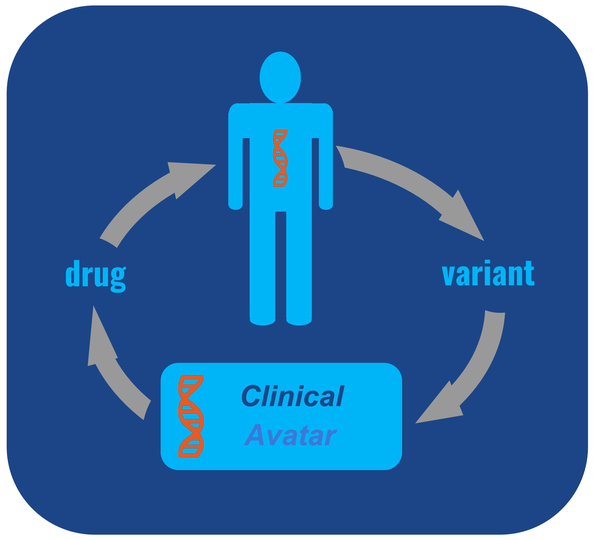
Humanized Animal Models for Detecting Pathogenicity, Interrogating MOA, and Enabling Targeted Drug Screening in Clinical Variants

Christopher Hopkins[1]* Kathryn McCormick[1] Trisha Brock[1] Bethan Jones[1] Gongping He[1]
Matthew Bainbridge[2] Adela Chicas Cruz[1] and Christine Kim[1]
[1] Nemametrix, 44 W 7th Ave, Eugene, OR 97401 *chris.hopkins@nemametrix.com
[2] Rady Children's Institute of Genomic Medicine, 3020 Children's Way, San Diego, CA 92123

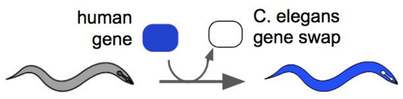
Synopsis: Genetic diagnosis of disease has opened the door into a **new era of personalized medicine**. Clinicians and geneticists are now engaged in trying to understand huge numbers of natural genetic variation through functional analysis. CRISPR technologies allow customization of animal models for probing variant biology. Using the power of gene replacement, Zebrafish and C. elegans are humanized into platforms for disease biology discovery.

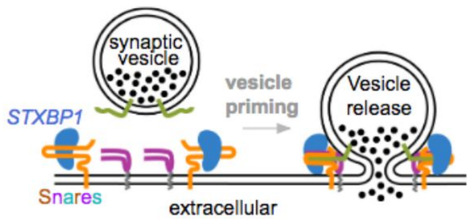
Clinical Avatar Platform



Fast and Precise System
Humanized animal model with clinical variant installed. Clinicians can use the platform to define gene function and uncover therapeutic approach.

STXBP1 HUMANIZATION





Objective: Replace unc-18 locus with human STXBP1 DNA.

Method: Sequence optimized cDNA inserted at start codon of unc-18. All endogenous coding is removed and human transgene uses native 3' UTR.

Result: Rescue is achieved in three phenotyping assays.

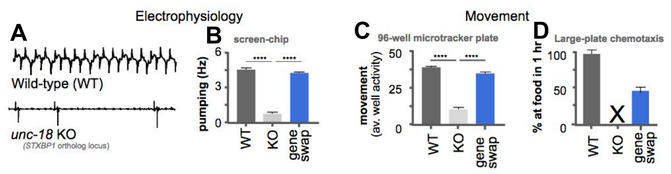
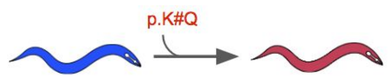


Figure 1. Rescue of function for *hSTXBP1* in *unc-18* locus. A) Example of pharynx pumping trace acquired by Ephys ScreenChip. B) Comparison of pumping frequency assay between N2 wildtype (WT), unc-18 knock-out (KO), and *hSTXBP1* gene insertion (gene swap). C) Movement assay measuring thrashing in liquid. D) Movement assay measuring chemotaxis to food source. **** p<0.0001

Conclusions: Conservation of biology occurs because human gene rescues. Sensitivity of rescue depends on type of assay used.

STXBP1 VARIANT INSTALL



Objective: Replace unc-18 locus with human STXBP1 DNA

Method: 3 pathogenic variants (R292H, R406H and R388X) are installed into STXBP1 humananized backbone. Variants are screened by 3 types of functional assays

Result: Installation of pathogenic variants in humanized locus leads to phenotypic defects.

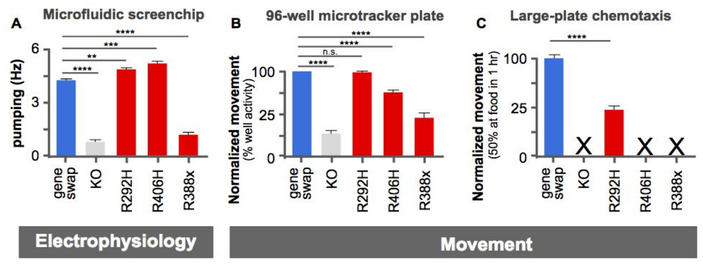
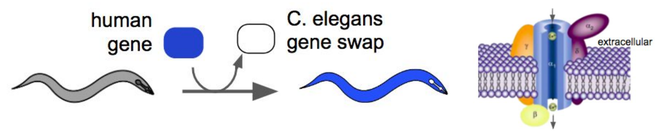


Figure 2. Change of function for pathogenic variants R292H, R406H and R388X installed in humanized *hSTXBP1* locus. A) Ephys ScreenChip assay. B) Microtracker thrashing assay. C) Chemotaxis assay. **** p<0.0001

Conclusions: Variant installation in humanized locus gives detectable phenotypes. Platform valid for probing disease-related variant biology in STXBP1 disorders.

CACNB4 HUMANIZATION



Objective: Replace unc-18 locus with human CACNB4 cDNA

Method: Sequence-optimized cDNA inserted at start codon. All endogenous coding is removed and human transgene uses eft-3 UTR

Result: Rescue achieved in ephys phenotyping assays

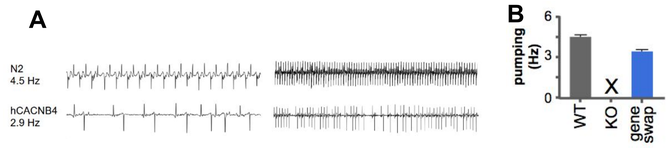
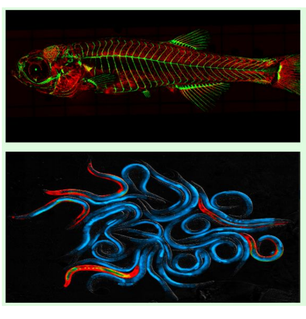


Figure 4. Electrophysiology. A) Example traces for N2, hKCNQ2 knock-in. B) Rescue of function for *hCACNB4* in *ccb-1* locus as measured by Ephys Screenchip measure of pharynx pumping frequency. ***** p>0.00005

Conclusions: Platform data suggest conservation of variant biology occurs and can be used to probe disease-related variant biology in CACNB4 disorders.



D. rerio zebrafish

C.elegans nematode

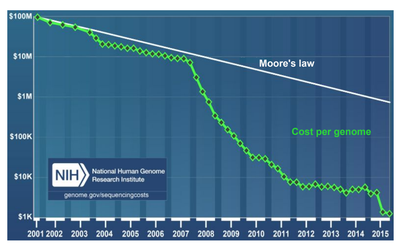
Planned Neurological Genes

ATP1A3, SCN1A, CACNA1A, CDKL5, MAPT, TARDBP, GRN, PSEN1, APP, LMNA, POLG, and others

Gene mutation in patient

benign or pathogenic?

Price per genome



Share of variant observations