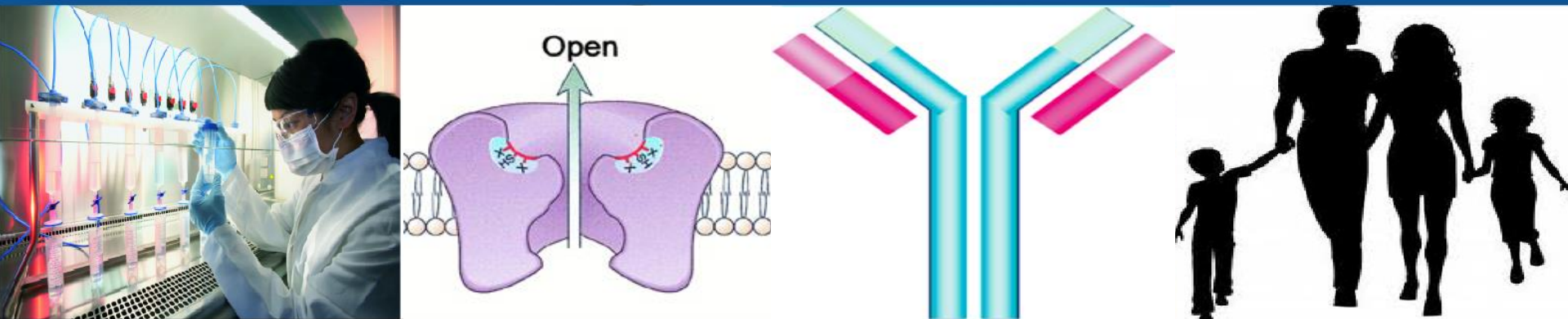


Tetragenetics



SionX™: A High-Yield Strategy for the Production of Recombinant Human Ion Channels for Antibody and Small Molecule Drug Discovery

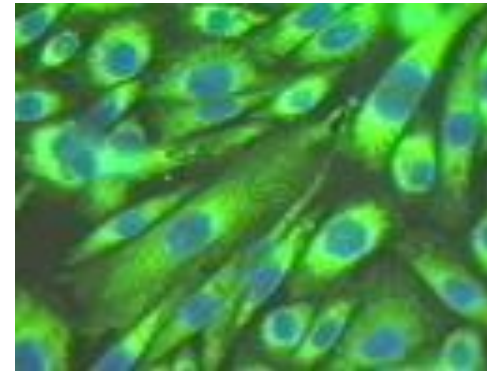
Aurora Ion Channel Retreat 6/25/14

Technology Overview: *TETRAHYMENA*

Microbial cells



Animal cells



Combining
both worlds

- Rapid, high cell density growth
- Inexpensive media
- Facile genetics
- Easily frozen for long-term storage



- Sophisticated eukaryote
- Mammalian-like PTMs
- No cell wall
- Enhanced features for production of **membrane** and **secreted** proteins

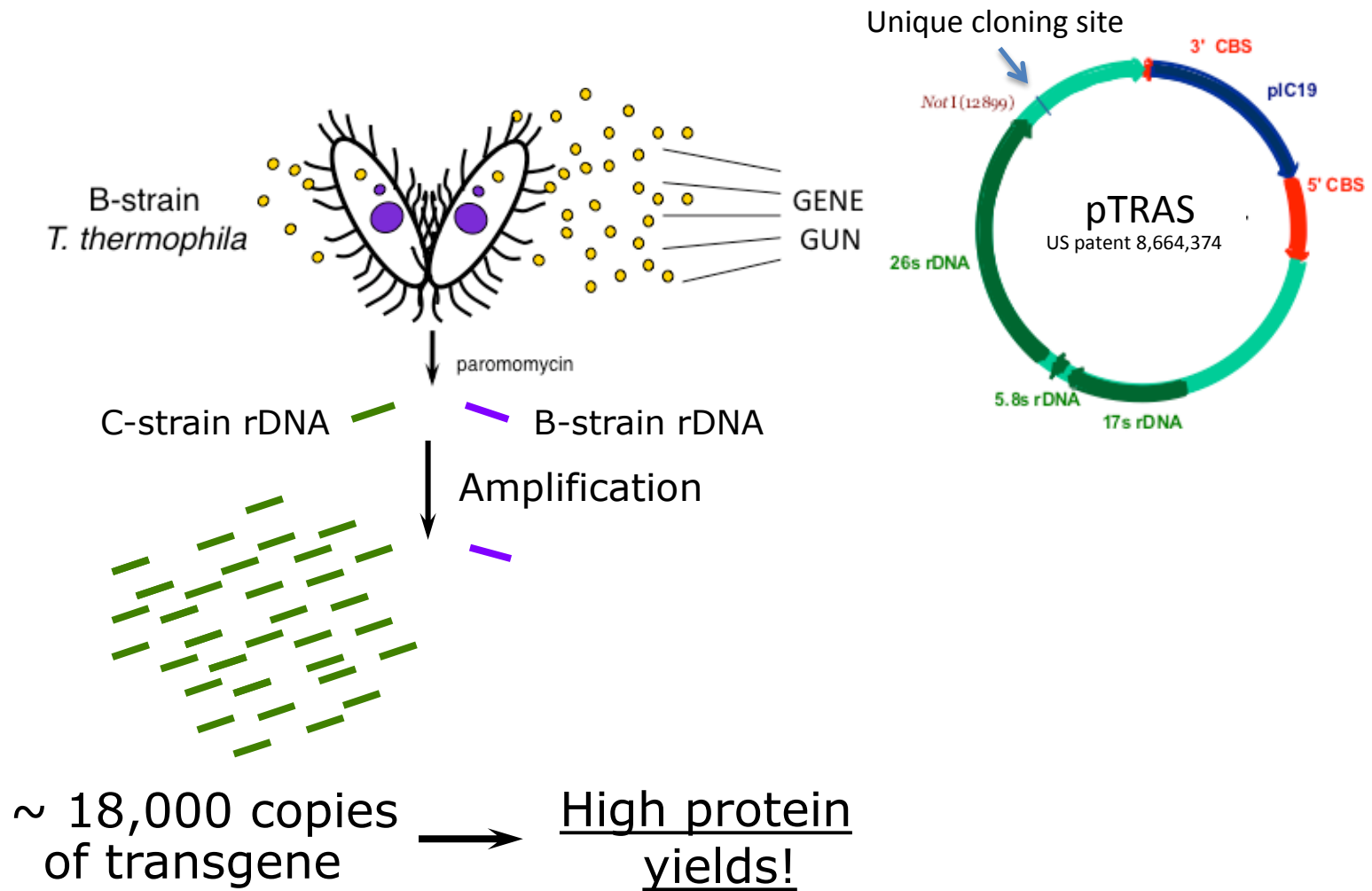
Tetrahymena thermophila as an Expression Host

- Common pond water ciliate
- First “animal-like” cell to be grown in pure culture
- Important model for cell biology & genetics for >50 years
 - Dynein as first microtubule-based motor
 - Self-splicing RNA (Nobel Prize – Cech)
 - Telomere structure and biosynthesis (Nobel Prize – Blackburn & Greider)
 - Elucidation of the histone code (Canada Gairdner Award - Allis)
 - Role of small RNAs in chromatin dynamics and DNA rearrangement (Gorovsky; Allis)
 - Large well-annotated genome (~24,000 genes)

Unique Features for Enhanced Production of Membrane and Secreted Proteins

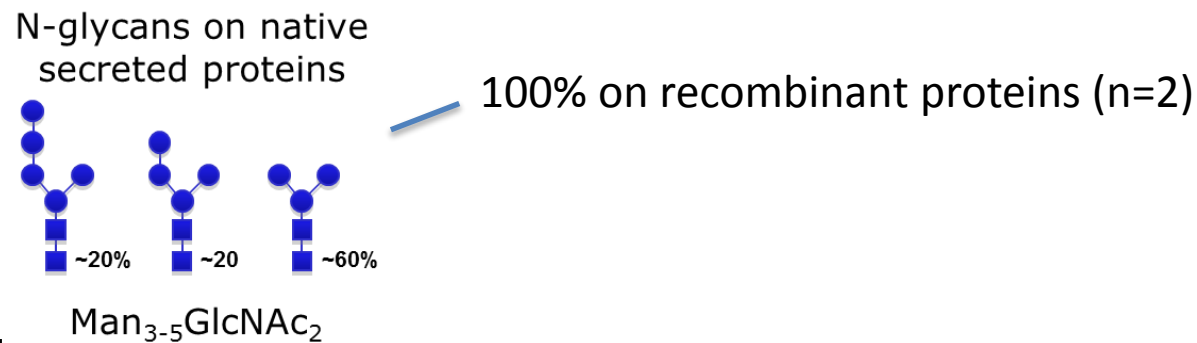
- Vector Biology
 - High copy number ribosomal DNA vectors
 - Constitutive and Inducible promoters
- Metabolism geared towards membrane protein production
 - Expanded cell surface due to hundreds of cilia
 - No cell wall (unlike yeast and bacteria)
- Near uniform N-glycosylation
- Ability to “humanize” PM lipids
- Expanded gene families for membrane transporters

Vector Biology



Post-translation Modifications

- Glycosylation
 - Minimal glycoform heterogeneity



- Lipidation
 - GPI-addition
 - Prenylation (farnesyl transferases; geranylgeranyl transferases; CaaX protease; methyltransferases)
- Phosphorylation
 - 1,069 predicted kinases including PI3 kinase

Highly Adaptive Physiology and Behavior

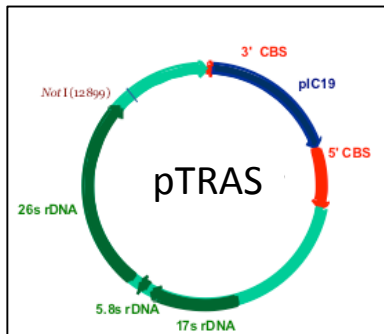
- Large gene families for each of the 4 major families of membrane transporters

Species	ABC	MFS	VIC	P-type ATPase
<i>T. thermophila</i>	161	125	332	91
<i>E. histolytica</i>	18	4	1	19
<i>D. discoideum</i>	61	27	3	24
<i>T. pseudonana</i>	55	42	22	22
<i>C. parvum</i>	13	8	2	9
<i>P. falciparum</i>	14	15	1	11
<i>Encephalitozoon cuniculi</i>	11	2	0	4
<i>N. crassa</i>	31	141	2	19
<i>S. cerevisiae</i>	24	85	2	16
<i>S. pombe</i>	9	58	1	13
<i>A. thaliana</i>	108	90	35	46
<i>C. elegans</i>	48	134	63	22
<i>D. melanogaster</i>	51	136	31	19
<i>H. sapiens</i>	47	81	89	32

Eisen et al. (2006). Macronuclear Genome Sequence of the Ciliate *Tetrahymena thermophila*, a Model Eukaryote. PloS One

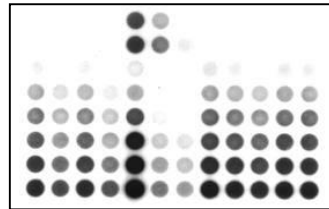
TETRAEXPRESS™: Standard Workflow for Production of Recombinant Proteins

Expression Plasmid Preparation



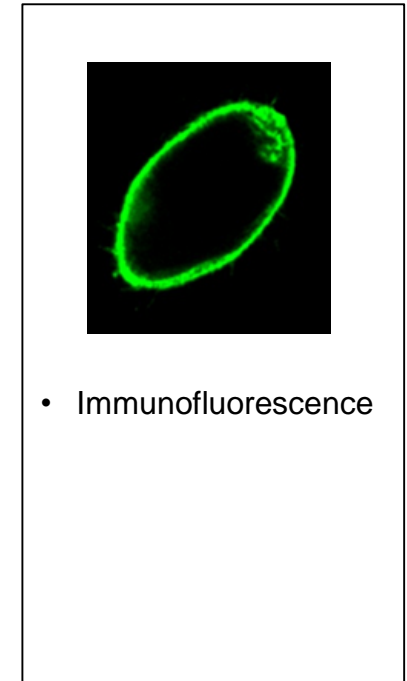
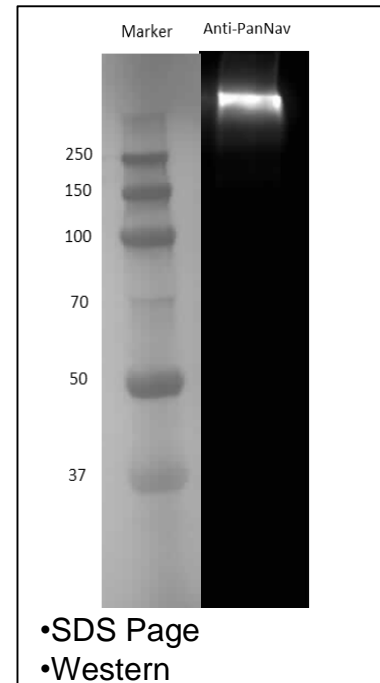
- Codon-optimize gene
- Gene synthesis
- High copy vector
- Epitope tag selection
- Promoter selection

Cell Line Screening and Selection (Dot Blot/ELISA)



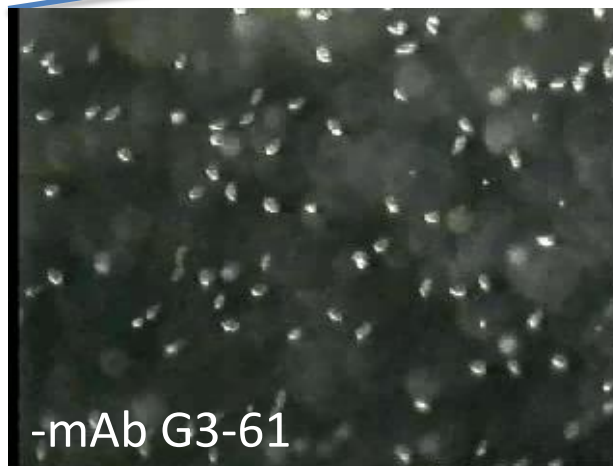
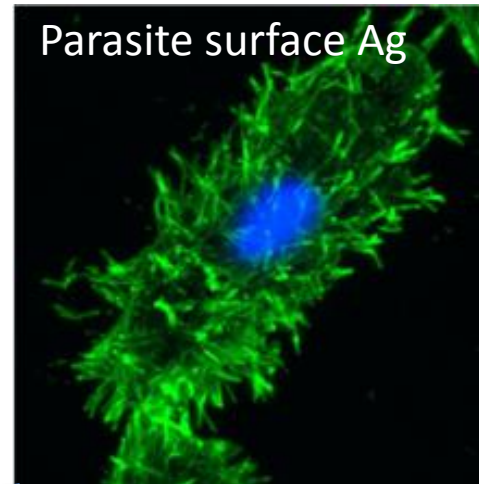
- Biolistic transformation
- 10-20 clones screened
- Identify high expressing clones
- Single cell isolates

Protein Expression Analysis Western Blotting & Localization



Total Cell Line Development Project: ~9-16 weeks

Recombinant MPs Localize to the Cell Surface



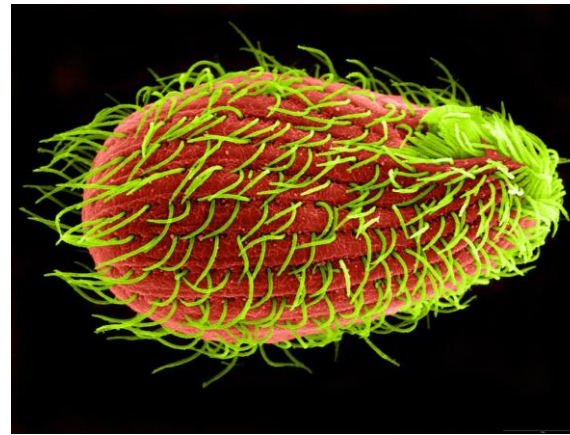
ION CHANNELS: Current State-of-the-Art

- Among the most difficult proteins to express in useful amounts
 - Typically produced in HEK or CHO cell lines
- Low level expression represents major bottleneck to therapeutic Ab discovery/development and structure-based drug design
- ***Even when expressed, a majority of recombinant protein fails to reach the plasma membrane and is likely improperly folded***

SionX™-TTG's Technology Suite for Ion Channel Production

Inducible growth cycle-dependent promoters

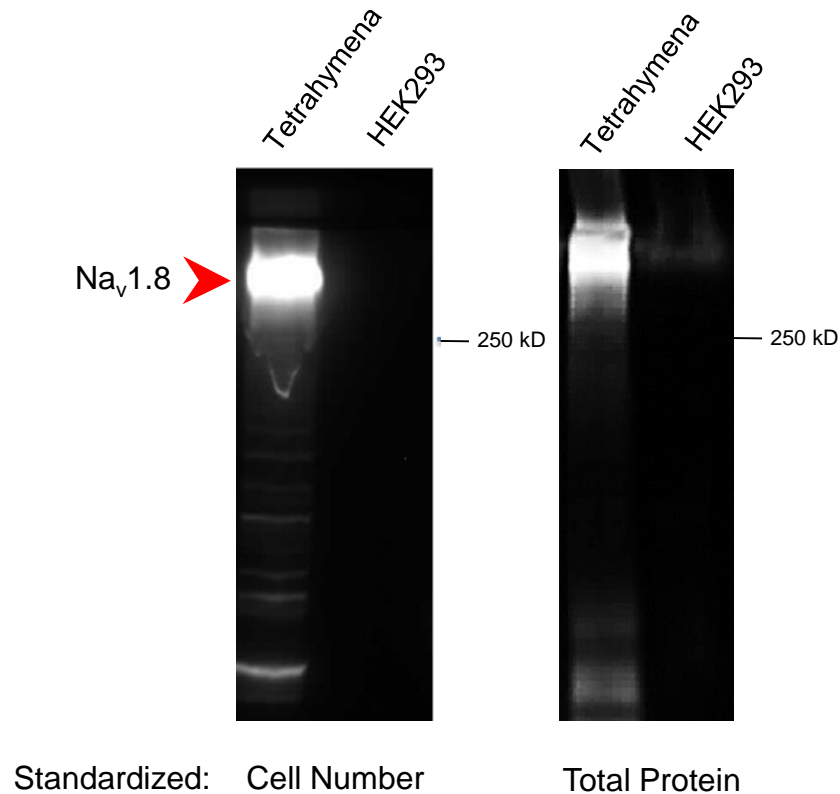
Proprietary growth conditions



- High-level expression
- Abundant cell surface localization
- Properly folded and functional

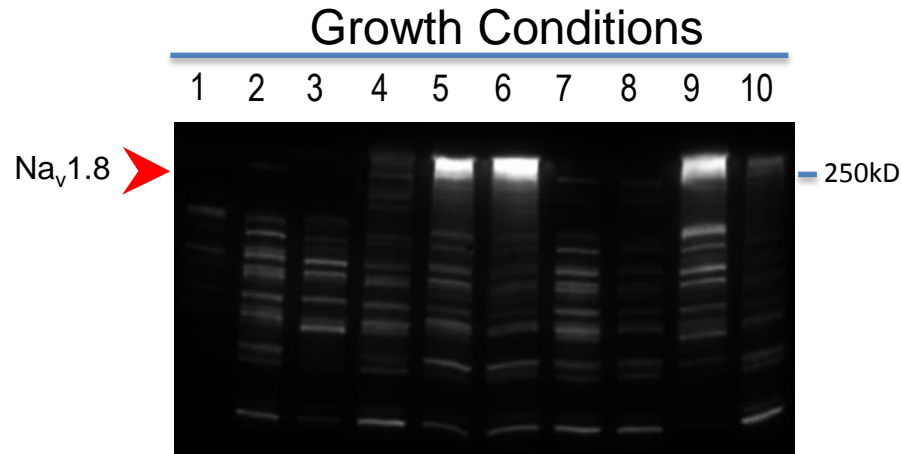
SionX™: Overexpression of Human Ion Channels

SionX™: Robust Expression



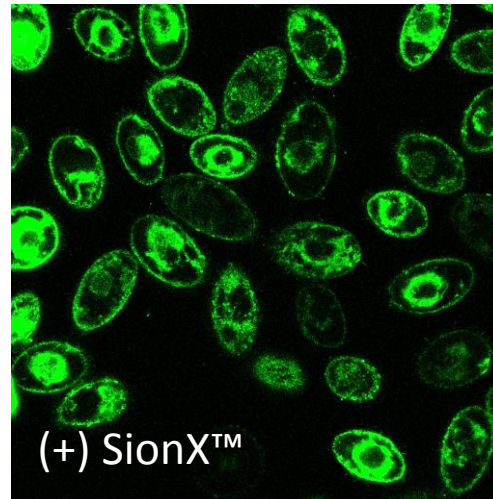
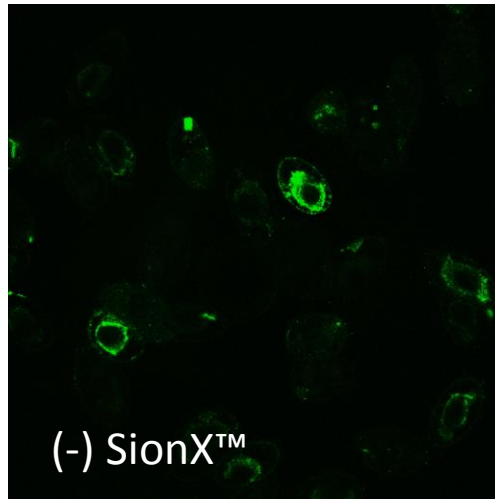
- >100 fold increase relative to HEK293 cells on a per/cell basis
- >50 fold when compared by total protein

SionX™: Expression Optimization



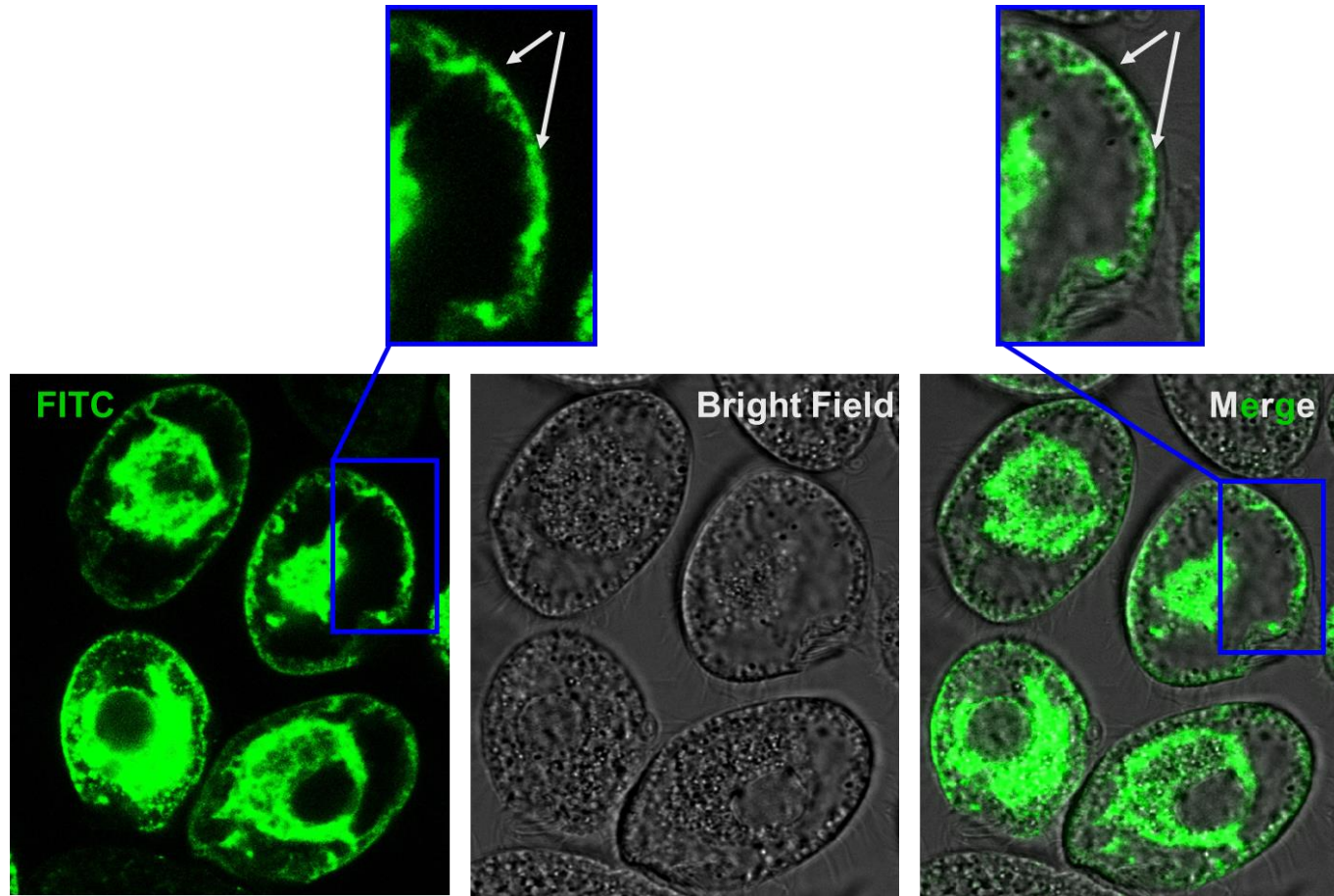
- Growth conditions

- *Expression levels highly dependent on culture conditions*
- *Certain conditions favor trafficking to plasma membrane*



SionX™: Cell Surface Trafficking

- Roughly 20-30% of protein at the plasma membrane



SionX™: Confirmation of Correct Fold



- Kv1.3 Voltage-gated K⁺ channels (Kv)
 - Tetrameric molecules--six putative α -helical transmembrane segments
 - Target for immunosuppression (multiple sclerosis, rheumatoid arthritis, type-1 diabetes mellitus and contact dermatitis)
- ShK Toxin
 - Blocks Kv⁺ channels by binding with high affinity ($K_d < 11 \text{ pM}$) to external vestibule formed by tetramer
 - Found to undergo conformational changes when bound to 'Shaker K' channel suggesting induced fit model may be present in toxin-channel interaction

SionX™: Confirmation of Correct Fold

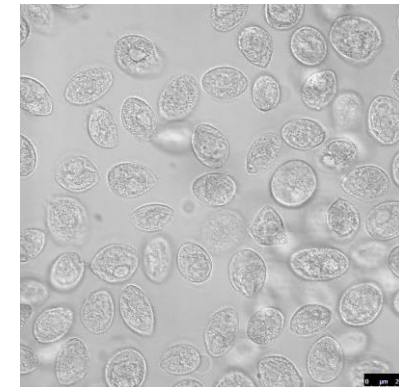
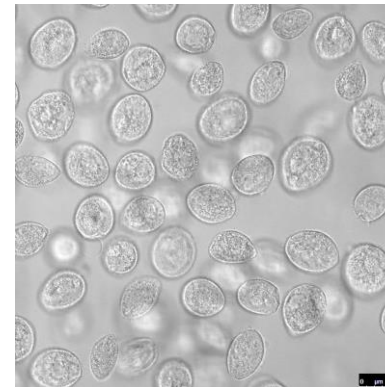
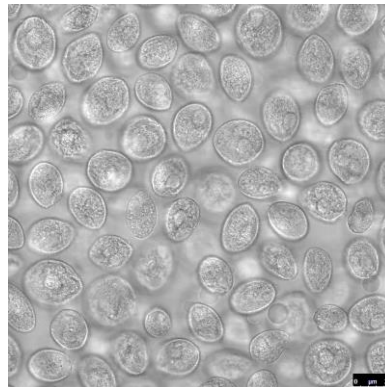
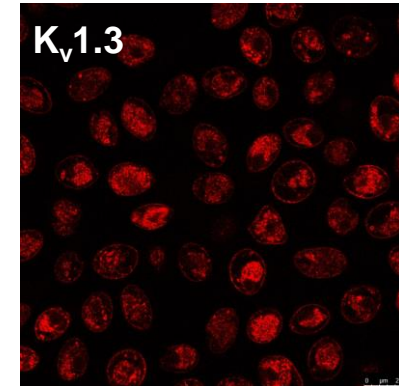
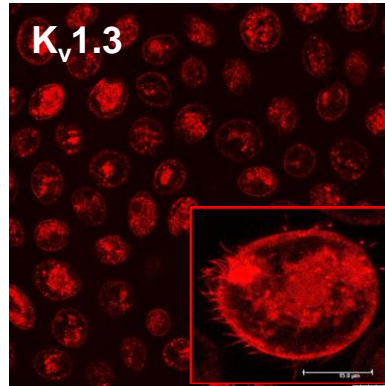
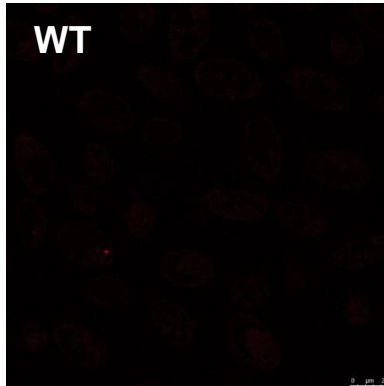
Pre-Incubation:

None

None

100 nM MgTx

100 nM IbTx

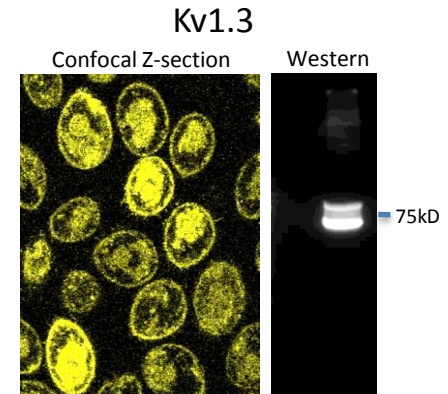
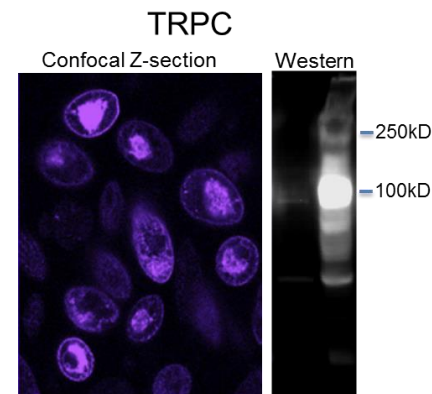
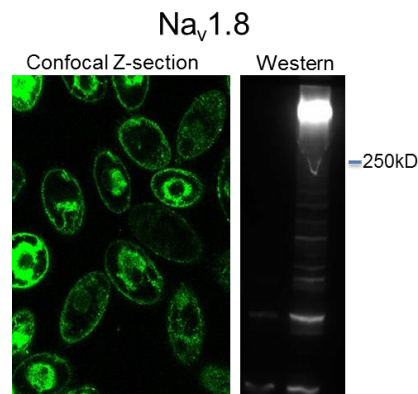
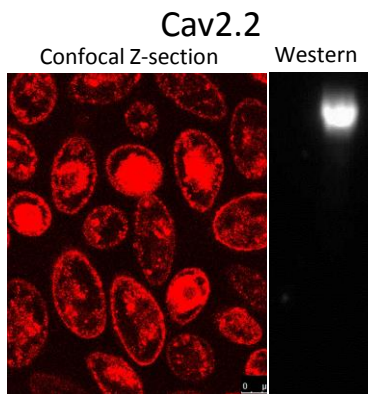


Specific Toxin: ShK-TAMRA (10 nM)
Competing Toxin: Margatoxin (MgTX)
Non-competing Toxin: Iberiotoxin (IbTX)

SionX™-Suitable for a Wide Range of Channels

	Expression	Plasma Membrane Localization	Purification	Correct Folding	Functionality
Nav1.x	X	X	X	X	X
Nav1.8	X	X	X	X	X
Kv1.3	X	X	X	X	TBD
Cav2.2	X	X	TBD	TBD	TBD
TRPCx	X	X	TBD	TBD	TBD
Kv2.1	X	X	TBD	TBD	TBD

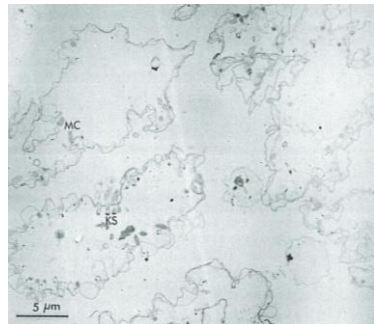
- Robust expression
- Plasma membrane trafficking
- Correctly folded
- Functional



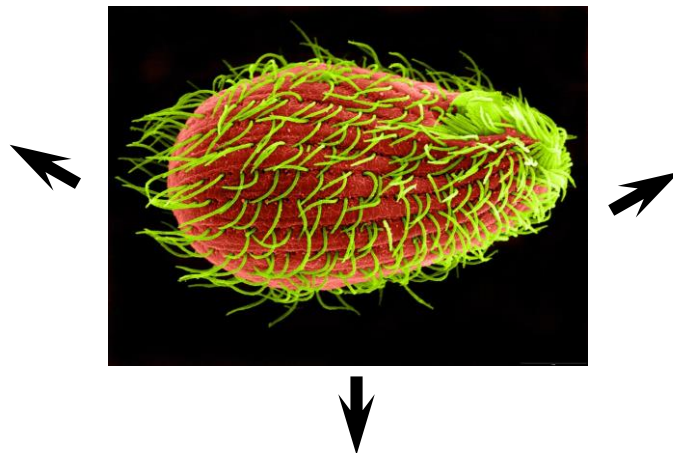
SionX™: Drug Discovery Toolbox

SionX™: Subcellular Fractions

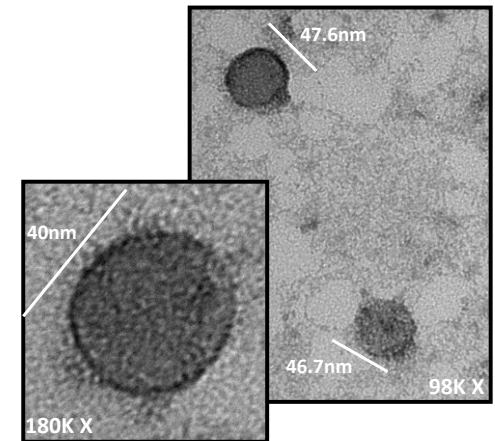
Enriched plasma membrane fractions – “pellicles”



Thin-section of isolated pellicles

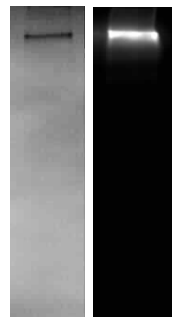


Plasma membrane vesicles



Negative stain EM

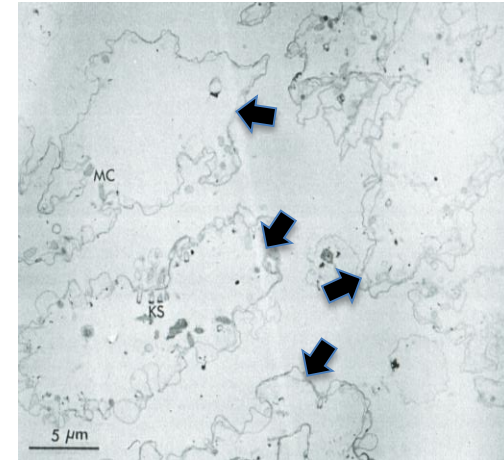
Nav1.8



Reconstituted purified protein (mg quantities)

SionX™: Whole-cell Ghosts (Pellicles)

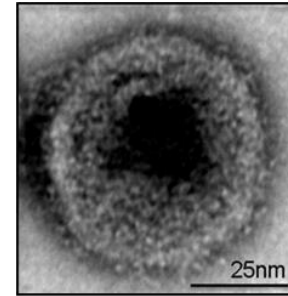
- Minimally processed “crude” plasma membrane fractions
- Enable therapeutic antibody discovery & production



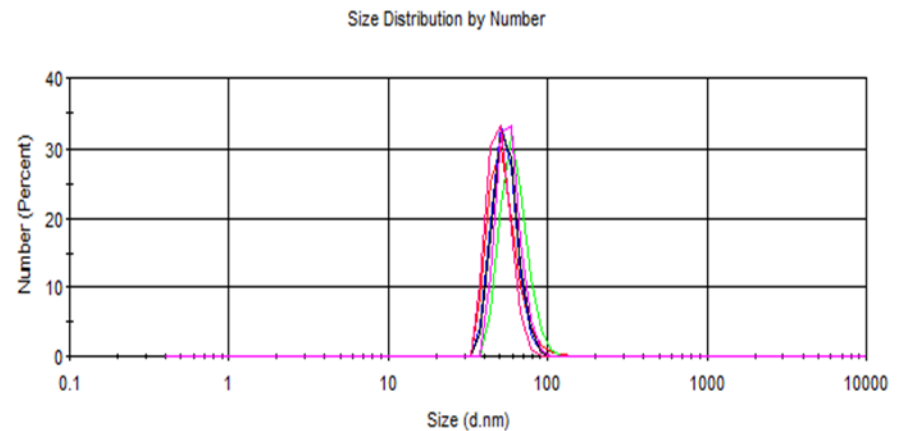
Isolated pellicles

SionX™: Plasma Membrane Vesicles

- Ideal for Antibody production
 - Uniform diameter
 - Repetitive surface display
 - 5-7 fold enrichment vs. pellicles
 - Up to 5% of total protein as recombinant target



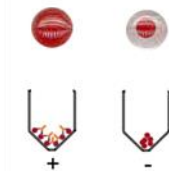
Nav 1.8 containing membrane vesicle
Negative stain EM



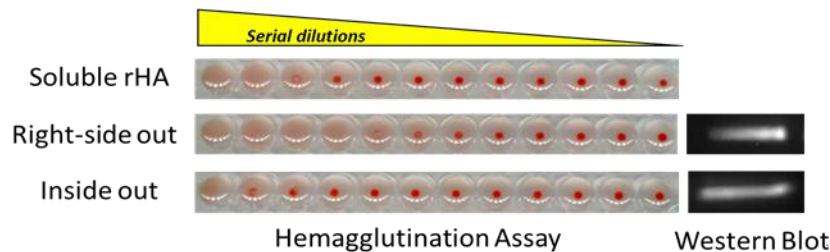
SionX™: Membrane Vesicle Validation

- Steps

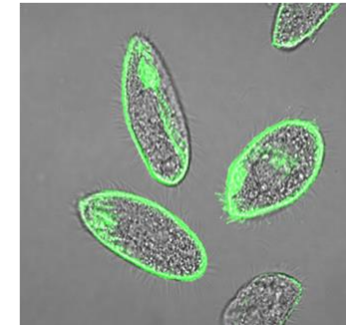
- Homogenization of cells
- Differential centrifugation
- Two phase enrichment & harvest of “right-side out” MVs



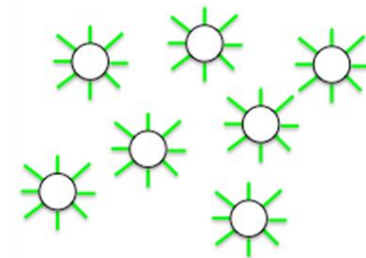
Hemagglutination



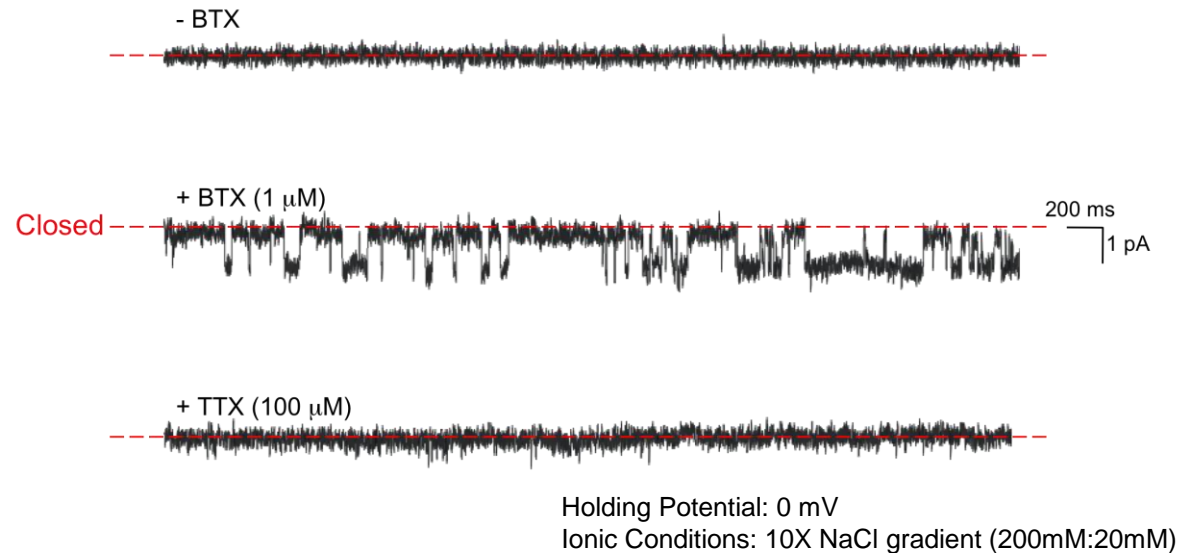
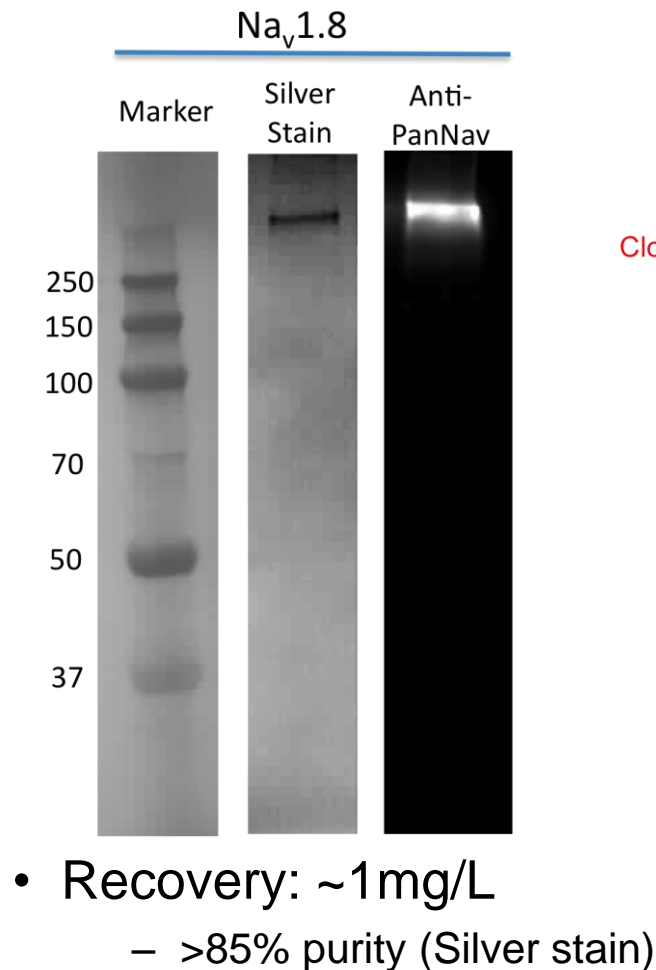
Cells expressing recombinant HA



Purified membrane vesicles

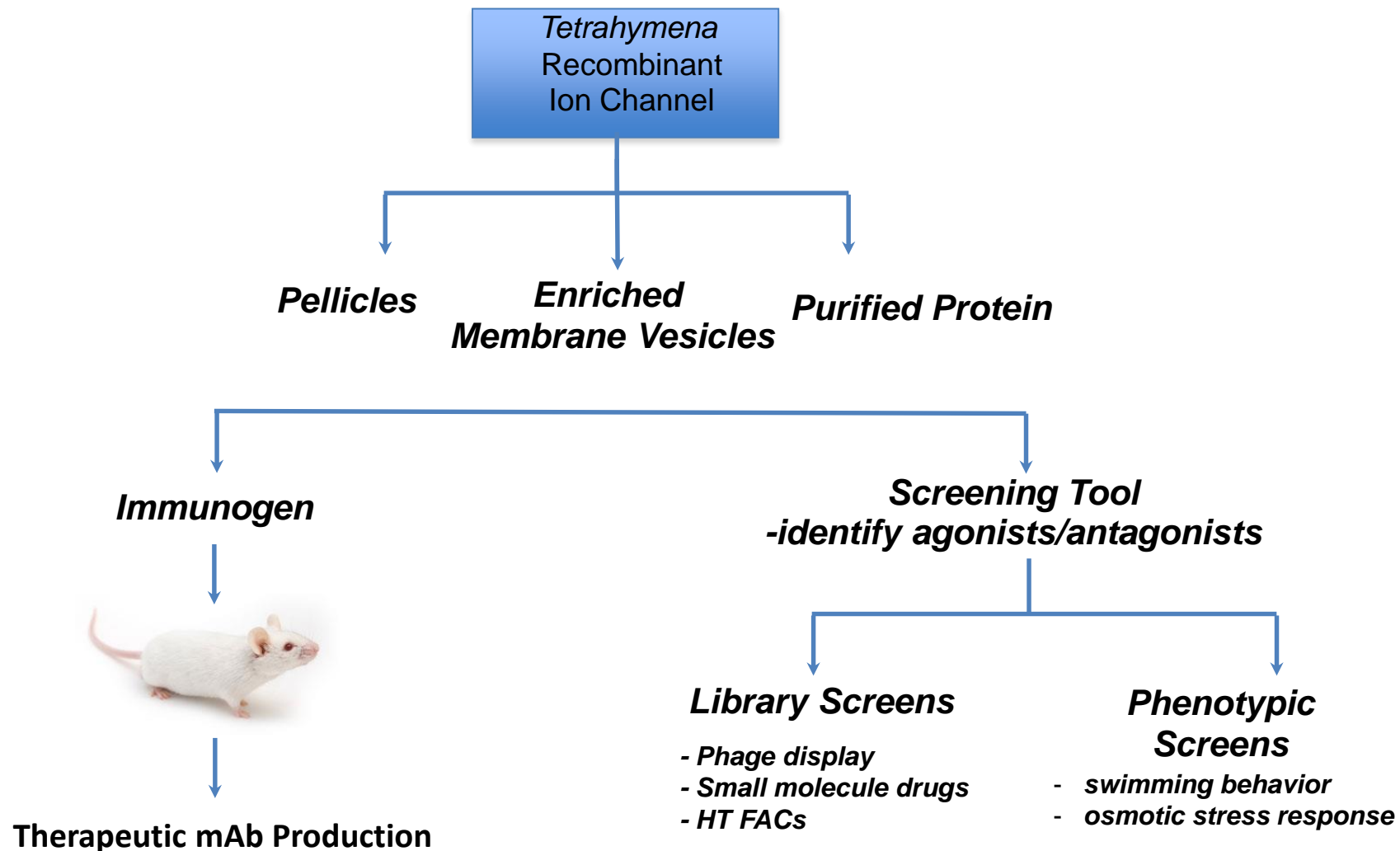


SionX™: Purified Proteins



- Ephys Analysis
 - Artificial Bilayer
 - Nanion Port-a-Patch
 - GUV/Nav1.8 Proteoliposome fusion

SionX™: Enabling Drug Discovery



Thanks

- Our Pharma Partners
- Tetragenetics Team
 - Paul Colussi
 - Ashot Papoyan
 - Yelena Bisharyan
 - Janna Bednenko
 - Alka Agarwal

Tetragenetics



Walden Sq. Science Park Cambridge, MA

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