

Reading The Ion Channel Library with the Qpatch-HT



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6/24/08

Aurora Biomed: 6th annual ion channel retreat

Stephen S. Smith, Ph.D
Study Director, ChanTest Corporation

The most trusted CRO for ion channels



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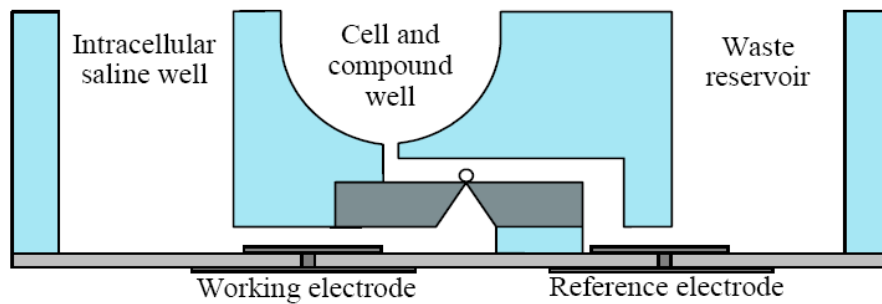
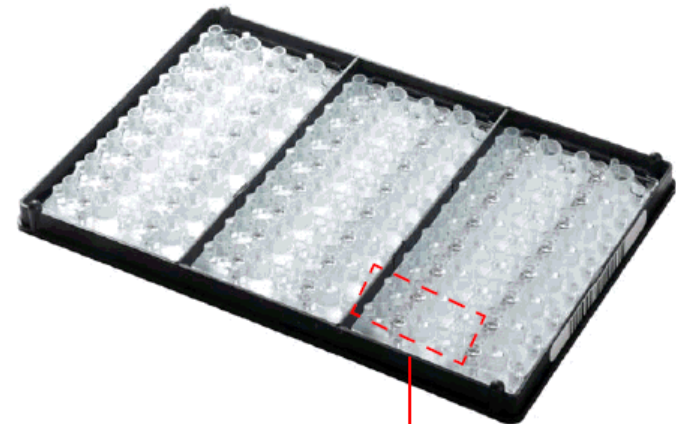
- Located in beautiful Cleveland, Ohio
- More than 40 ion channel cell lines, and new ones appearing regularly
- Manual Patch clamp, sharp electrode, Ussing Chambers, trafficking assays.
- Multiple PatchXpress
- IonWorks Quattro
- FLIPRs
- **The Qpatch-HT**

The Qpatch-HT



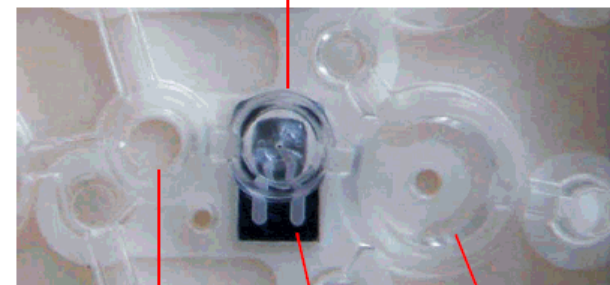
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Cell and compound well

A single measurement site



Intracellular saline well

Silicon Chip

Waste Reservoir

The screening station console



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Job: #810 - MKJ_HEK Nav1.2 IV n=1

Barcode: 0058533000045

QPlate

A	1	2	A	3	4	A	5	6
B	●	●	B	●	●	B	●	●
C	●	●	C	○	●	C	●	●
D	●	●	D	●	●	D	●	●
E	●	●	E	●	●	E	○	●
F	●	●	F	●	●	F	●	○
G	●	●	G	●	○	G	●	●
H	●	●	H	●	●	H	●	●

- Not used
- Ready
- Cell applied
- Cell positioned
- Gigaseal obtained
- Whole-cell obtained
- Test Experiment
- Experiment Tested
- Failed

	R [MΩ]	P [mbar]	V-hold [mV]	R-series [MΩ]	C-slow [pF]
A5	2273.33	-20	-90	4.5	6.2
A6	1276.54	-20	-90	4.5	14.8
B5	208.04	-20	-90	4.6	7.9
B6	2009.26	-20	-90	6.5	6.7
C5	262.64	-20	-90	3.2	7.0
C6		0	0		
D5	9213.56	-20	-90	4.2	8.0
D6	1220.62	-20	-90	2.9	18.4
E5	61.80	0	-90		
E6	1064.02	-20	-90	5.5	7.9
F5	4452.06	-20	-90	3.5	7.8
F6	339.70	0	-90	54.5	5.1
G5	4508.83	-20	-90	2.9	9.5
G6	13.71	0	-90	5.6	12.1
H5	319.59	-20	-90		
H6	873.67	-20	-90	3.2	17.6

MTP 2 MTP 1 MTP 3 **QPlate**

Job Summary

	QPlate	Total
Gigaseals obtained:	11	11
Whole-cells obtained:	34	34
Experiments completed:	0	0
Job start time:	14:23	More...

Sweep Response

Current scale: 100 pA per division QPlate position: **All**

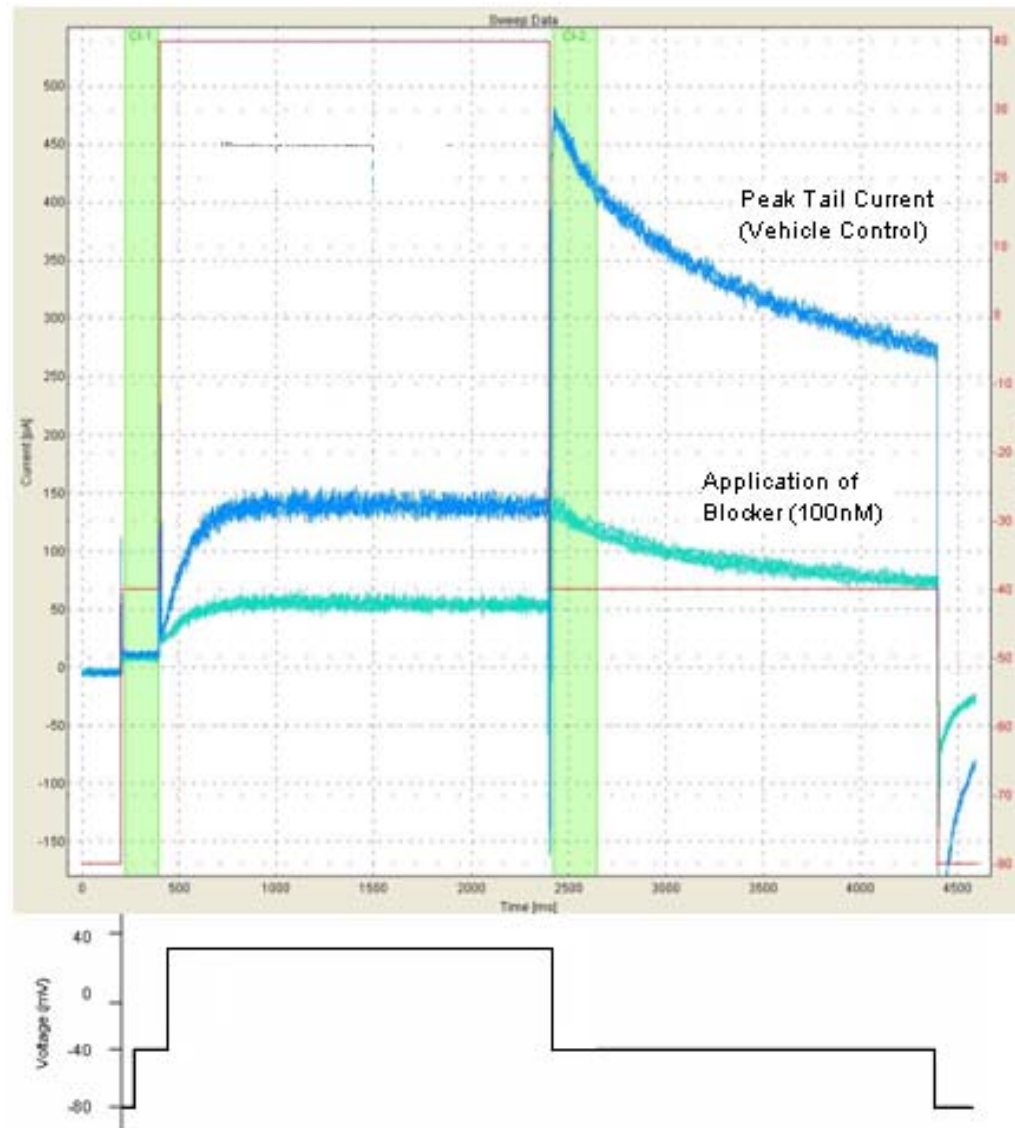
Time scale: 10 ms per division

hERG: The Petroski protocol



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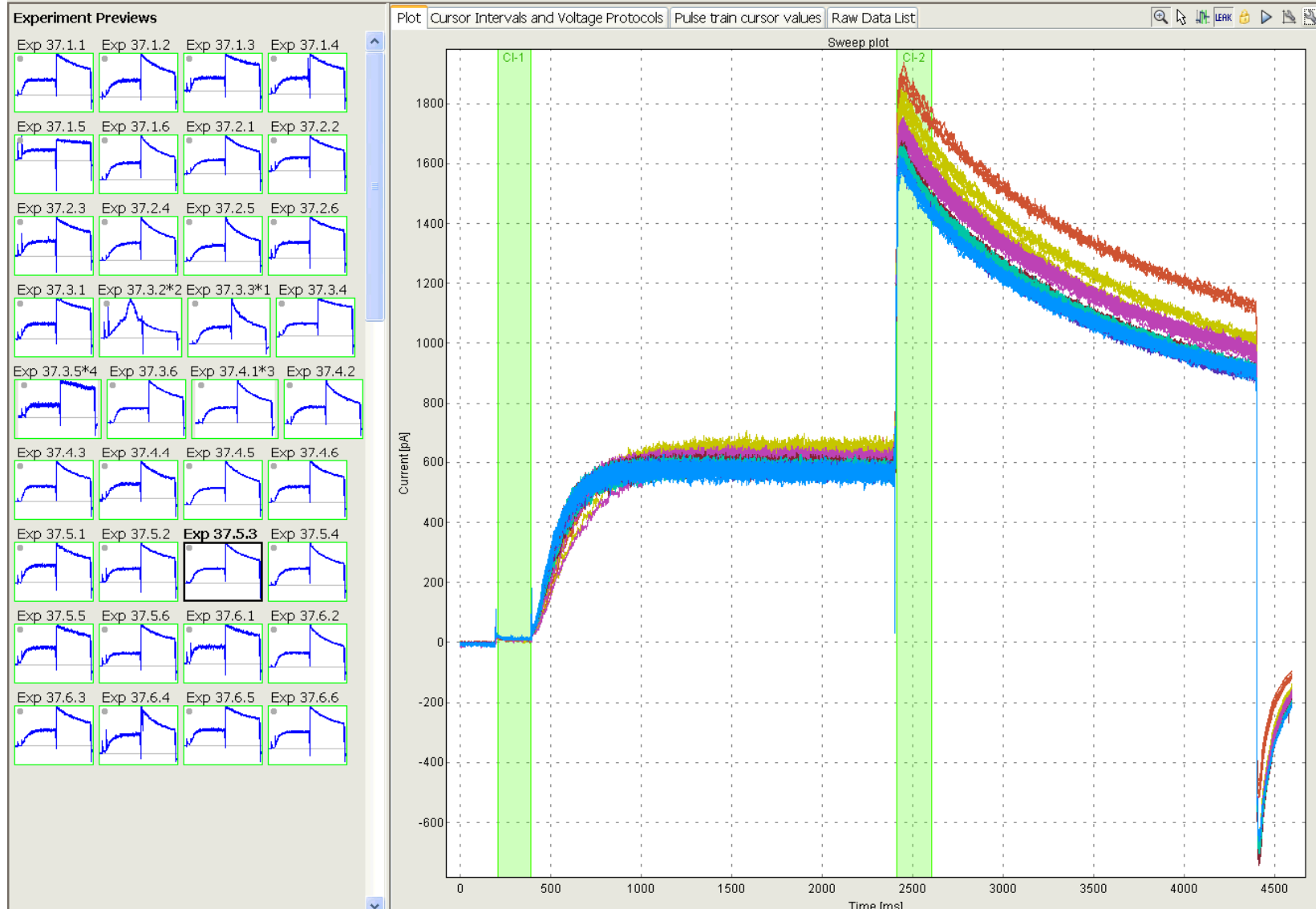


Not too bad out of the box



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Performance metrics



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QPlate '00965535001532'

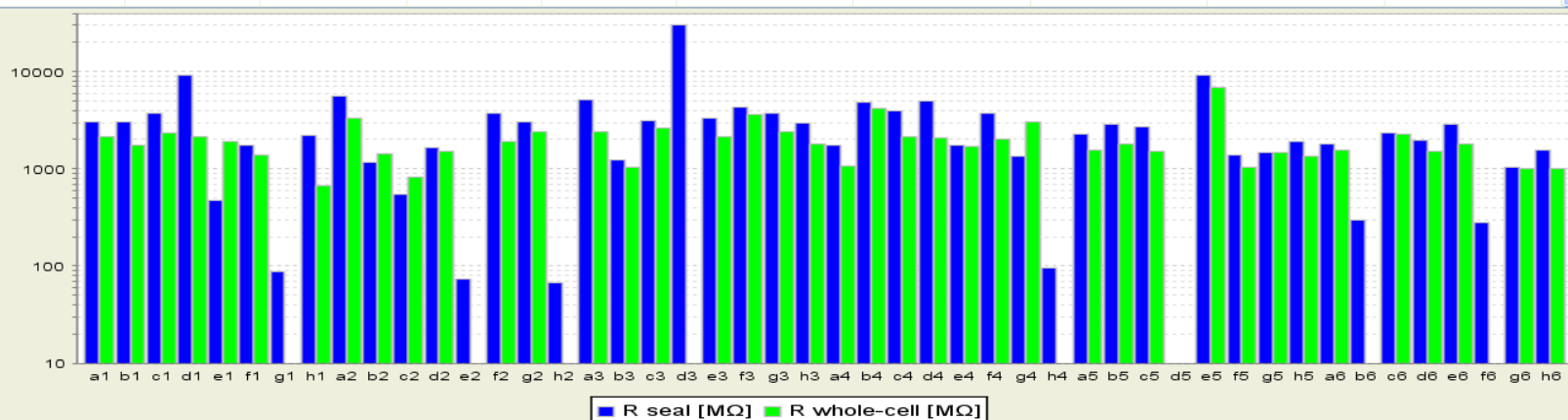
Used in job: #37 - GZHU_Terfenadine 11192007

Start of use: 2007-11-19 14:11:38

Pos.	Primed	Cell attached	Seal	Whole-cell	R chip [MΩ]	R seal [MΩ]	R whole-cell [MΩ]	WC duration [sec]	Completed exp.
C4	✓	✓	✓	✓	2.57	3916.2	2142.5	1378	1
D4	✓	✓	✓	✓	2.86	5033.6	2098.5	1513	1
E4	✓	✓	✓	✓	2.51	1755.7	1679.4	1323	1
F4	✓	✓	✓	✓	2.50	3697.1	2010.3	1297	1
G4	✓	✓	✓	✓	2.56	1330.2	3071.0	695	0
H4	✓	✓	✓	✓	2.47	96.7	0.0	0	0
A5	✓	✓	✓	✓	2.47	2250.9	1579.8	1476	1
B5	✓	✓	✓	✓	2.47	2875.5	1797.2	1379	1
C5	✓	✓	✓	✓	2.53	2730.8	1528.7	1520	1
D5	✓	✓	✓	✓	0.00	0.0	0.0	0	0
E5	✓	✓	✓	✓	2.63	9241.6	6858.1	1332	1
F5	✓	✓	✓	✓	2.51	1389.7	1025.9	1329	1
G5	✓	✓	✓	✓	2.52	1474.1	1464.0	1328	1

	Primed	Cell attached	Seal	Whole-cell	R chip [MΩ]	R seal [MΩ]	R whole-cell [MΩ]	WC duration [min]	Completed exp.
Total	47	46	45	39	2.4	4693.0	999.1	19.7	33
Success rate	98%	96%	94%	81%					70%

Total	47	47	42	40					32
Success rate	98 %	98 %	88 %	83 %					



Print... Export Cursor Values...

Go to QPlate position Go to Job

Initial validation performance



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Qplate	Primed	Cell attached	Seal	Wholecell	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

Average Seal Resistance = 1940 M Ω

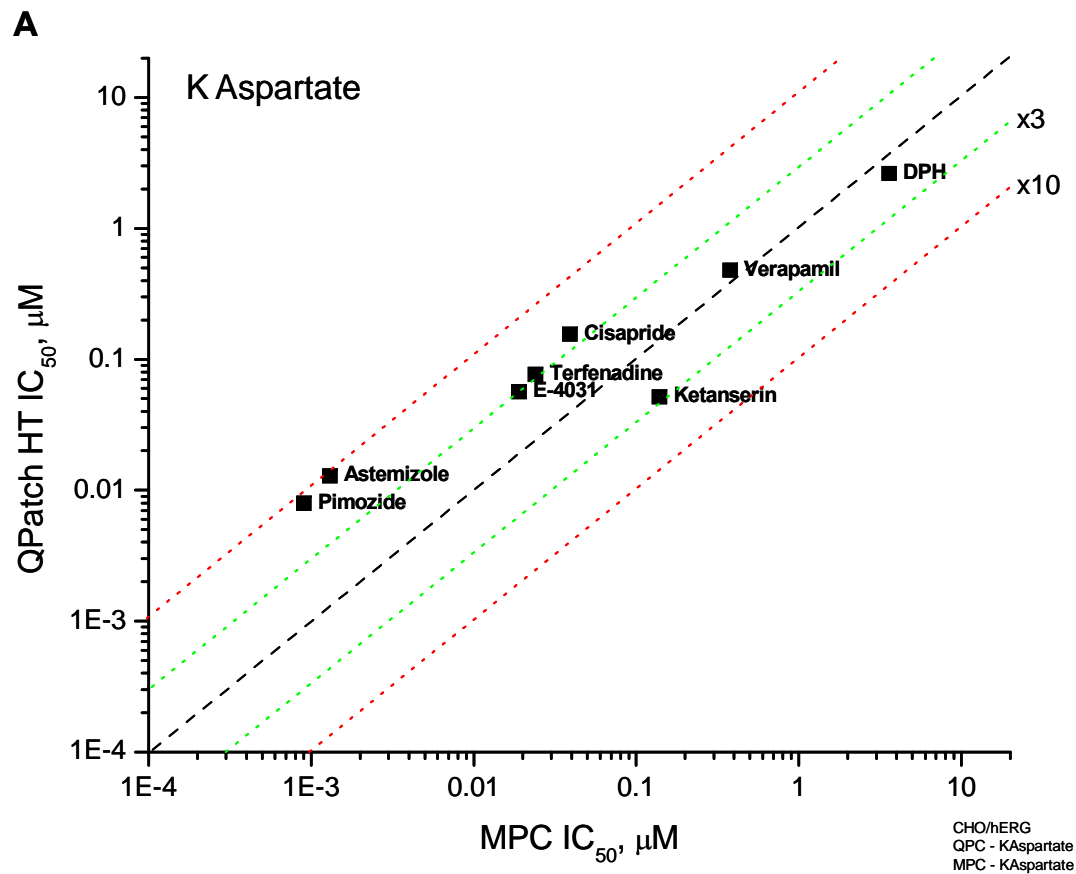
Average Membrane Resistance = 1170 M Ω

Validation Pharmacology



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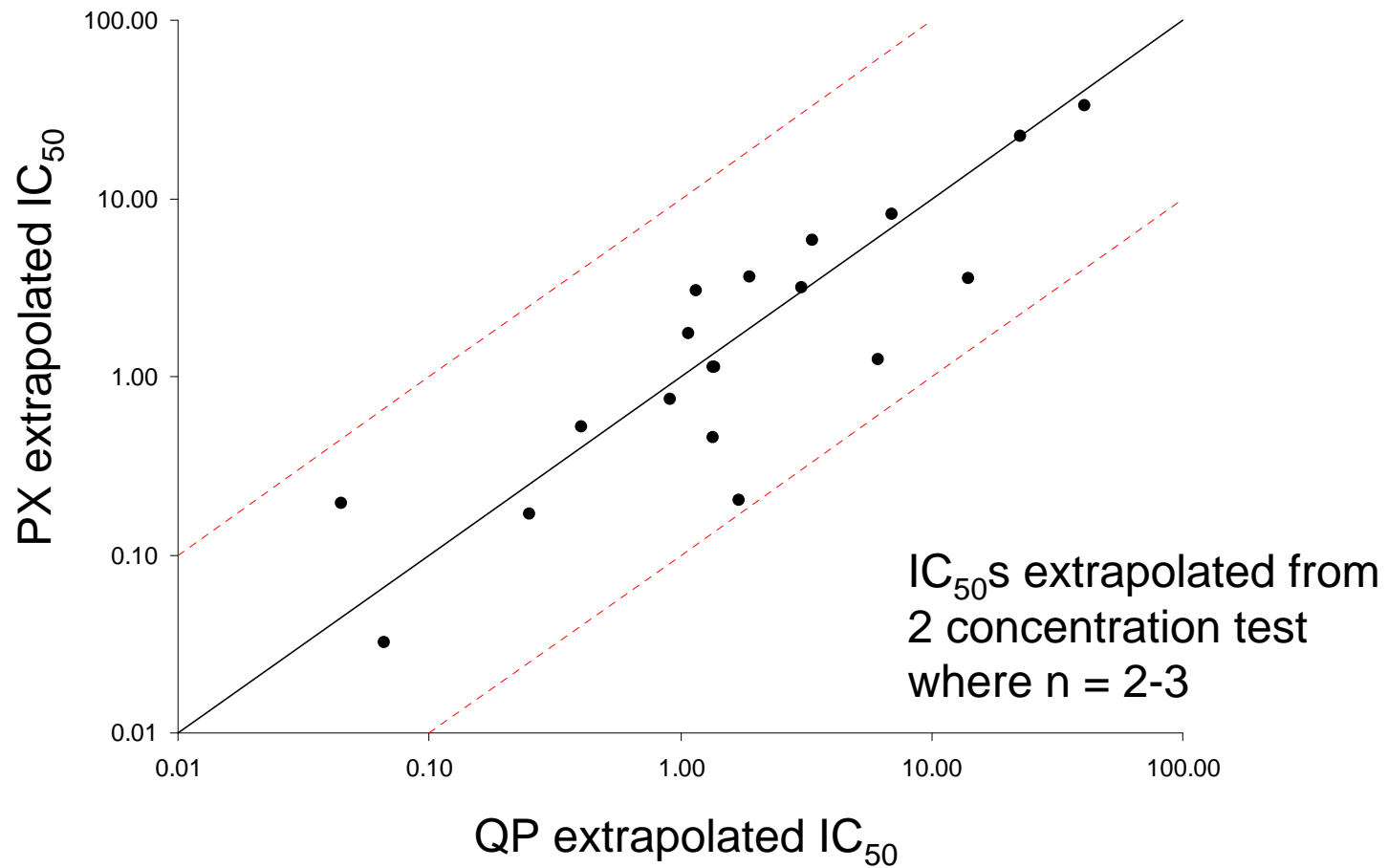


Comparative study



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Myth: Tuesdays are bad for hERG



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Study design specified 176 data points including controls

Expected requirements: 6 Qplates, 5 hrs instrument time, 3 hrs prep time

Chambers	Primed	Cell attached	Seal	Whole-cell	Completed exp.	Analyzed
256	247	241	226	209	160	112
	96%	94%	88%	82%	63%	44%
		98%	91%	85%	65%	45%

R chip [MΩ]	R seal [MΩ]	R whole-cell [MΩ]	WC duration [min]
2.7	3213	2264	20

Used 5 1/3 Qplates, 6 hrs of instrument time and 3 hrs of prep time

Myth: CHO work better than HEK



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QPlate '00965535001736'

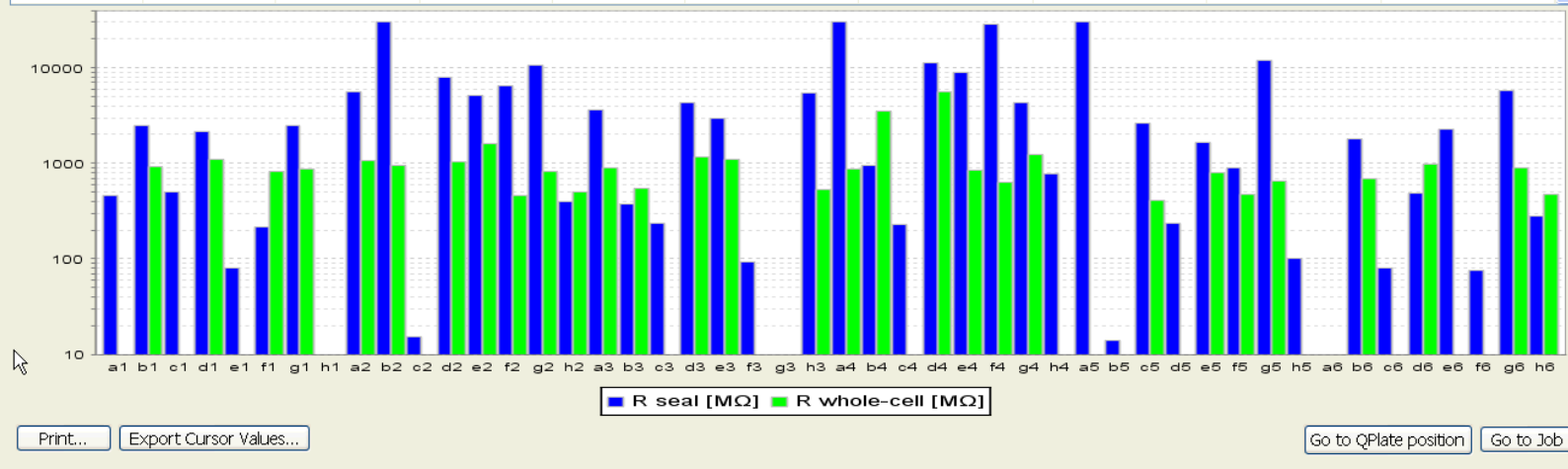
Used in job: #142 - GZHU_Cav3.2 03122008b

Start of use: 2008-03-12 13:43:30

Pos.	Primed	Cell attached	Seal	Whole-cell	R chip [MΩ]	R seal [MΩ]	R whole-cell [MΩ]	WC duration [sec]	Completed exp.
C4	✓	✓	✓			1.99	230.7	0.0	0
D4	✓	✓	✓	✓		1.97	11127.2	5600.2	1707
E4	✓	✓	✓			2.00	9041.9	839.7	1712
F4	✓	✓	✓	✓		1.98	28339.4	636.0	1701
G4	✓	✓	✓	✓		2.04	4348.6	1220.3	1708
H4	✓	✓	✓			2.00	778.9	0.0	0
A5	✓	✓	✓			2.06	30000.0	0.0	0
B5	✓	✓	✓			2.06	14.1	0.0	0
C5	✓	✓	✓			2.09	2670.3	405.5	1738
D5	✓	✓	✓	✓		2.05	234.0	0.0	0
E5	✓	✓	✓	✓		2.03	1660.8	799.2	1672
F5	✓	✓	✓	✓		2.09	898.0	473.8	1693
G5	✓	✓	✓	✓		2.10	11792.2	659.7	1695

	Primed	Cell attached	Seal	Whole-cell	R chip [MΩ]	R seal [MΩ]	R whole-cell [MΩ]	WC duration [min]	Completed exp.
Total	47	45	39	30	2.0	3323.0	571.3	17.4	28
Success rate	98%	94%	81%	63%					60%

Total	47	45	39	30					28
Success rate	98 %	94 %	81 %	63 %					

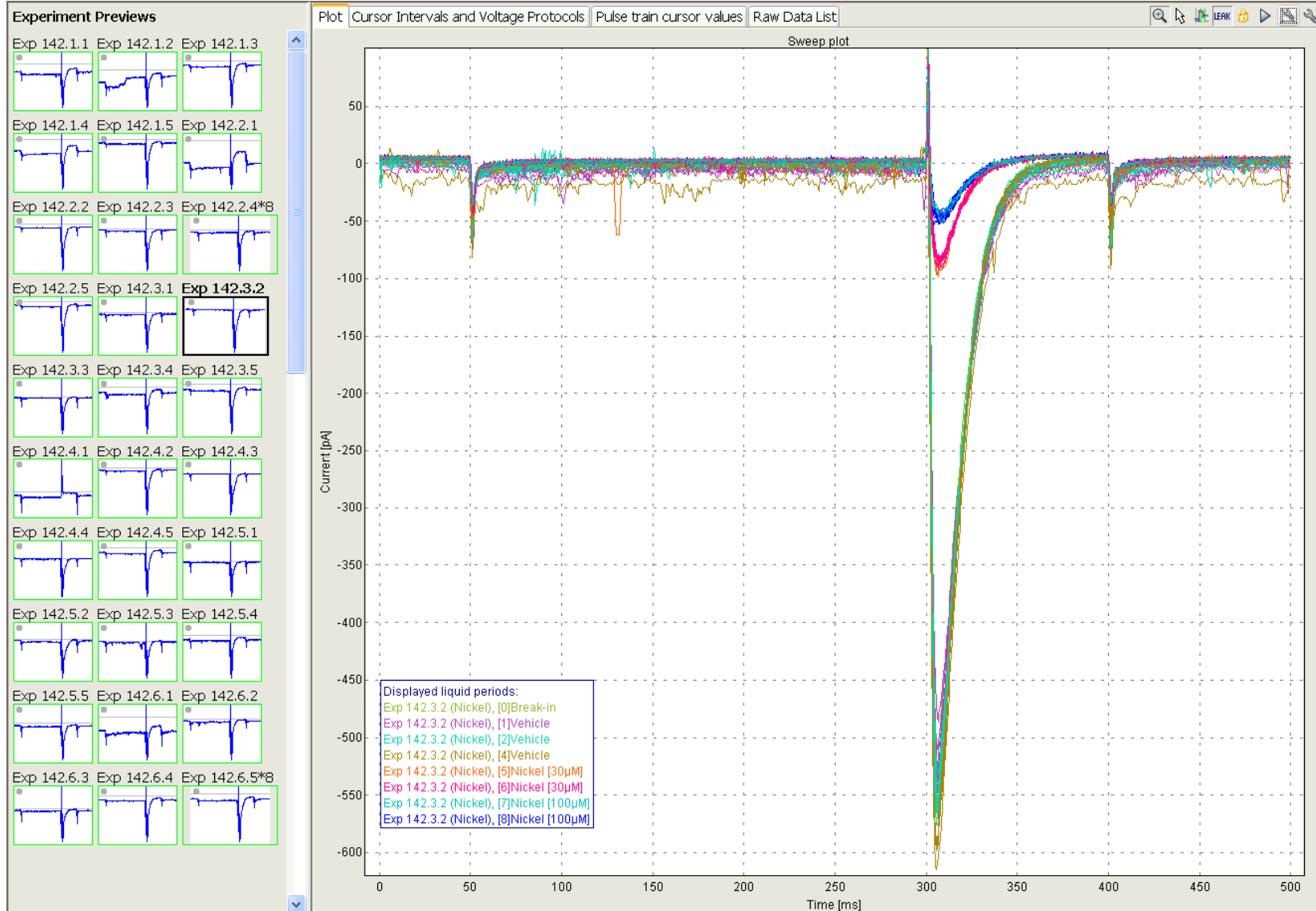


HEK Cav3.2 (T-type Ca channel)



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Cell lines validated on Qpatch-HT



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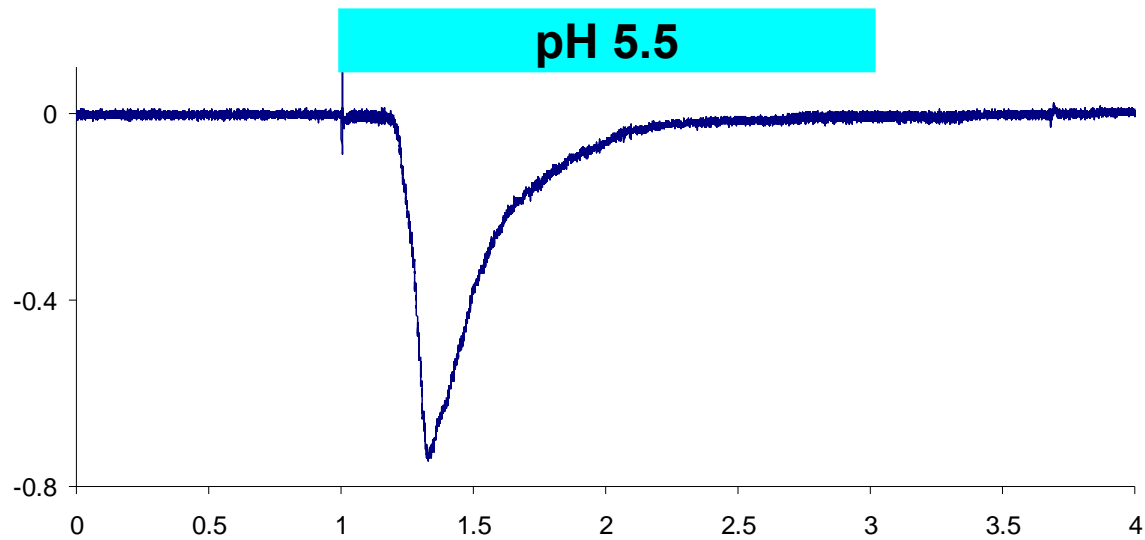
ASIC2A	Kv1.5
BK (KCa1.1)	Kv4.3
Cav1.2/(2/(2(KvLQT1-minK
Cav2.2/(3/(2(Nav1.1
Cav3.2	Nav1.4
CFTR	Nav1.5
HCN2	Nav1.6
HCN4	Nav1.7
hERG	Nav1.8
IK (KCa3.1)	SK2 (KCa2.2)
Kir2.1	SK3 (KCa2.3)
Kv1.2	TRPA1
Kv1.4	TRPM8

The ASIC2A current



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Starting the cell line



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- Confirmed construct and current with transient transfection.
- Transfect CHO T-REX cell
- Co-transfected with GFP
- FACS sorted for individual clones
- Approximately 50 clones expanded
- Characterization and selection

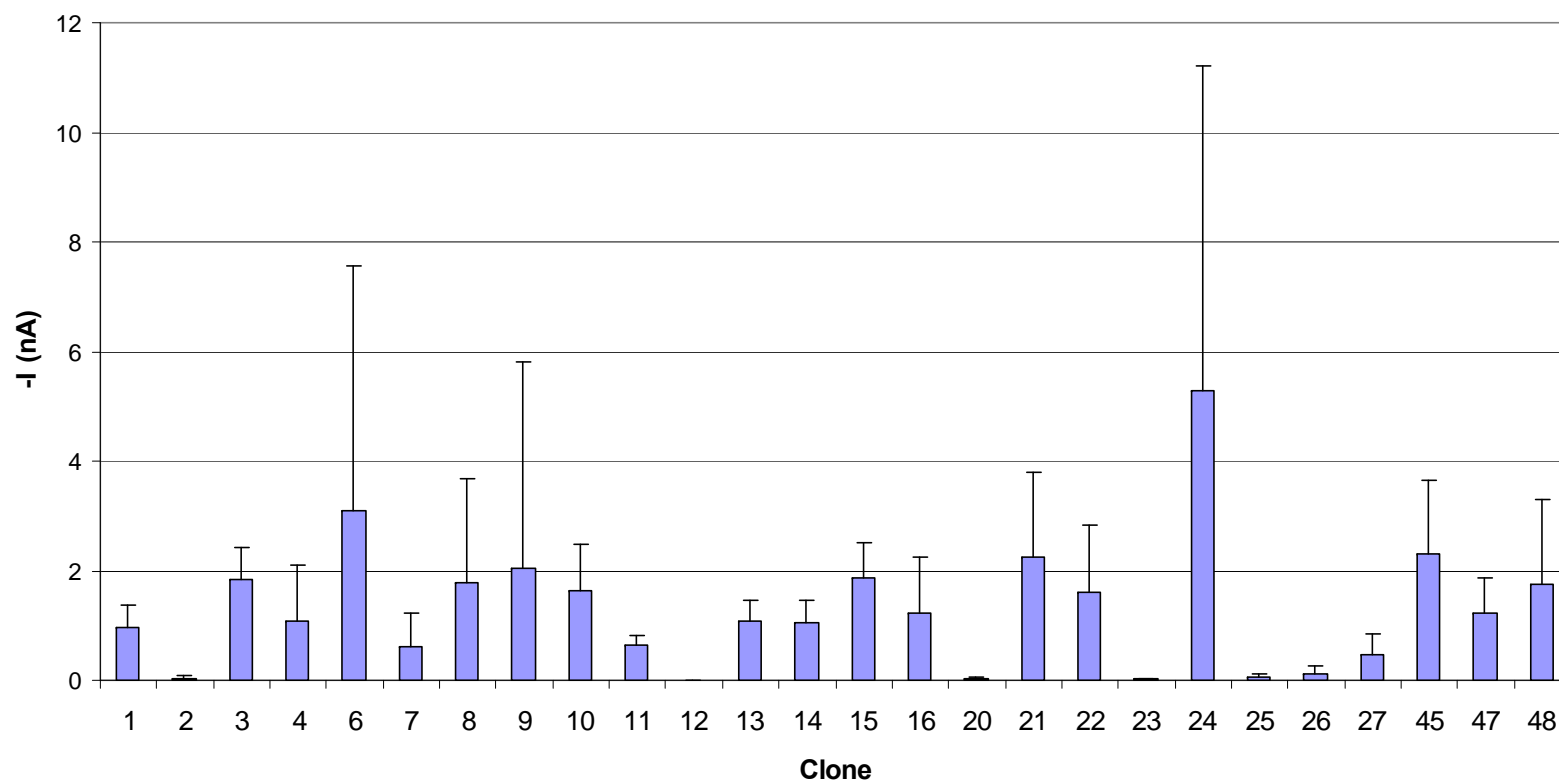
Send in the clones



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Mean Current +/- Standard deviation

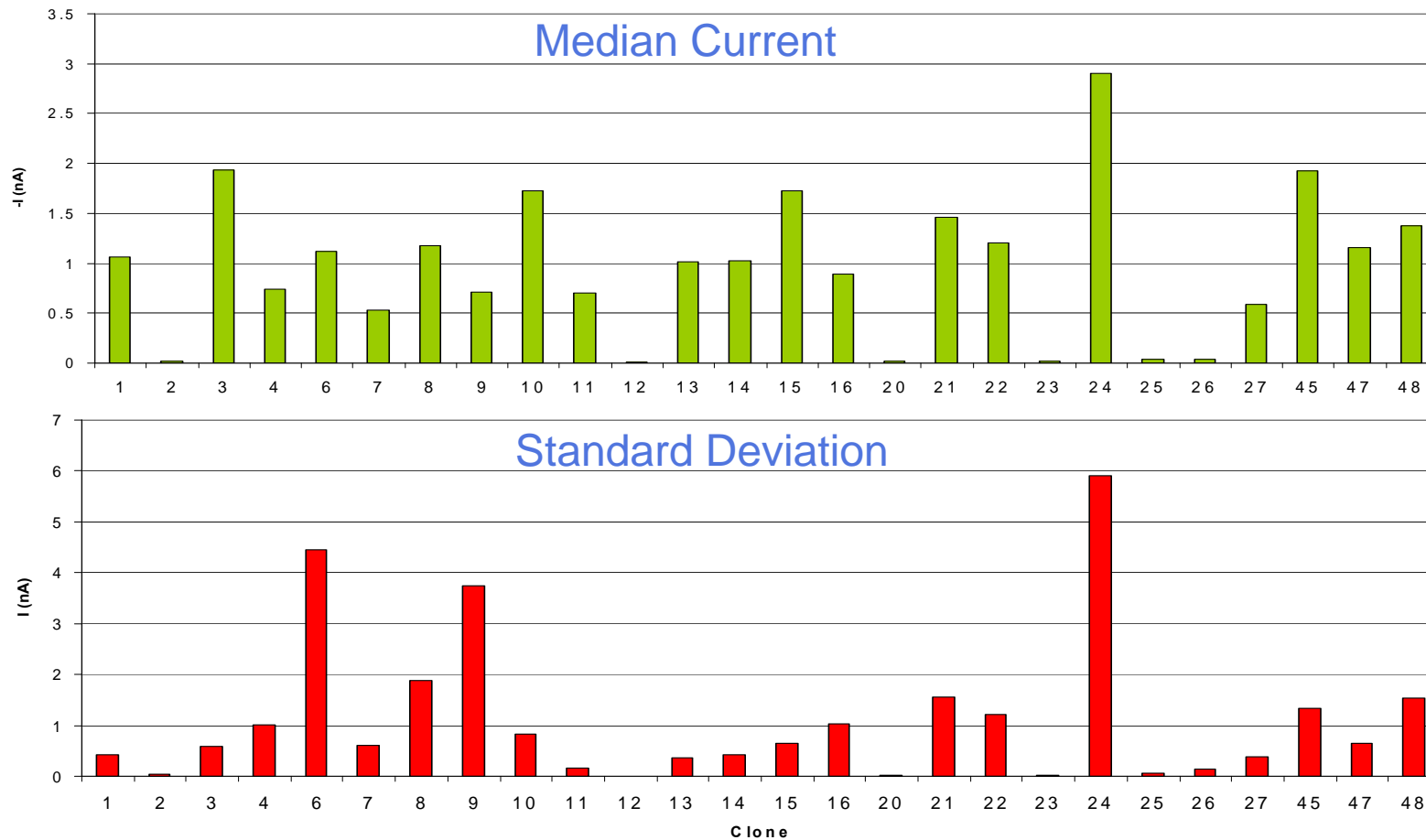


Picking a clone



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Picking a clone



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Clone	Mean	SD	Median	n	Mean > 1.5	Med > 1.5	Mean < 0.5	Med < 0.5
1	-0.95184	0.419061	-1.06659	5	0	0	0	0
2	-0.03745	0.048294	-0.01767	5	0	0	1	1
3	-1.83959	0.592007	-1.93787	9	1	1	0	0
4	-1.07384	1.014221	-0.74152	10	0	0	0	0
6	-3.09733	4.455149	-1.1208	8	1	0	0	0
7	-0.62272	0.59967	-0.52915	8	0	0	0	0
8	-1.78254	1.889068	-1.17995	14	1	0	0	0
9	-2.05106	3.746279	-0.71091	7	1	0	0	0
10	-1.64682	0.825826	-1.73009	7	1	1	0	0
11	-0.65431	0.169517	-0.7028	3	0	0	0	0
12	-0.00841	0.005118	-0.01057	7	0	0	1	1
13	-1.08538	0.366083	-1.01935	11	0	0	0	0
14	-1.03877	0.425119	-1.02372	6	0	0	0	0
15	-1.87848	0.640276	-1.72588	6	1	1	0	0
16	-1.22227	1.022864	-0.88976	8	0	0	0	0
20	-0.02732	0.024297	-0.01678	5	0	0	1	1
21	-2.25114	1.55259	-1.46468	7	1	0	0	0
22	-1.60865	1.214562	-1.20636	13	1	0	0	0
23	-0.01853	0.012235	-0.01621	8	0	0	1	1
24	-5.29692	5.913851	-2.89954	10	1	1	0	0
25	-0.05469	0.062457	-0.03506	7	0	0	1	1
26	-0.11124	0.145408	-0.03734	5	0	0	1	1
27	-0.4538	0.38508	-0.59126	3	0	0	1	0
45	-2.31073	1.333055	-1.92887	9	1	1	0	0
47	-1.21811	0.653508	-1.16106	8	0	0	0	0
48	-1.75833	1.529058	-1.37266	5	1	0	0	0
				26	11	5	7	6

The ASIC blocker experiment

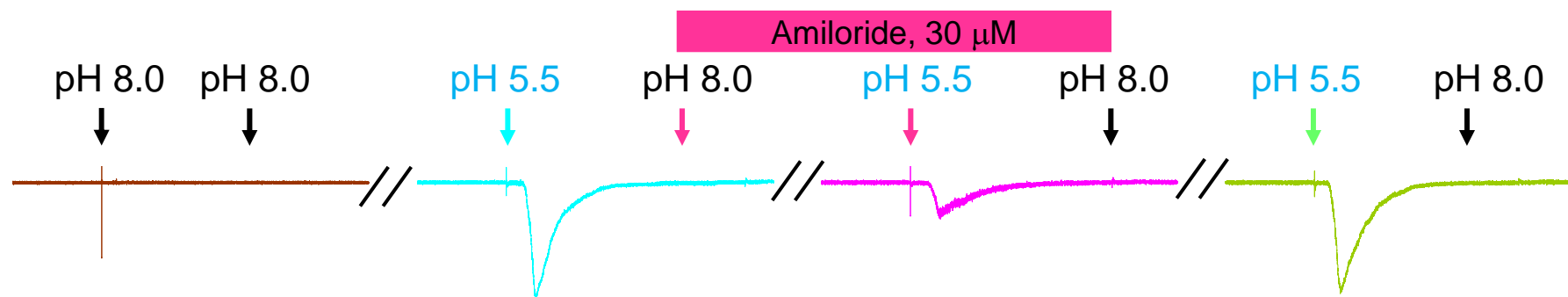
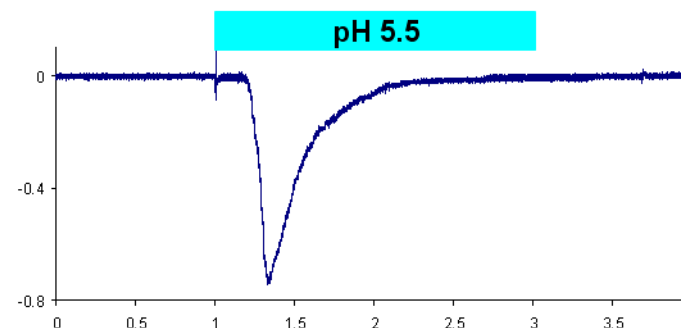


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The Compound plate layout

	1	2	3	4	5	6
A	■	■	■	■	□	□
B	□	□	□	□	□	□
C	□	□	□	□	□	□



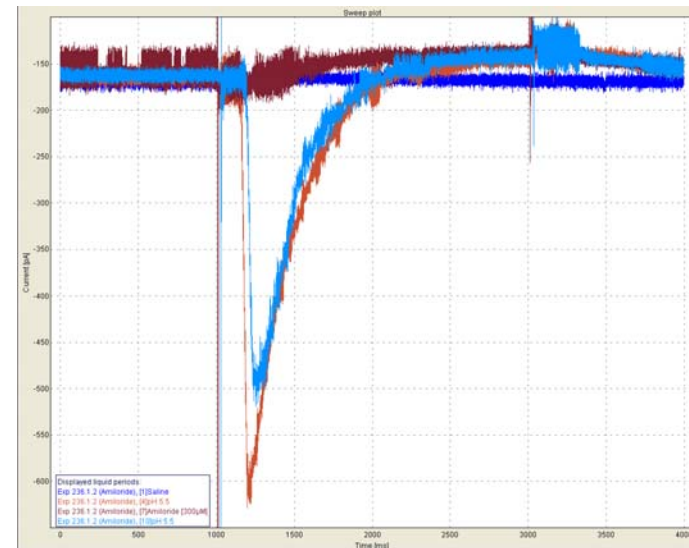
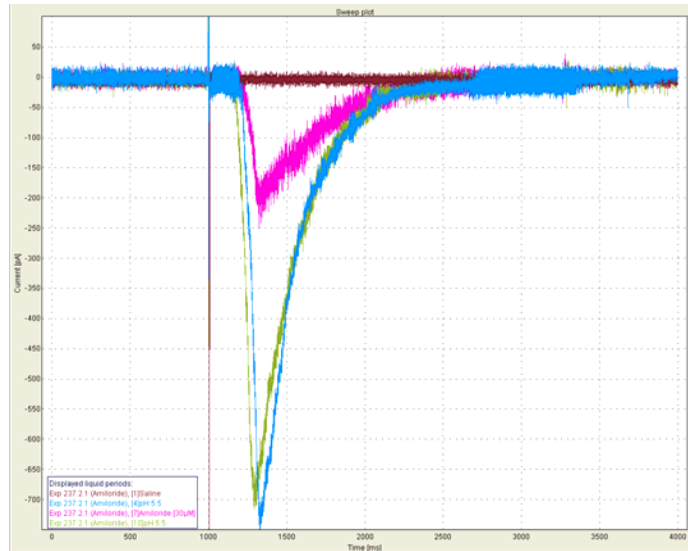
// = 1-4 minutes

ASIC2A amiloride inhibition

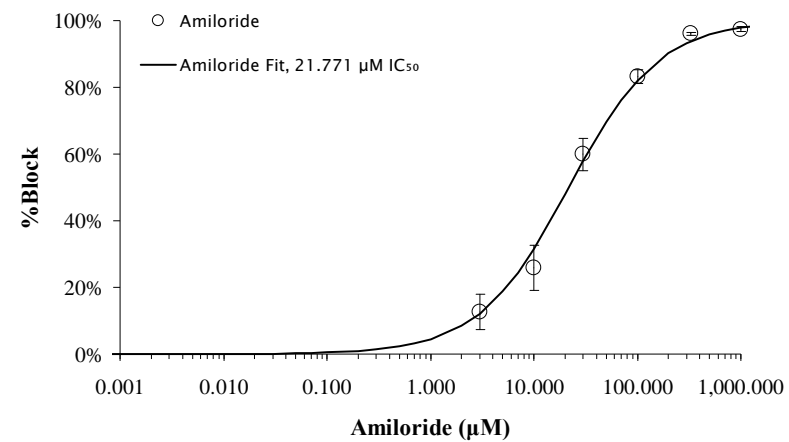


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Amiloride ASIC2A Concentration-Response

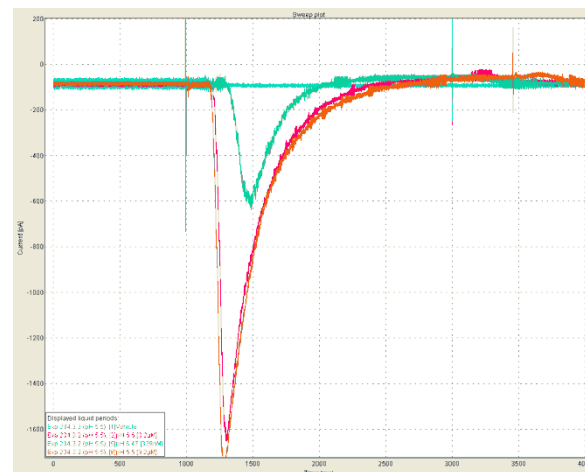
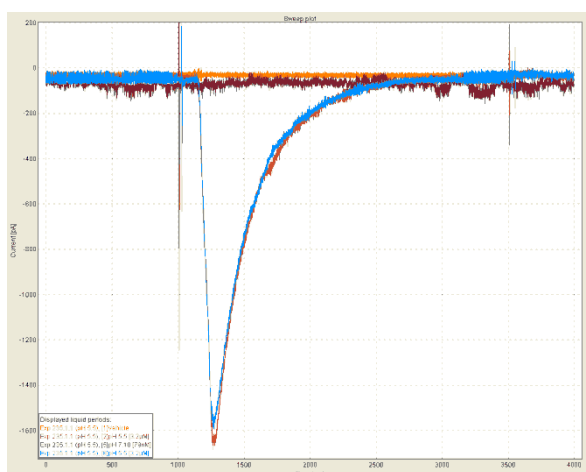


ASIC2A activation

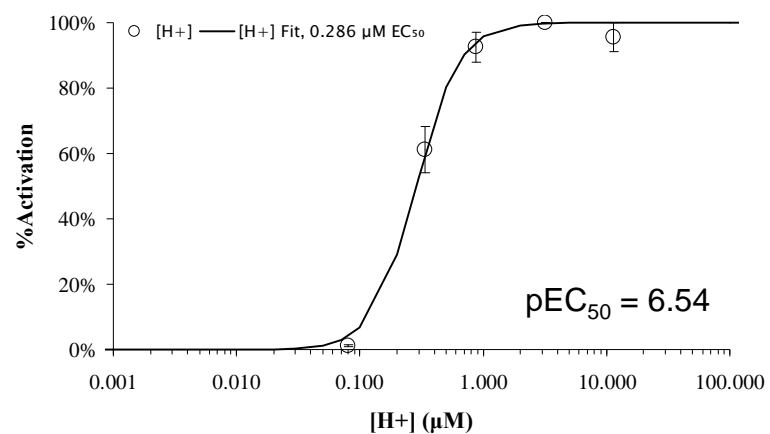


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[H⁺] ASIC2A Concentration-Response



Optimization in process



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A study which required a minimum of 22 good cells

Based on validation runs, planned for 44 cell recordings, using 3-5 Qplates

Accutase lift, 1 hr recovery

	Primed	Cell attached	Seal	Whole-cell	R chip [MΩ]	R seal [MΩ]	R whole-cell [MΩ]	WC duration [min]	Completed exp.
Total	46	37	17	8	2.5	851.5	695.4	8.2	8
Success rate	96%	77%	35%	17%					17%

Accutase lift, 15 min recovery

	Primed	Cell attached	Seal	Whole-cell	R chip [MΩ]	R seal [MΩ]	R whole-cell [MΩ]	WC duration [min]	Completed exp.
Total	46	44	33	23	2.5	2946.9	907.1	14.2	21
Success rate	96%	92%	69%	48%					46%

Accutase lift, no additional recovery

	Primed	Cell attached	Seal	Whole-cell	R chip [MΩ]	R seal [MΩ]	R whole-cell [MΩ]	WC duration [min]	Completed exp.
Total	47	45	37	27	2.7	4360.0	956.9	21.0	21
Success rate	98%	94%	77%	56%					45%

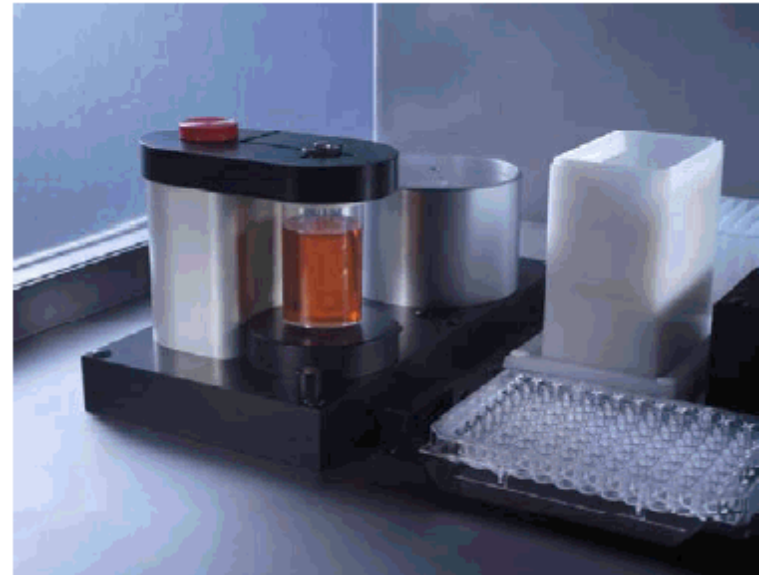
Continuation of the optimization process



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- Determine best lift and recovery conditions
- Determine best experimental paradigm
- Determine highest useable passage number



Summary



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- The Qpatch-HT has performed well with cell lines tested to date
- Key advantages
 - Increased throughput
 - Less effort per datapoint
 - Easier to transfer to staff scientist

Press Release



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Sophion and ChanTest announce ion channel cell line collaboration at the 6th Annual Ion Channel Retreat

Vancouver, BC (June 24, 2008). Today, Sophion Bioscience and ChanTest Corporation announced a landmark agreement regarding ion channel cell lines for ion channel drug discovery and safety testing at the 6th Annual Ion Channel Retreat. Under the terms of this agreement, Sophion has the right to provide ion channel cell lines from ChanTest that are optimized for Sophion's QPatch automated patch clamp systems.

Colleagues



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