

ChanTest's Ion Channel Safety, Discovery and Supplies Services



The Ion Channel Company

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Adjunct Professor, Physiology & Biophysics
Case Western Reserve University

About ChanTest

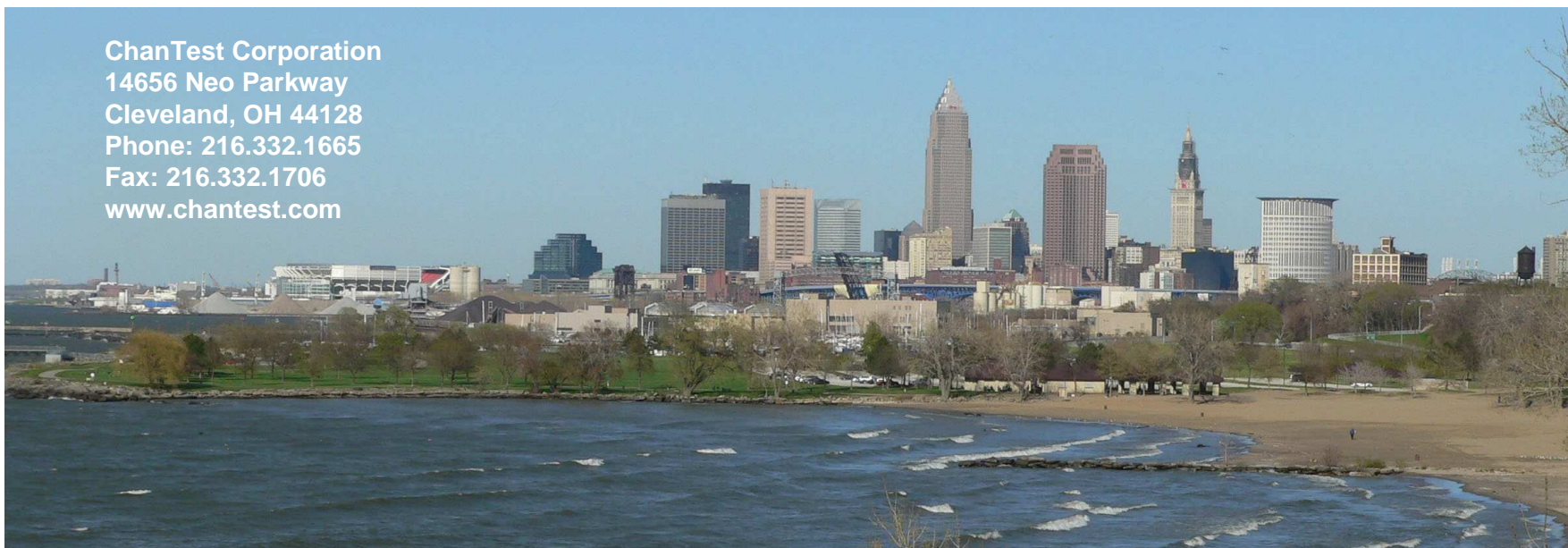


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- **Contract Research Organization**
 - Since 1998 - specializing in ion channel safety and discovery testing for the pharmaceutical industry
 - 75 employees (22 PhDs)
- **Molecular and Cell Biology**
 - cloning, cell line construction/optimization
- **Pharmacology & Physiology**
 - *in vitro* manual and automated electrophysiology
- **Analytical & Med Chem**
 - real time HPLC dose sample analysis
 - automated solubility assessment
 - SAR

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Prologue



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- ChanTest is constructing a major Ion Channel Library of ~ 120 cell lines (64 now) each of which will be validated and optimized on the principal, automated EPhys platforms: PX, QPatch, IW Q and FLIPR^{TETRA}.
- Library “books” are arranged into panels by tissue (Cardiac Channel PanelTM), therapeutic area (Pain PanelTM) and IC family (Nav1.x Panel).
- Channel panels are FDA Critical Path Tools for understanding the 1^o and 2^o ion channel PD for safety and efficacy.

ChanTest Services



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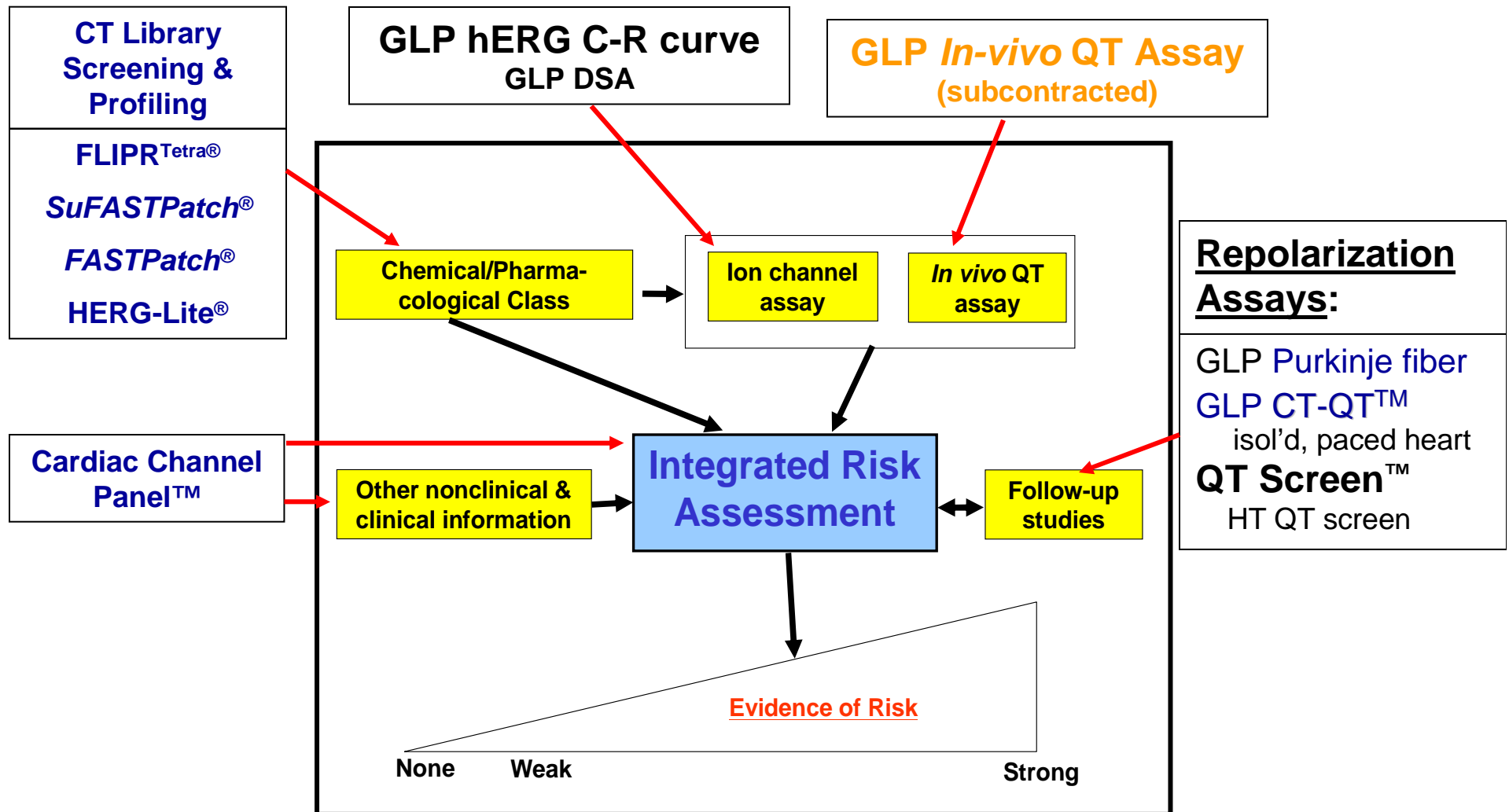
- Part 1: Development of a preclinical cardiac safety assessment program based on understanding drug effects on cardiac ion channels**
- Part 2: Construction of ChanTest's Ion Channel Library; validation on automated EPhys platforms**
- Part 3: Using the ChanTest Library/automated EPhys platform for drug safety and discovery**
- Part 4: Optimization of ion channel cell lines on automated EPhys platforms**

ICH S7B Guidelines Nonclinical Testing Strategy



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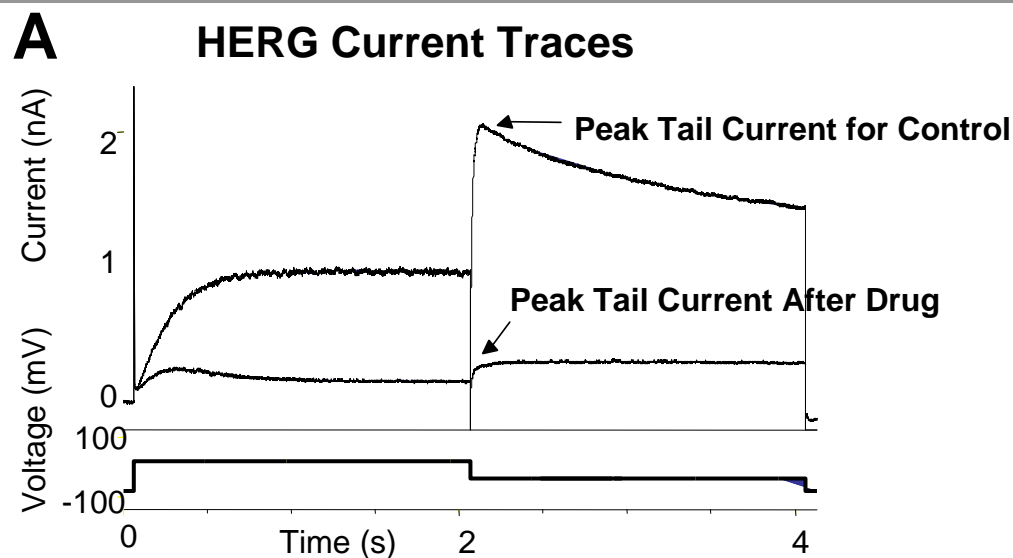


Measuring the IC_{50} of Direct Block of HERG

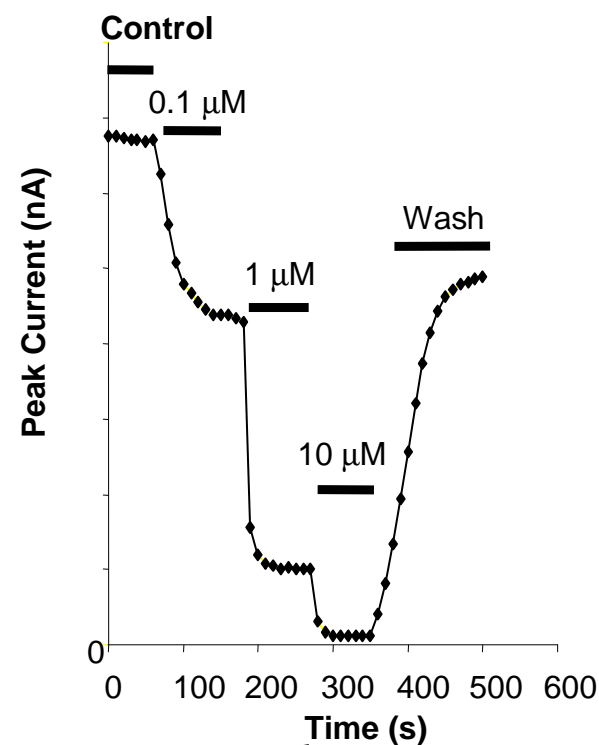


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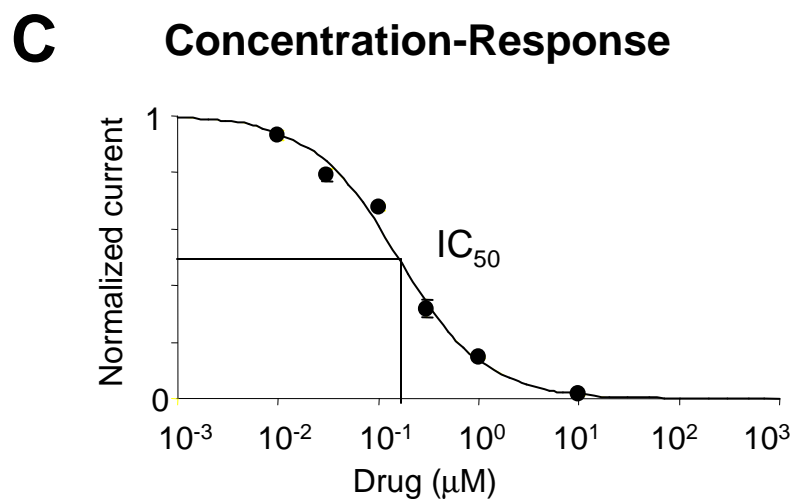
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B Time Course Of Peak Tail Current



Note Time Scale

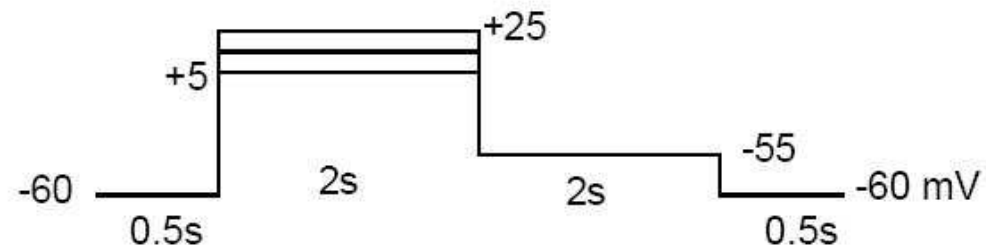
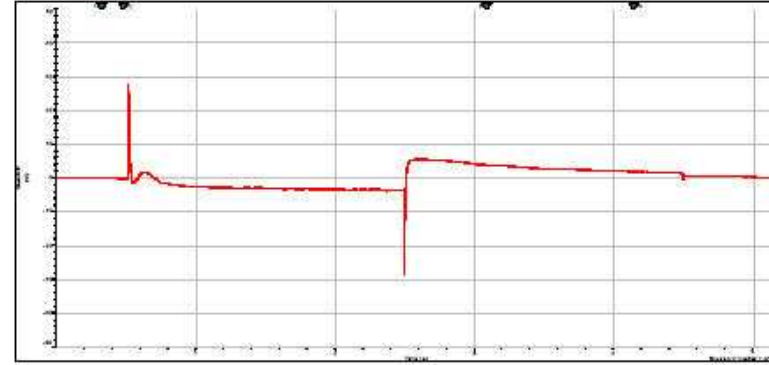
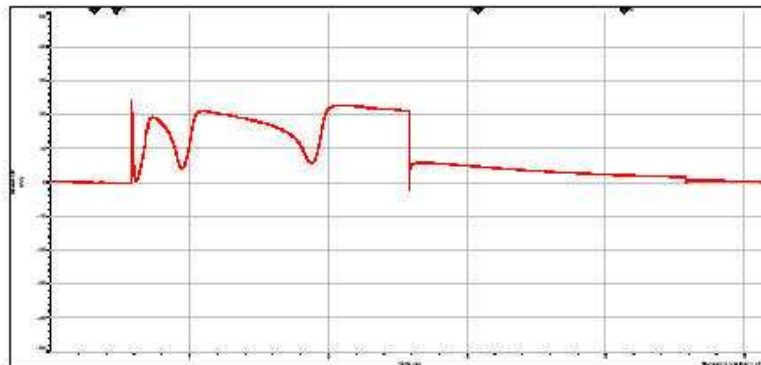
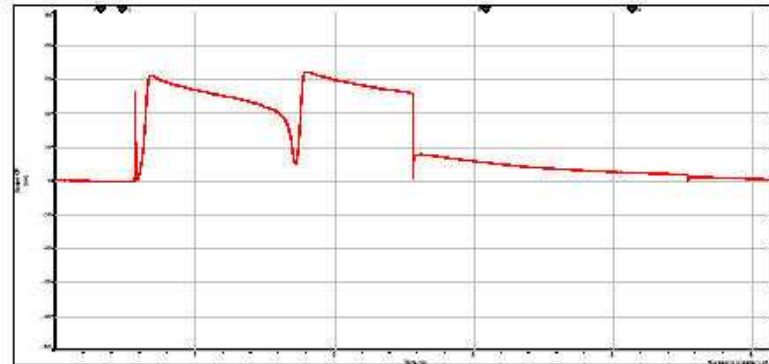
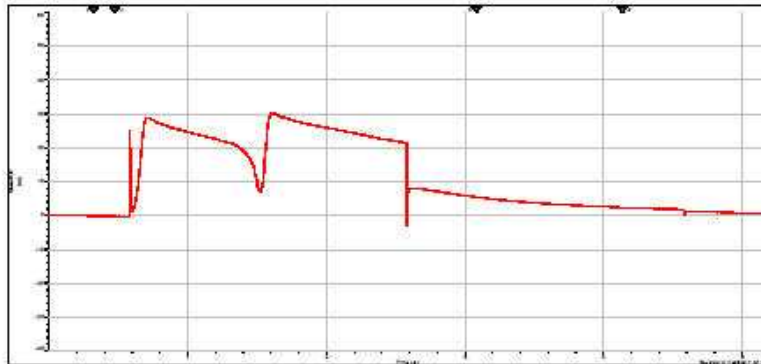


HEK hERG: Space Clamp Inadequate



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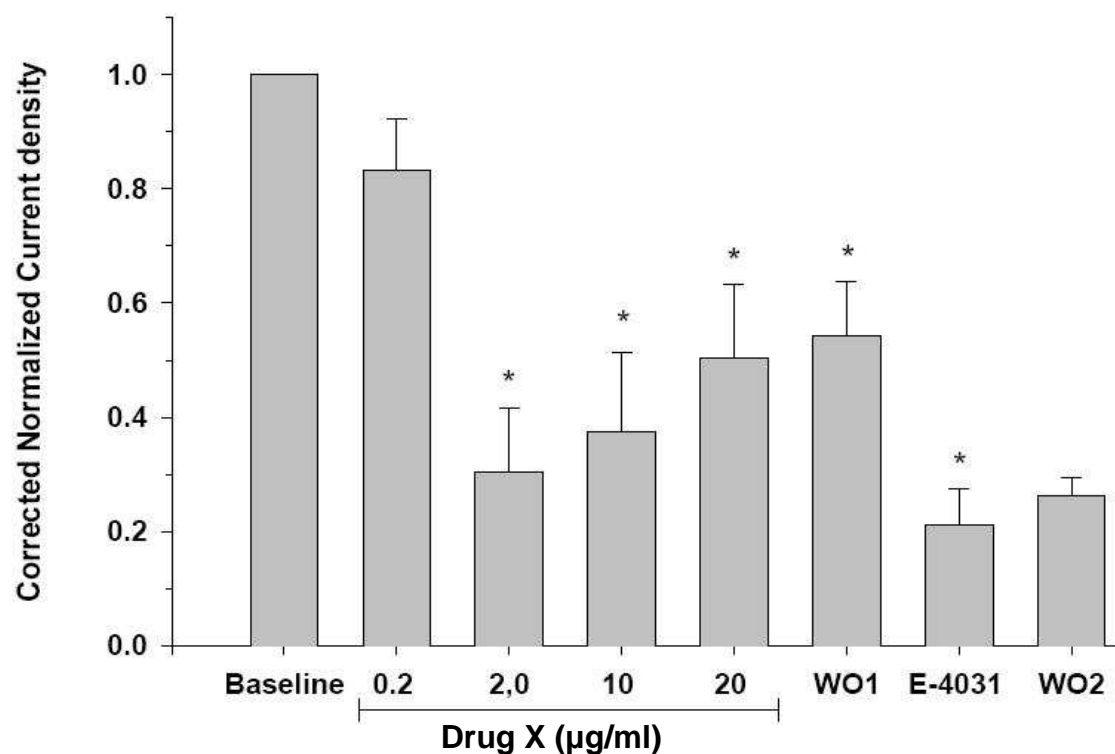


Effects of Drug X on HERG



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*: $p \leq 0.05$; error bars, S.E.M.; WO, washout

Conclusion: The results of this study demonstrated that Drug X at concentrations between 2.0 and 20.0 $\mu\text{g/mL}$ cause a significant inhibition in the hERG current density recorded from HEK293 cells stably transfected with hERG. This suggests that Drug X produced a functional inhibition of the I_{Kr} current produced by HEK293 cell transfected with the hERG gene. The response of the positive control (E-4031) verified the sensitivity of the assay.

Cardiac Arrhythmic Risk: HERG, APD/QT, TdP



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- \uparrow QT or \uparrow APD is associated with hERG block or inhibition of hERG trafficking.
- CT **HERG-Lite** detects drug effects on trafficking.
- HERG block \neq \uparrow QT or \uparrow APD, e.g., verapamil, tolterodine, citalopram.
- TdP is associated with \uparrow QT, but rarely.
- **QT is the biomarker regulators use for TdP.**
- CT **QT Screen** has \ll 1% variance and is a rapid, inexpensive assay for QT risk.



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Part 2: Construction of ChanTest's ion channel library; validation on automated EPhys platforms

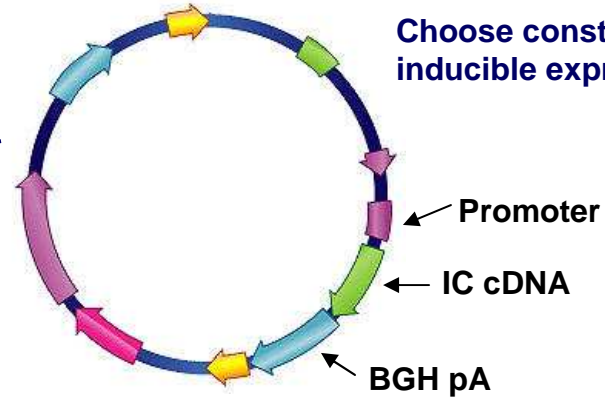
Construction of ChanTest's Catalog of Ion Channel Cell Lines



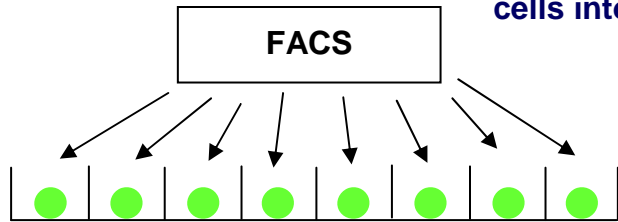
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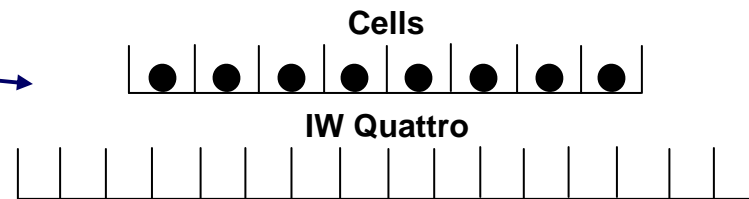
Stable transfection via nucleofection into HEK293, CHO, etc. cells; co-transfection with GFP



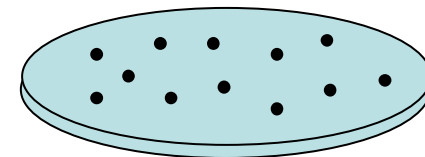
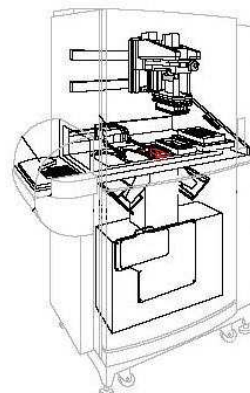
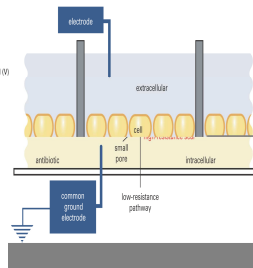
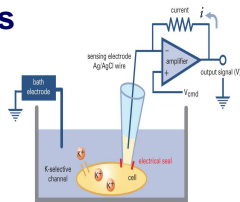
Sorting of GFP-positive cells into 96-well plates



Clonal selection by MT screening



Validation of cell line on ChanTest platforms



HERG Validation/Confirmation

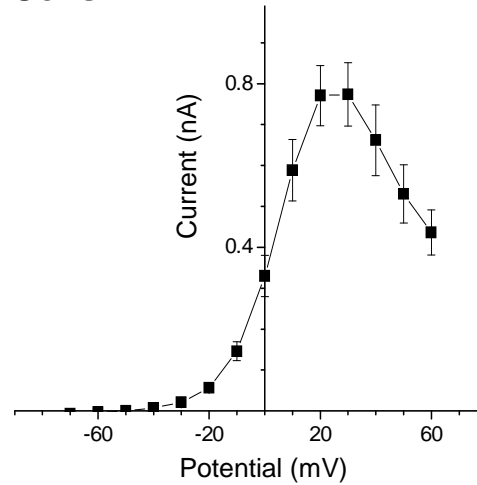
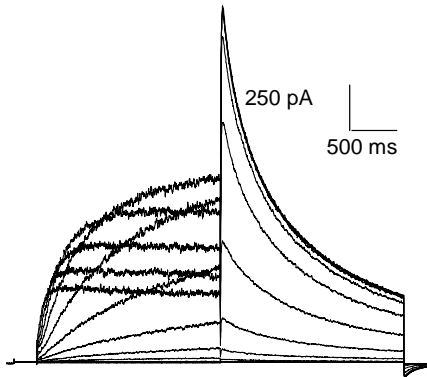


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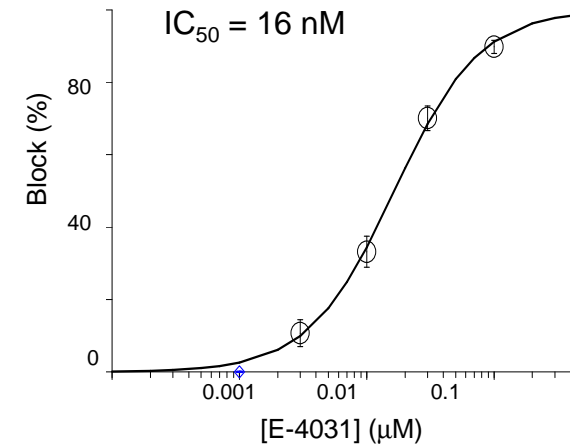
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A. FASTPatch[®]

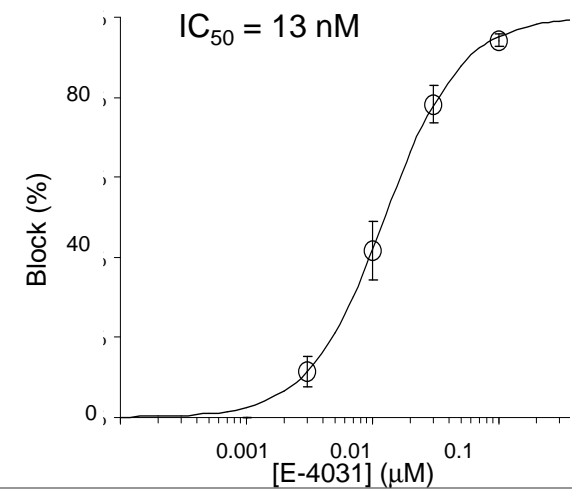
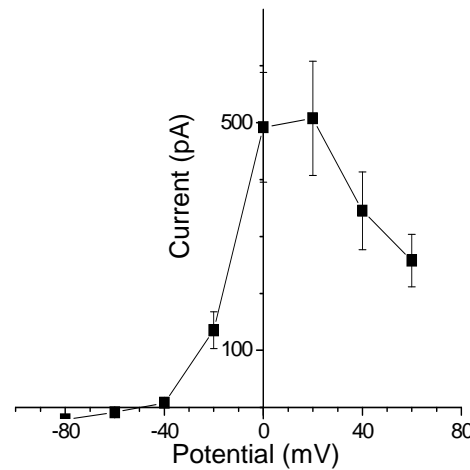
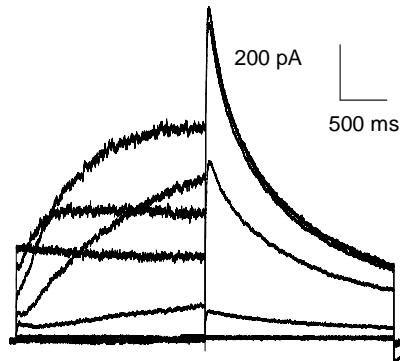
HERG Current in HEK Cells



E-4031 Concentration-Response



B. Manual Patch Clamp

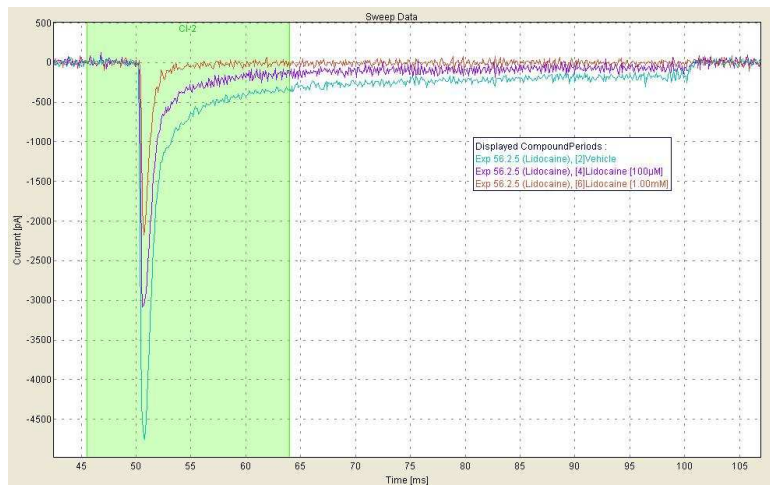


HNav1.5-CHO QPatch

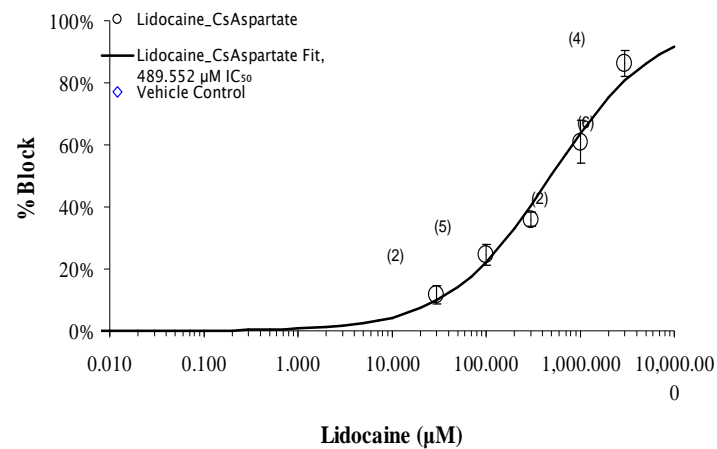


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Lidocaine_CsAspartate Concentration-Response

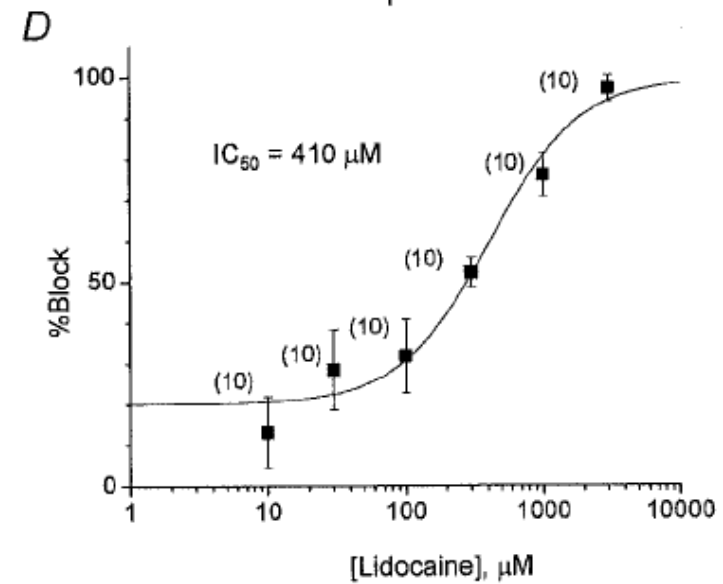
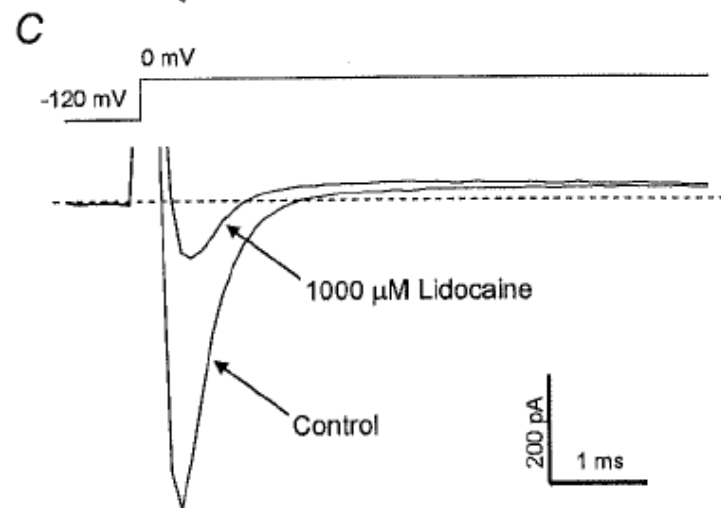
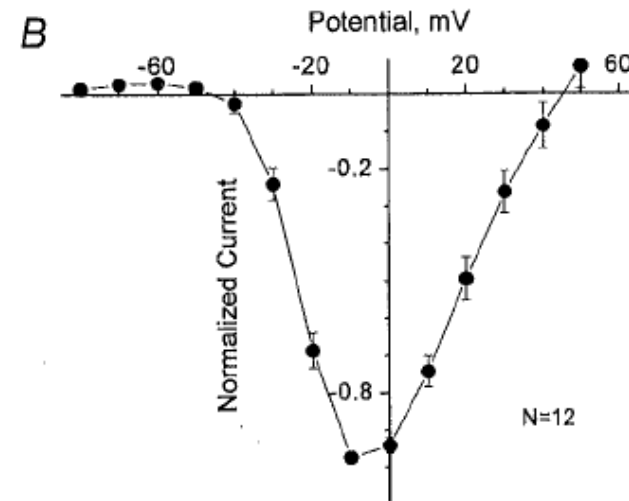
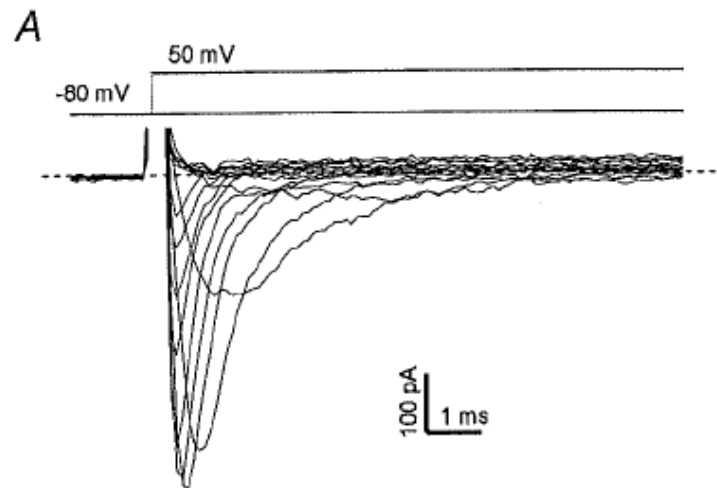


HNav1.7-CHO IW Q

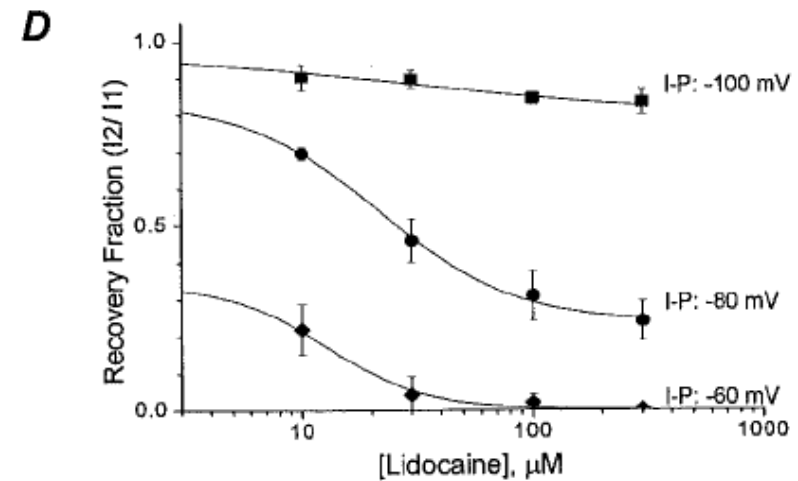
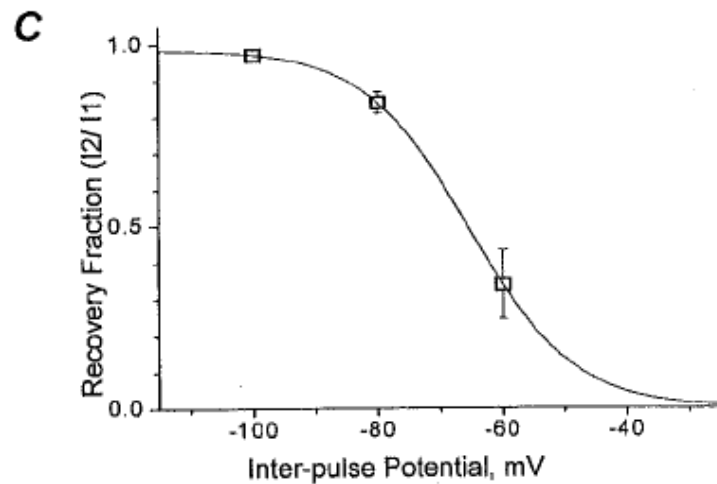
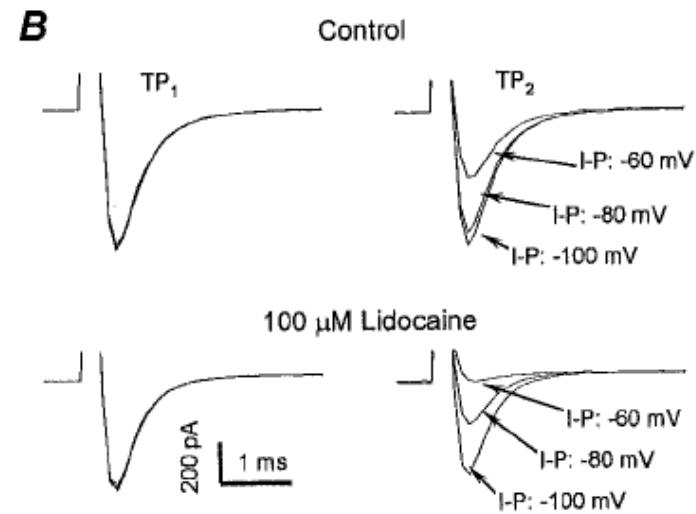
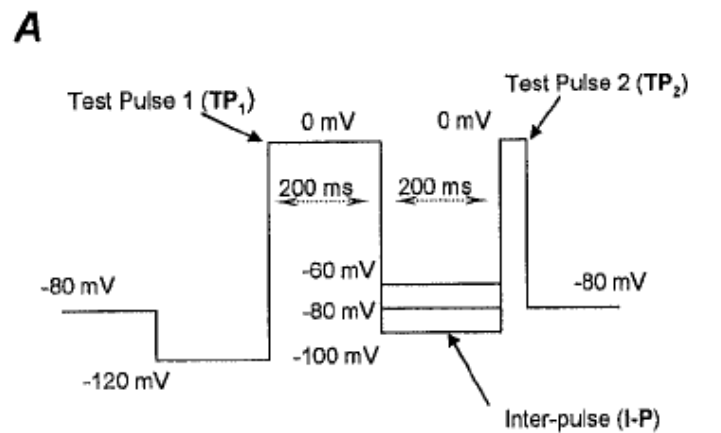


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HNav1.7 Use-Dependence IW Q

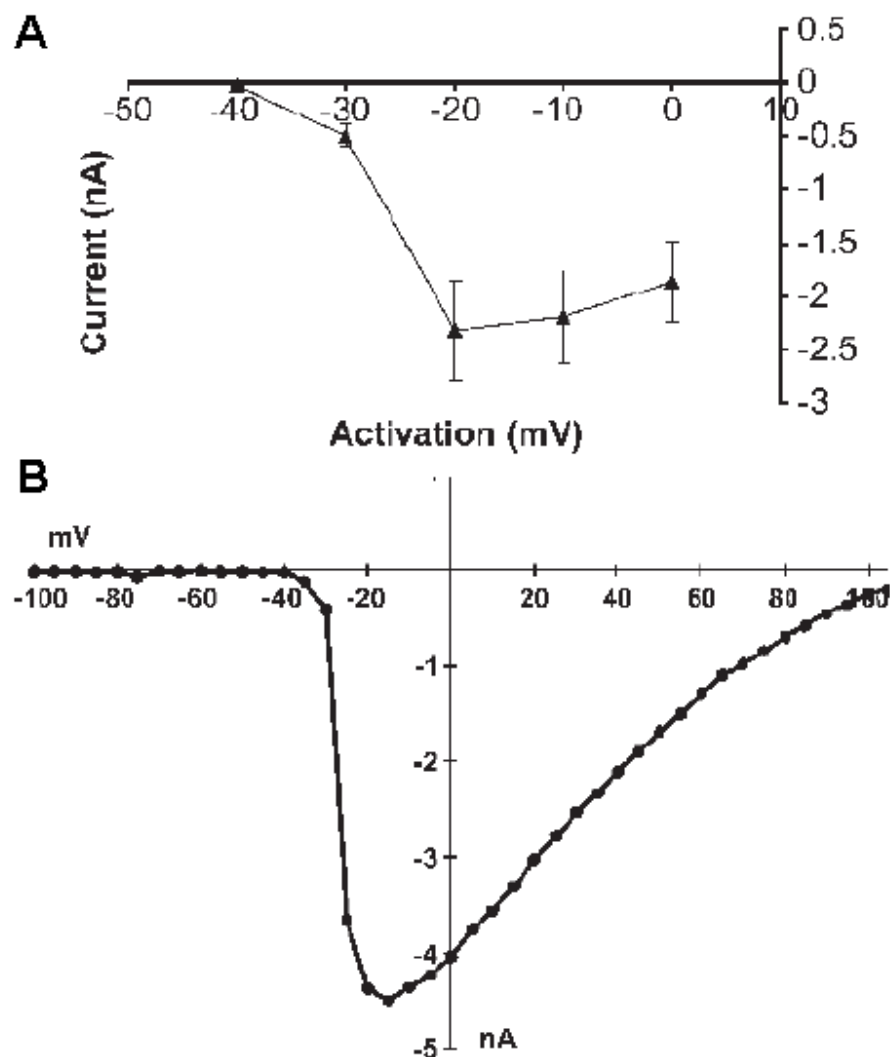


HEK Nav1.7 Improper Voltage Control



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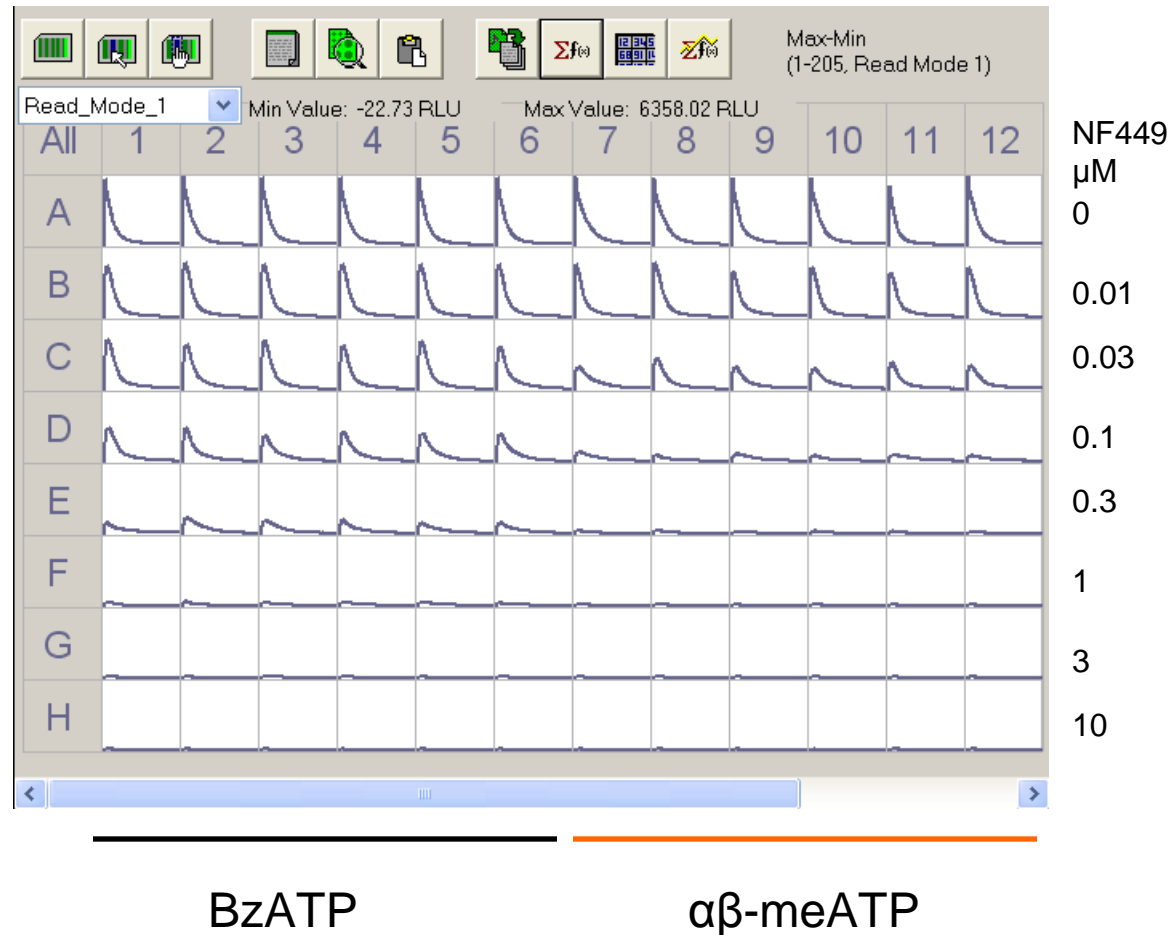
From Trivedi et al. 2008 Assay Drug Dev Technol

Validation of P2X1-HEK by FLIPR^{Tetra} Assay



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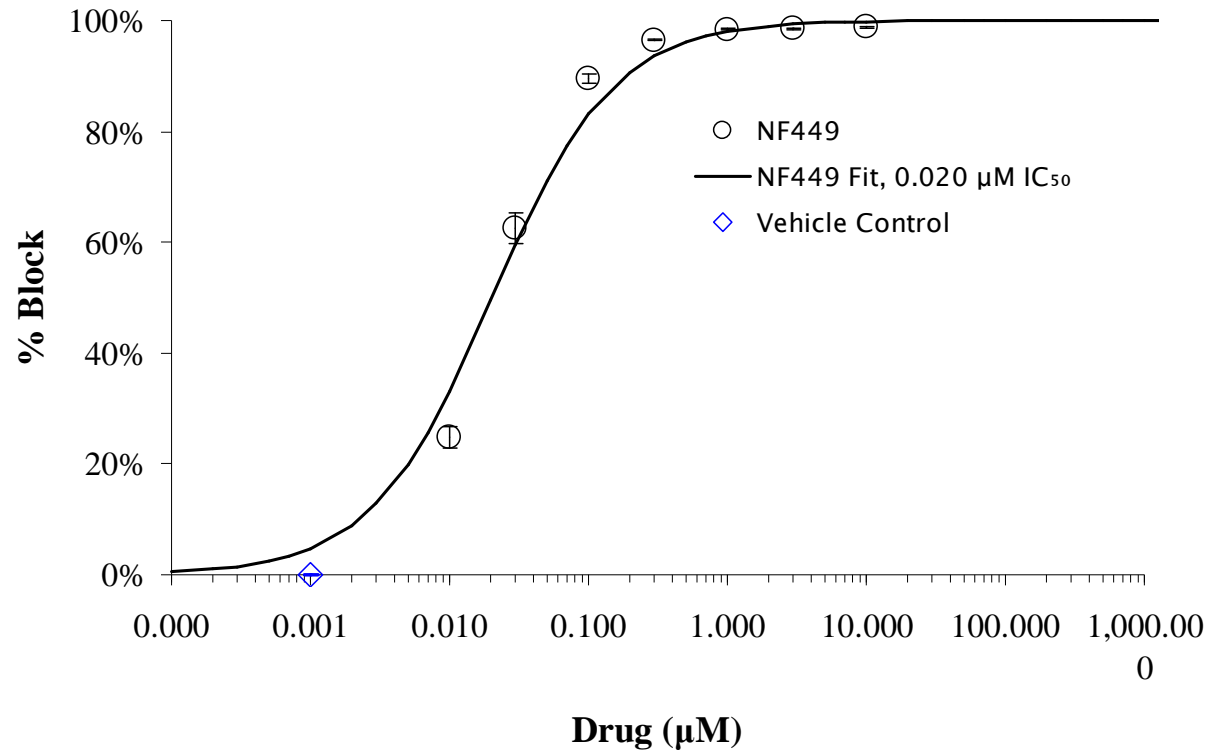
Dose-dependent block of αβ-meATP and BzATP (1μM each) - induced Ca²⁺ responses by NF449.

Concentration-Response of NF 449 on P2X1



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Concentration-response curve of NF449 effects on 1 μM $\alpha\beta\text{-meATP}$ - induced Ca^{2+} response in HEK/P2X1.

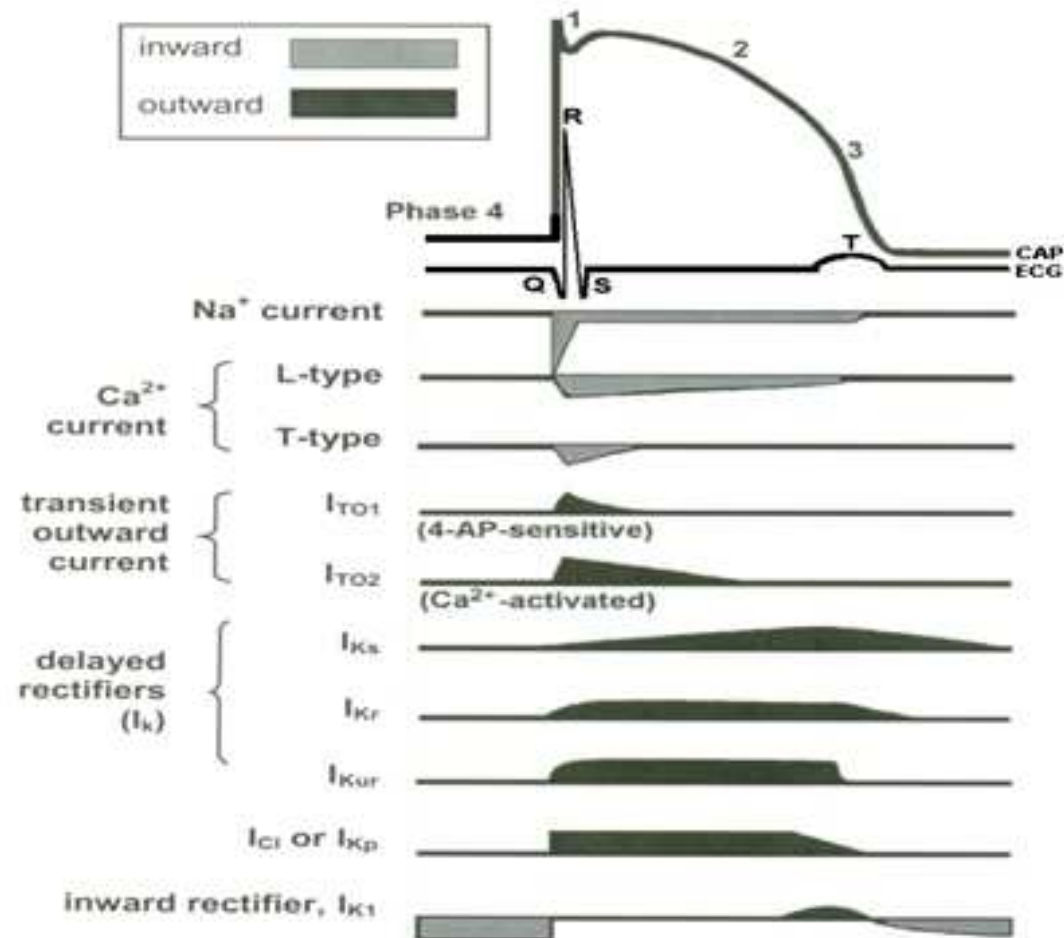
The results were averages of 6 individual replicates.



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Part 3: Using the ChanTest library/automated EPhys platform for drug safety and discovery



Cardiac Channel Panel™



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hERG

KvLQT1/minK

Kv4.3

Kv1.5

Kir2.1

Kir6.2/SUR2A

Kir3.1/3.4

Nav1.5

Cav1.2, $\alpha 2$ - δ , $\beta 2$ (L-type)

Cav3.2 (T-type)

HCN2

HCN4

Biomarker: “Characteristic that is objectively measured as an indicator of normal or pathogenic processes or pharmacologic response to a therapeutic intervention” *Clin Pharm Ther.* 69: 89, 2001

Lessons from HERG-APD/QT Discordance



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- hERG	9/98 (9.2%)	59/98 (60.0%)
+ hERG	10/98 (10.2%)	20/98 (20.4%)
	+ APD	- APD

Lessons from Screening a Cardiac Channel Panel



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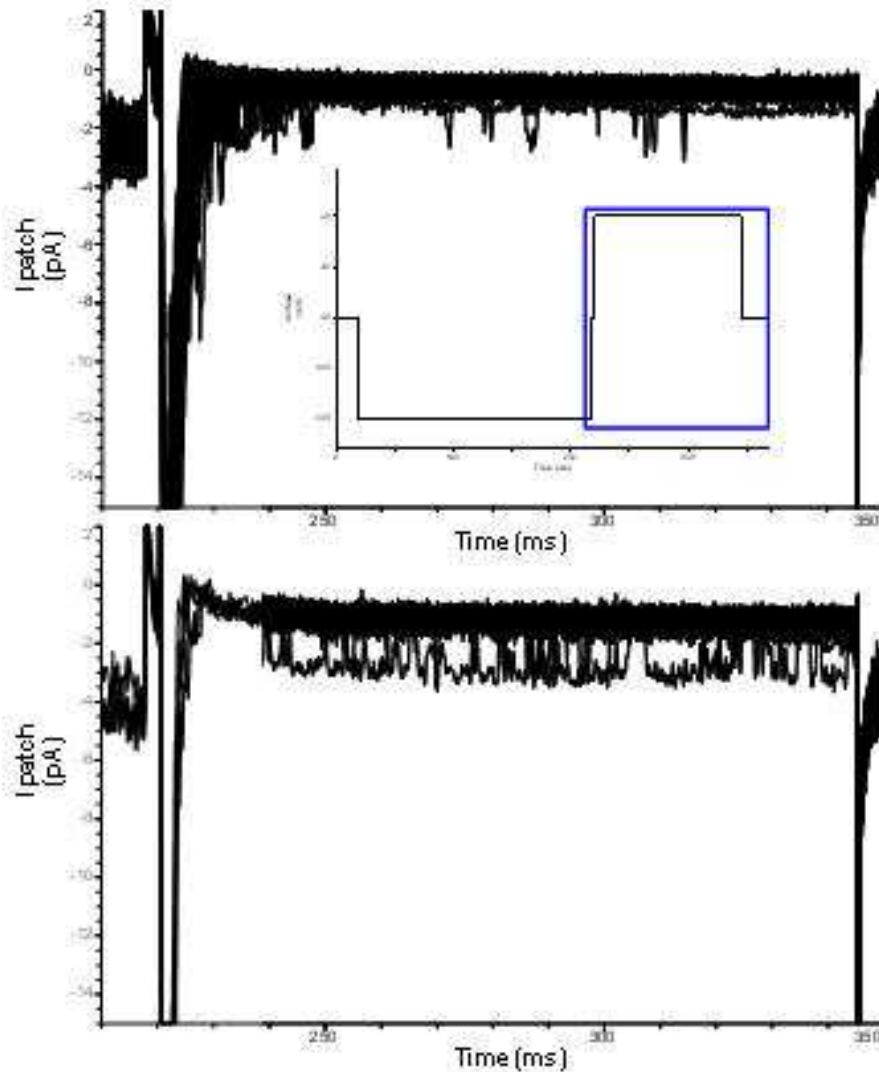
	Cav3.2	Cav1.2	Nav1.5	HCN4	Kv1.5	Kv4.3	hERG
E-4031*	4.6	4.0	5.3	11.1	3.2	5.7	99.3
Verapamil		65.4					97.8
Vanoxerine	83.2	92.8	62.9	46.4		59.5	98.7
Alfuzosin**	9.9	0.5	-5	8.4		2.9	39.3
	20% < block <50%						
	50% < block						
	5% increase						
Data presented as mean of the block (n=2-3); 10 μM; 0.1 Hz; FASTPatch							
* 1 μM							
** Lacerda et al. 2008 JPET.							

Type 2 Discordance: LQT without HERG Block, Nav1.5 Gain of Function



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Alfuzosin Increases Opening
Probability of Late Cardiac
Sodium Channels

(Lacerda *et al.* 2008 JPET, 324: 427-433)

Type 2 Discordance: LQT and Inward Rectifier Block



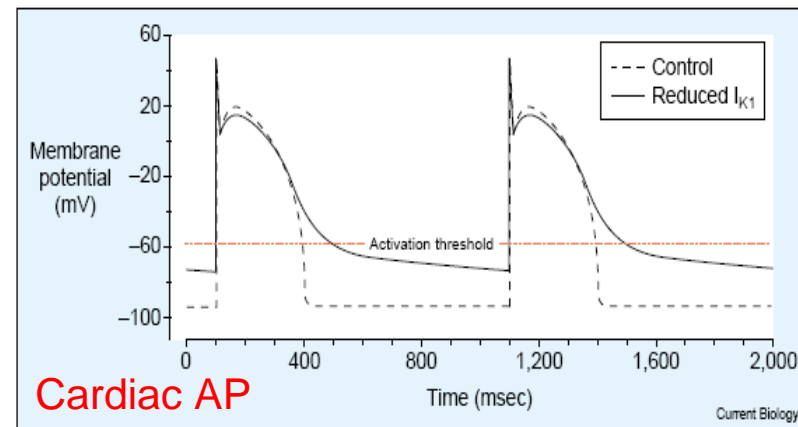
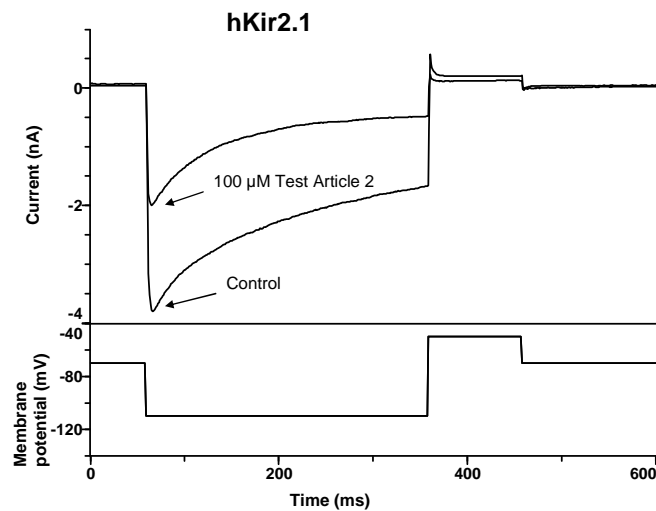
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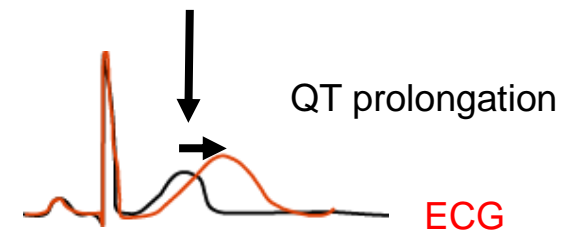
Channel:	hERG	hNav1.5	hKvLQT1 / hminK	hKv4.3	hKv1.5	hCav1.2	hCav3.2	hKir2.1	hHCN4
Conc. (μM)									
Test Article 1	1								
	10								
	100								
Test Article 2	1								
	10								
	100								

Color code

≤ 20%
20 - 40%
> 40%



Jongsma HJ, *Current Biology*, Vol. 11 pvR747-R750 (2001)



Complex Type 2 Discordance



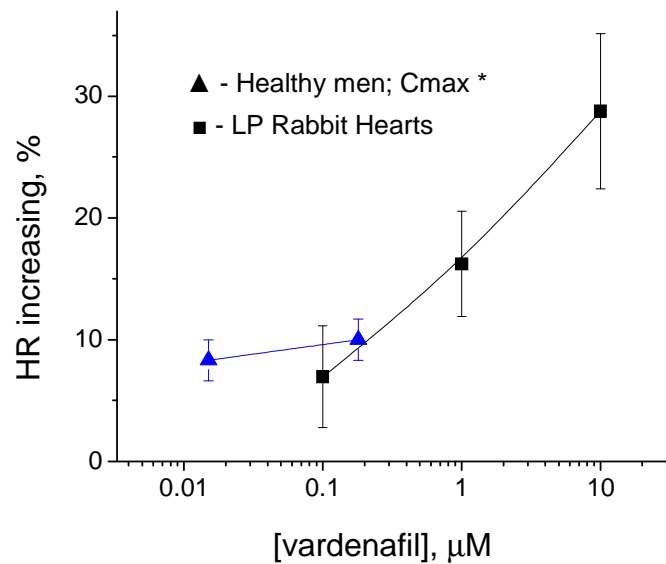
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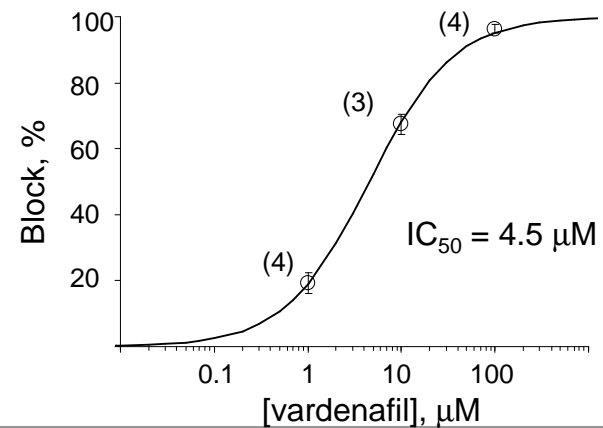
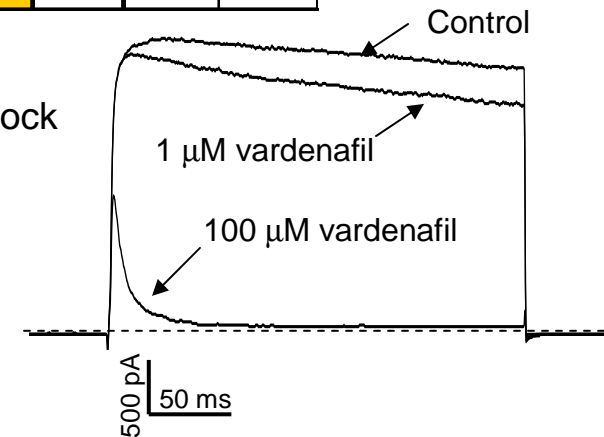
A. Channel Panel™

	Mean Block, %									
	Cav3.2	Cav1.2-beta2	Nav1.5	HCN2	HCN4	Kv1.5	Kv4.3	LQT1-minK	hERG	Kir2.1
Vardenafil (10 μ M)	7.5	10.9	1.1	-6.6	-12.2	67.4	92.3	2.8	21.1	3.8

B. Heart Rate



C. Kv1.5 Block



* Morganroth et al, Am J Cardiol, 2004

Pain Channel Panel



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Channels available now	
ASIC1a	P2X1
ASIC2a	P2X2
Cav1.2/ β 2 (L-type)	P2X3
Cav2.1/ β 4 (P/Q-type)	P2X4
Cav2.2/ β 3/ α 2 δ (N-type)	TRPA1
Cav3.2 (T-type)	TRPC1
I _K (Kca3.1)	TRPC4
Nav1.3	TRPV1
Nav1.7	Channels available later in 2008
Nav1.8	Nav1.9
NMDA receptor (NR1/NR2A)	P2X7
NMDA receptor (NR1/NR2B)	

Ion Channel Family Panel



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Voltage-gated Sodium Channel Family

Nav1.1

Nav1.2

Nav1.3

Nav1.4

Nav1.5

Nav1.6

Nav1.8

Nav1.9



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Part 4: Optimization of ion channel cell lines on automated EPhys platforms

QPatch Success^{°*}



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Qplate	Primed	Cell-attached	Seal	Whole cell	Completed experiment	Analyzed experiment
1538	47	46	46	43	29	28
1537	46	46	43	36	27	21
1539	47	47	41	39	25	23
1540	48	48	43	30	21	18
1531	47	46	45	39	33	26
1532	47	47	42	40	32	27
1533	48	48	48	44	28	20
Success Rate (%)	98.2	97.6	91.7	80.7	58.0	48.5

[°]best practice

*CHO-HERG

IW Quattro Success^o



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Cell Line	Hole #	Seal Test*	At start*	At end**
CHO/Nav1.1	374	358	368	323
CHO/Nav1.4	369	345	365	333
CHO/Nav1.5	334	297	317	301
CHO/Nav1.7	342	292	340	325
CHO/Nav1.8	368	335	303	186
CHO/hERG	372	339	356	321
CHO/Kv _{LQT1} /minK	381	377	371	365
Success Rate (%, mean \pm SD)	93.8 \pm 4.7	86.9 \pm 8.1	91.3 \pm 5.2	85.4 \pm 5.0

^o best practice

*Rs > 30 M Ω ; hole # increased

**Rs > 30 M Ω ; Δ Rs < 25%; Δ v < 5 mV; 25 mins

PX Success



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Cell Line	Hole #	Verified Seal #	Percent Sealed	Average Seal (MΩ)	Whole -Cell #	Percent Whole-Cell	Rm Initial (MΩ)	Rm Final (MΩ)
CHO Cav1.2	577	413	71.6%	1109.0	376	65.2%	547.5	531.1
HEK Cav1.2	379	303	79.9%	766.7	281	74.1%	540.1	547.0
HEK Cav2.1	252	161	63.9%	613.7	151	59.9%	419.2	501.2
HEK Cav2.2	166	115	69.3%	636.2	108	65.1%	498.2	632.8
HEK Kv1.5	71	47	66.2%	724.3	43	60.6%	386.1	433.8
HEK Kv4.3	90	50	55.6%	1407.6	43	47.8%	436.7	305.4
CHO KvLQT/minK	44	39	88.6%	760.0	37	84.1%	345.1	361.6
HEK Kir3.1/3.4	41	31	75.6%	1528.9	27	65.9%	409.0	593.4
HEK Kir6.2/SUR2A	39	33	84.6%	3306.4	29	74.4%	1448.4	510.4
HEK Nav1.5	465	295	63.4%	917.3	281	60.4%	465.6	243.4

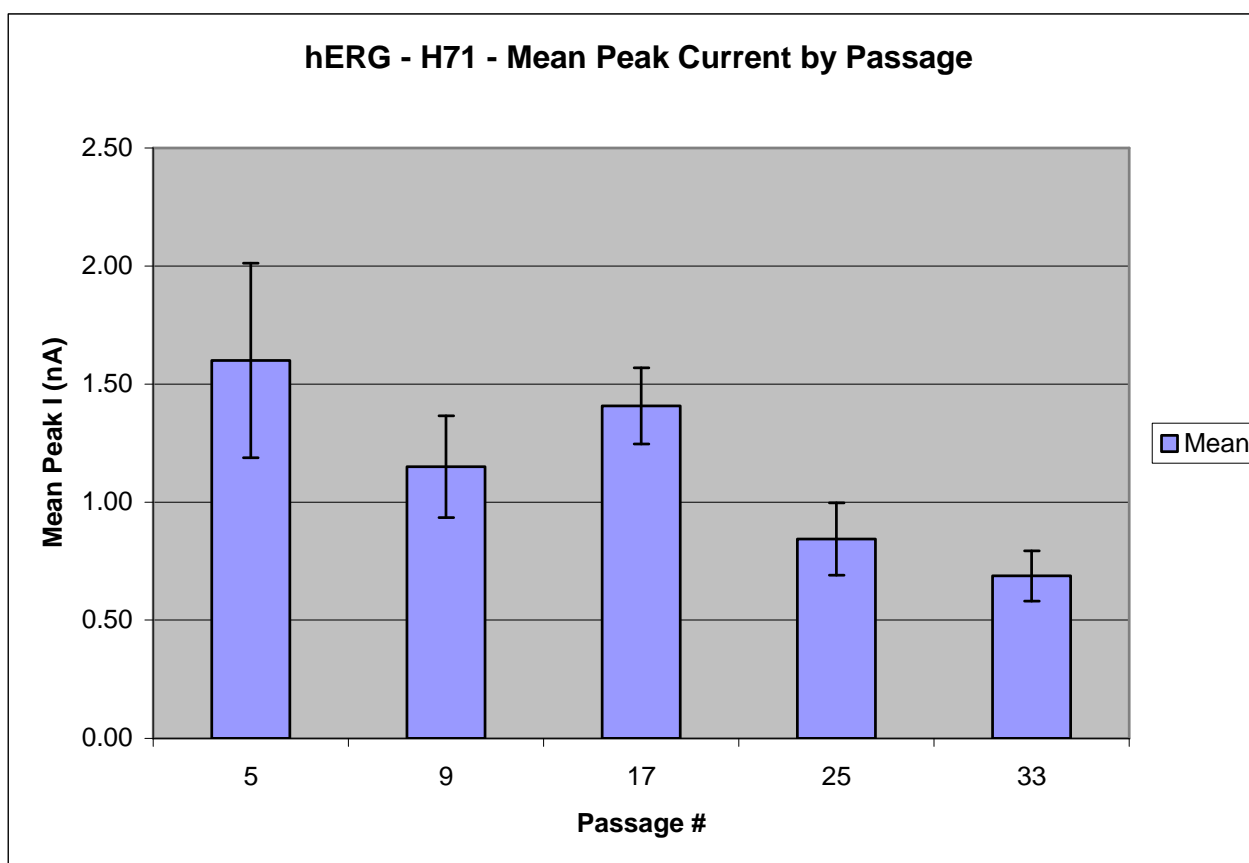
HERG Stability - HEK



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Passage #	Mean	STDEV	SEM	n
5	1.60	0.92	0.41	5
9	1.15	0.61	0.22	8
17	1.41	0.84	0.16	27
25	0.84	0.63	0.15	17
33	0.69	0.60	0.11	32



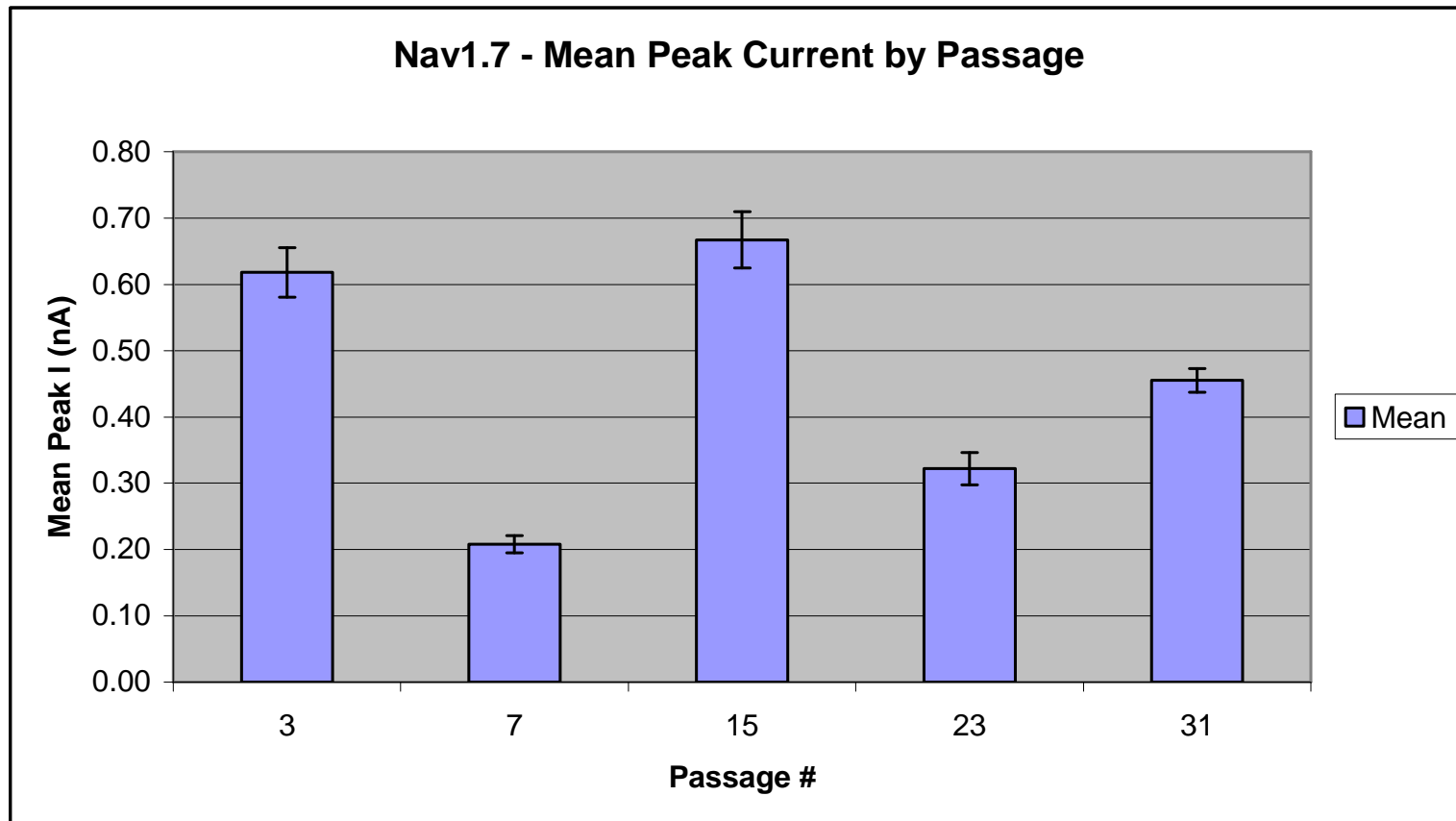
Nav1.7 Stability - CHO



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Passage	Mean	SEM	n
3	0.62	0.04	114
7	0.21	0.01	112
15	0.67	0.04	111
23	0.32	0.02	89
31	0.46	0.02	327



Ion Channel Cell Lines for Sale*



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Cell Line	Validated Platforms
Kv1.5-HEK	MPC, PX
Kv4.3-HEK	MPC, PX
Kv1.3-HEK	PX
HCN4-HEK	MPC, PX
hERG-CHO	MPC, PX, QPatch, Quattro
KvLQT1/minK-CHO	MPC, PX, QPatch, Quattro
Nav1.7-CHO	PC, QPatch, Quattro

Cell Line	Validated Platforms
TRPA1-CHO	QPatch, FLIPR
Nav1.4-CHO	MPC, PX, Quattro
Cav3.2-HEK	MPC, PX
Nav1.1-HEK	MPC, PX
Nav1.3-HEK	MPC, PX
Nav1.6-HEK	MPC, PX
TRPV1-HEK	MPC, PX, FLIPR

* Validation summary; cell husbandry and preparation

Non-Instrument Factors Affecting Assay Success



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- Cell husbandry
 - passage
 - density
- Cell preparation
 - cell-lift reagents
 - spin prior to final resuspension
 - trituration of pellet
- Compound preparation
 - Minimal-binding compound plates
 - glass vials
 - DMSO dilution

Conclusions



The Ion Channel Company

www.chantest.com

- ChanTest provides the highest quality, preclinical cardiac risk assessment service including molecular, cellular, tissue and whole heart assays.
- The Cardiac Channel Panel™ is the most comprehensive ion channel screen and biomarker for cardiac arrhythmia risk assessment.
- ChanTest is developing the world's largest and most completely validated Automated Patch Clamp-Ephys/Ion Channel Library platform.
- The APC-Ephys/ICL platform enables rapid, accurate screening of drugs against channel panels arranged by tissue, therapeutic area and channel family. Panels test 1° and 2° ion channel PD effects (potency, selectivity and off-target profiling).
- The APC-Ephys/ICL platform identifies toxicity/safety indicators at early stages of drug development quickly and economically.
- ChanTest will make its cell lines optimized for automated Ephys instruments available for purchase.

Acknowledgement



The Ion Channel Company

www.chantest.com

Thanks to my colleagues at ChanTest.

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Cell Line Validation



The Ion Channel Company

www.chantest.com

HEK Cell Lines: Available Assays				CHO Cell Lines: Available Assays					
Channels	Assays			Channels	Assays				
	Manual patch	FastPatch (PatchXpress)	FLIPR		Manual patch	FastPatch (PatchXpress)	FastPatch (Qpatch)	SuperFastPatch (IW Quattro)	FLIPR
5-HT3A	✓	✓							
				ASIC1a					
				ASIC2a		✓			
				BK	✓		✓		
				Cav1.1 (SKM L-type)					
Cav1.2/β2 (L-type)	✓	✓	✓	Cav1.2/β2/α2δ (L-type)	✓	✓	✓	✓	
Cav2.1/β4 (P/Q-type)	✓	✓		Cav2.1/β4 (P/Q-type)					
Cav2.2/β3/α2δ (N-type)	✓	✓	✓	Cav2.2/β3/α2δ (N-type)	✓	✓	✓	✓	✓
Cav3.2 (T-type)	✓	✓		Cav3.2 (T-type)	✓	✓	✓	✓	
				CFTR	✓	✓	✓	✓	
				CLC-1					
				CLC-2					
				CLC-KA/barttin					
				CLC-KB/barttin					
				CNGA1/CNGB1					
				CNGA3/CNGB3					
EAG1									
				GABA-A (a2b2g2)		✓			
				GABA-A (a5b2g2)		✓			
HCN1	✓	✓							
				HCN2			✓	✓	
				HCN3					
HCN4	✓	✓		HCN4	✓	✓	✓	✓	
HERG1	✓	✓		HERG1	✓	✓	✓	✓	
				IK			✓		
KCNQ2/KCNQ3	✓	✓							
KCNQ3/KCNQ5	✓	✓							
				KCNQ4					
Kir2.1	✓	✓		Kir1.1					
				Kir2.1	✓	✓	✓	✓	
				Kir3.1/Kir3.2					
Kir3.1/Kir3.4	✓	✓		Kir3.1/Kir3.4					
				Kir6.1/SUR2B					
Kir6.2/SUR1									
Kir6.2/SUR2A	✓	✓							
				Kir6.2/SUR2B					
Kv1.1	✓	✓							
Kv1.2	✓	✓		Kv1.2	✓	✓	✓	✓	
Kv1.3	✓	✓							
Kv1.4	✓	✓		Kv1.4	✓	✓	✓	✓	
Kv1.5	✓	✓		Kv1.5	✓	✓	✓	✓	
				Kv2.1/Kv9.3					
Kv3.4	✓	✓		Kv3.4	✓	✓	✓	✓	
				Kv4.1					
Kv4.2/KChiP2.2	✓	✓							
Kv4.3	✓	✓		Kv4.3	✓	✓	✓	✓	
KvLQT1/minK	✓	✓		KvLQT1/minK	✓	✓	✓	✓	

Cell Line Validation



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HEK Cell Lines: Available Assays				CHO Cell Lines: Available Assays					
Channels	Assays			Channels	Assays				
	Manual patch	FastPatch (PatchXpress)	FLIPR		Manual patch	FastPatch (PatchXpress)	FastPatch (Qpatch)	SuperFastPatch (IW Quattro)	FLIPR
Nav1.1	✓	✓ (Use dep)		Nav1.1	✓	✓ (Use dep)	✓	✓ (Use dep)	
Nav1.2	✓	✓ (Use dep)		Nav1.2	✓	✓ (Use dep)		✓ (Use dep)	
Nav1.3	✓	✓		Nav1.3					
Nav1.5	✓	✓		Nav1.4	✓	✓ (Use dep)	✓	✓ (Use dep)	
Nav1.6	✓	✓ (Use dep)		Nav1.5	✓	✓ (Use dep)	✓	✓ (Use dep)	
Nav1.7	✓	✓ (Use dep)		Nav1.6					
Nav1.8	✓	✓		Nav1.7	✓	✓ (Use dep)	✓	✓ (Use dep)	
Nav1.9				Nav1.8	✓	✓ (Use dep)	✓	✓ (Use dep)	
NCX1	✓			Nav1.9					
NMDA receptor (NR1/NR2A)	✓			Nicotinic receptor (alpha7)					
NMDA receptor (NR1/NR2B)	✓			NMDA receptor (NR1/NR2A)					
P2X1	✓	✓	✓	NMDA receptor (NR1/NR2B)					
P2X2			✓						
P2X2/P2X3			✓						
P2X3	✓	✓							
P2X4			✓						
P2X7									
				SK1					
				SK2			✓		
				SK3		✓	✓		
				TRPA1			✓		✓
TRPC1	✓								
TRPC4	✓								
TRPC6	✓		✓	TRPC6					
				TRPM2	✓				
				TRPM4	✓				✓
				TRPM8	✓		✓		✓
				TRPP1/TRPP2					
TRPV1	✓	✓	✓						
				TRPV4	✓				✓
TRPV6	✓	✓							

validation document; ready for studies	✓
validation data but no document; for studies	✓
validation in progress	
cell line under construction	

cell line available for study now (6-13-08)
cell line in progress; to be completed in 2008
cell line to be completed by mid-2009