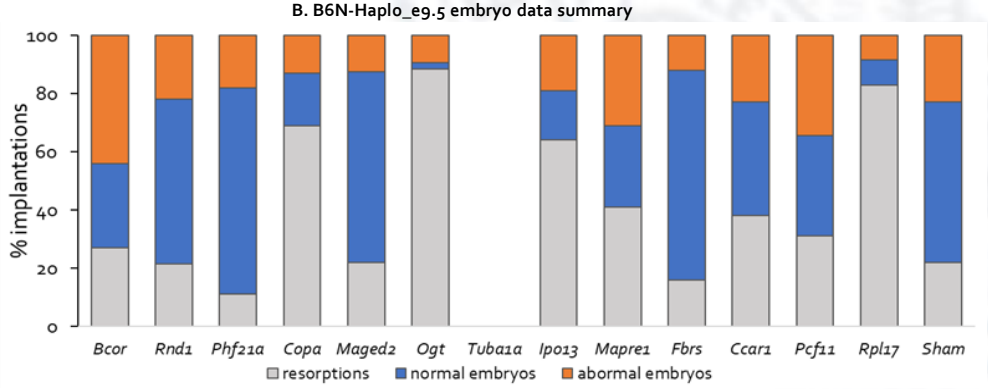
Comparing the preimplantation and postimplantation embryo development without genotype





Bar graphs of (A) preimplantation embryo data. Different colors within bars are yields of 2- cell, 3-8-cells, morula and blastocysts (expressed as % total embryos) recovered after 4.5 days of culture. (B) 9.5 embryo data. Different colors are resorption, normal and abnormal embryos (expressed as % implantations).

Irrespective of genotype, there was a strong concordance between preimplantation and postimplantation viability: lines that yielded a large proportion of viable blastocysts resulted in a greater proportion of e9.5 live embryos and lines with poor preimplantation viability tended to have high resorption rates at e9.5. There were some exceptions (*Copa, Ogt, Ipo13*,and *Tuba1a*). There we no embryos recovered for *Tuba1a*.

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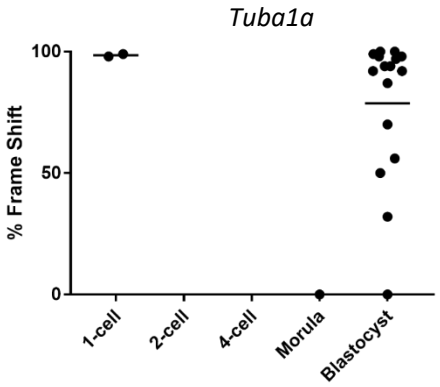
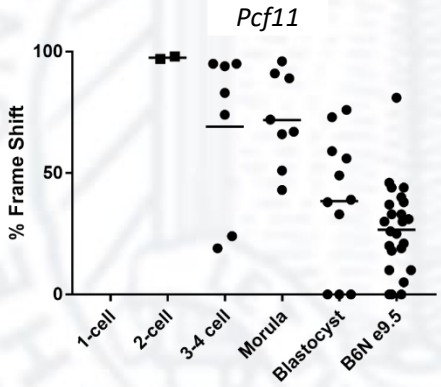
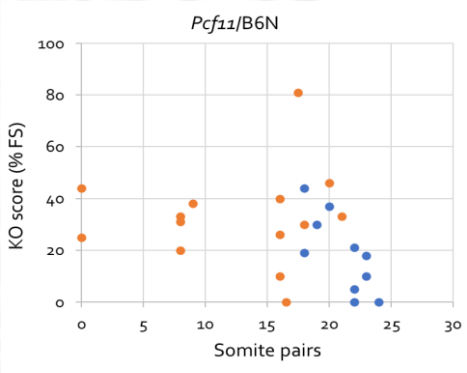
Comparing the preimplantation and postimplantation embryo development w. genotype







Pcf11	KO score Median	KO score Mean ± SD	Somites Mean ± SD
Normal embryos	18	18 ± 15	21 ± 2.2
Abnormal embryos	33	33 ± 19	13 ± 6.2

Representative examples of discordant and concordant findings between *in vitro* culture and e9.5 collection. *Tuba1a* presented normal blastocyst culture and a high frameshift mutation rate. However, we could not recover any viable embryos at e9.5 across all four genetic backgrounds. Examination of the uterus suggests severe inflammatory response. In contrast *Pcf11*, demonstrated a correlation between developmental arrest and frameshift mutation rates both in *in vitro* testing and at e9.5. Further, the %Frameshift correlated to abnormal somite pair counts with a trend toward higher KO scores in embryos with abnormal development at e9.5.

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Summary of e9.5 development of haploessential genes (B6N)

Gene	Embryo somite pairs (mean ± SD)			Abnormal embryos (% total live embryos)		
	0%FS[a]	1-49%FS	>50%FS	0%FS	1-49%FS	>50%FS
Orc6	20 ± 6.2	15 ± 9.7	--	27	47	--
Exoc2	22 ± 4	21.6 ± 6.3	17 ± 0	29	27	100
Bcor	19 ± 7.1	19.5 ± 6.2	10.6 ± 8	38	46	93
Ccar1	21.5 ± 2.1	21.6 ± 2.4	12.2 ± 4.2	0	29	100
Copa	9.6 ± 7.3	18 ± 9.2	0 ± 0	75	25	100
Fbrs	23 ± 4	23.5 ± 2	23 ± 1.4	22	7	0
Ipo13	17 ± 3.6	20 ± 5.3	17.5 ± 0.7	71	42	50
Maged2	17.4 ± 4.8	19 ± 6	22 ± 2.6	33	33	0
Mapre1	26	21 ± 4	20 ± 2.7	0	38	58
Ogt	12 ± 4	--	12 ± 3.8	50	0	100
Pcf11	21 ± 3.9	15 ± 7.2	17.5 ± 0	0	45	100
Phf21a	16 ± 0	20 ± 3.0	20 ± 8.1	0	20	22
Rnd1	15 ± 7.7	22 ± 3.9	19 ± 3.9	50	12.5	40
Rpl17	21 ± 5.6	--	--	50	--	--
Tuba1a	--	--	--	--	--	--

Data show limited association between editing efficiency (grouped into no editing (0%FS), moderate editing (1-49%FS), and extensive editing (>50%FS) and embryonic viability and somite stage. In contrast, e9.5 embryos with greater than 50% frameshift editing tended to be abnormal for most genes examined.

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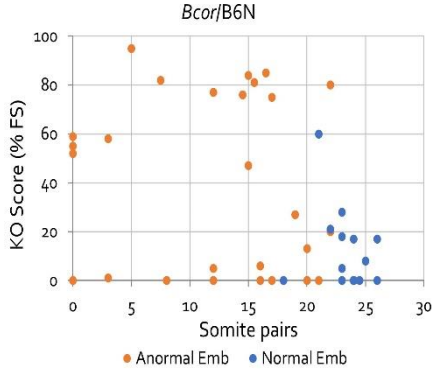
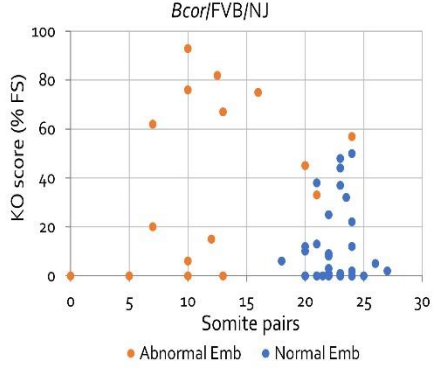
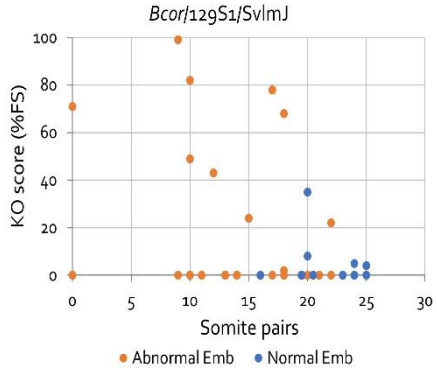
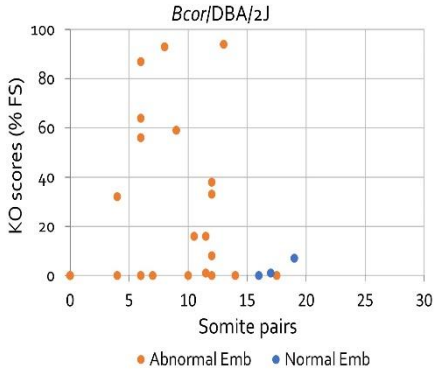
Genetic background may influence e9.5 phenotype









In *Bcor* frameshift mutants, plots show the association between frameshift mutations (%FS), developmental stage (somite pairs) and morphology (normal vs abnormal) electroporated into 4 different genetic backgrounds. *Bcor* normally developing embryos reached ~22 somite pairs using B6N, FVB and 129S1 backgrounds and ~15 somites on the DBA background. Developmental delay was also present in sham-control DBA embryos (data not shown). Independent of backgrounds, normally developing embryos tended to show lower frameshift mutation rates than abnormal embryos. However, for abnormal embryos the association between frameshift editing and development is a more complex relationship but does not appear to vary by genetic background. Dots are individual viable embryos.

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Conclusions:

The goal of this pilot program was to study the feasibility of analyzing haploessentiality in a high throughput pipeline.

- In vitro preimplantation embryo culture and phenotype evaluation at e9.5 are informative for high throughput evaluation of haploessential genes and produced a number of novel findings.

However, the following limitations and challenges need to be acknowledged and addressed if we are to apply this system to a high throughput pipeline:

- Genotyping – limited genetic material leads to inability to read some samples and early arrest lead to poorer genotyping outcomes. This impacts statistical power and leads to analysis bias.
- Mosaicism – different transcriptional patterns in founder embryos likely affected phenotyping outcomes.
- More studies are needed to determine the relationship between strain background, abnormal morphology, and frameshift mutation rates.

Larger sample sizes are needed to address the above limitations.