



# Peer Network Resource Centers Wisconsin

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National New Disorders In-Person Meeting  
Washington, DC  
June 21-23, 2017



# Wisconsin Status

## *Pompe NBS Pilot*

- **Pompe NBS Pilot**
  - Enzyme/tandem mass spectrometry assay
  - GAA sequencing data as supplementary information to metabolic physicians
  - Starting date: July 10, 2017



	Median	10% of Median	15% of Median	# of Samples	# < 10%	# 10% to 15%	Min	Max
Day1	16.03	1.60	2.40	363	0	0	4.58	36.96
Day2	15.73	1.57	2.36	186	0	0	5.61	29.22
Day3	16.72	1.67	2.51	127	0	0	6.47	40.87
Day4	16.40	1.64	2.46	234	1	0	0.90	79.95
Day5	16.93	1.69	2.54	616	0	1	2.33	45.72
Day6	16.71	1.67	2.51	361	0	0	2.91	40.68
Day7	17.30	1.73	2.59	157	0	0	3.75	38.03
Day 8	16.08	1.61	2.41	223	0	1	2.30	35.25
Day9	16.91	1.69	2.54	563	0	0	4.31	52.68
Day10	16.70	1.67	2.51	473	0	0	3.96	61.10
Day 11	16.61	1.66	2.49	147	0	0	6.55	58.57
Day12	16.78	1.68	2.52	265	0	0	2.93	39.94
Day13	17.27	1.73	2.59	527	0	0	5.6	53.56
Day 14	17.00	1.70	2.55	309	0	0	5.45	40.88
Day15	15.92	1.59	2.39	90	0	0	0.26	40.54
Day16	16.88	1.69	2.53	413	0	0	0.91	78.67
Day 17	16.14	1.61	2.42	601	0	0	4.26	57.23
Day 18	15.99	1.60	2.4	395	0	0	5.58	51.17
Day 19	15.53	1.56	2.33	250	0	0	2.72	42.79
Day 20	15.73	1.57	2.36	198	0	0	3.8	41.37
Day 21	15.9	1.59	2.39	136	0	0	4.15	36.08
Mean		<b>1.64</b>	<b>2.47</b>					
SD		<b>0.054</b>	<b>0.080</b>					
				6634.00				

- Note: 1. One sample in Day4 is an unsatisfactory sample.  
2. The MO samples were included in Day 15, and there was a blind QC sample in Day 16 run.



# GAA Assay Clinical Performance

Specimen ID	GAA Activity		Assessment		Notes
	WI GAA_MSMS	MO GAA_DMF	WI	MO	
Missouri #1	15.30	Not Provided	Normal	Normal	
Missouri #2	9.74	Not Provided	Normal	Inconclusive (multiple low enzymes)	Repeat NBS Normal
Missouri #3	19.96	Not Provided	Normal	Normal	
Missouri #4	0.87	Not Provided	Abnormal	Pompe – later onset	
Missouri #5	10.05	Not Provided	Normal	Normal	
Missouri #6	1.73	Not Provided	Possible Abnormal	Pompe carrier	
Missouri #7	3.04	Not Provided	Normal	Pompe pseudodeficiency	
Missouri #8	15.82	Not Provided	Normal	Normal	
Missouri #9	2.54	Not Provided	Normal	Inconclusive (multiple low enzymes)	Repeat NBS Normal
Missouri #10	0.26	Not Provided	Abnormal	Pompe – classical infantile	



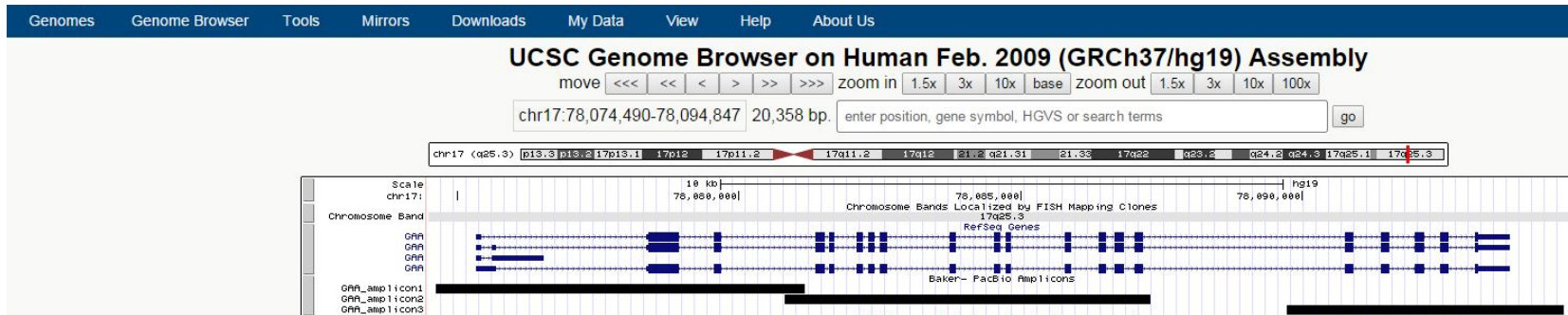
# Wisconsin Status

*Molecular assay for Pompe, MPS I and X-ALD*

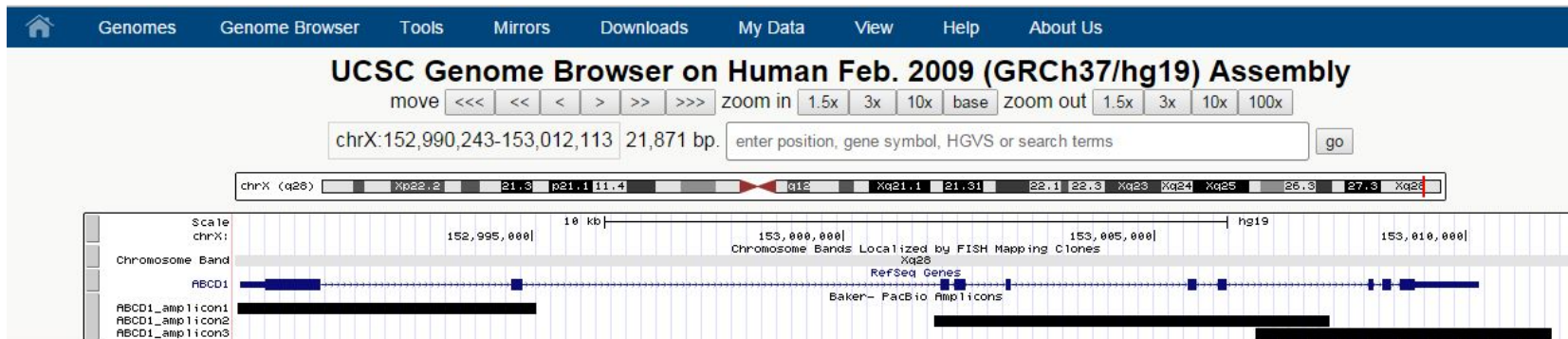
- **Molecular testing for Pompe, X-ALD and MPS I**
  - Sanger sequencing
  - Next generation sequencing: PacBio SMRT



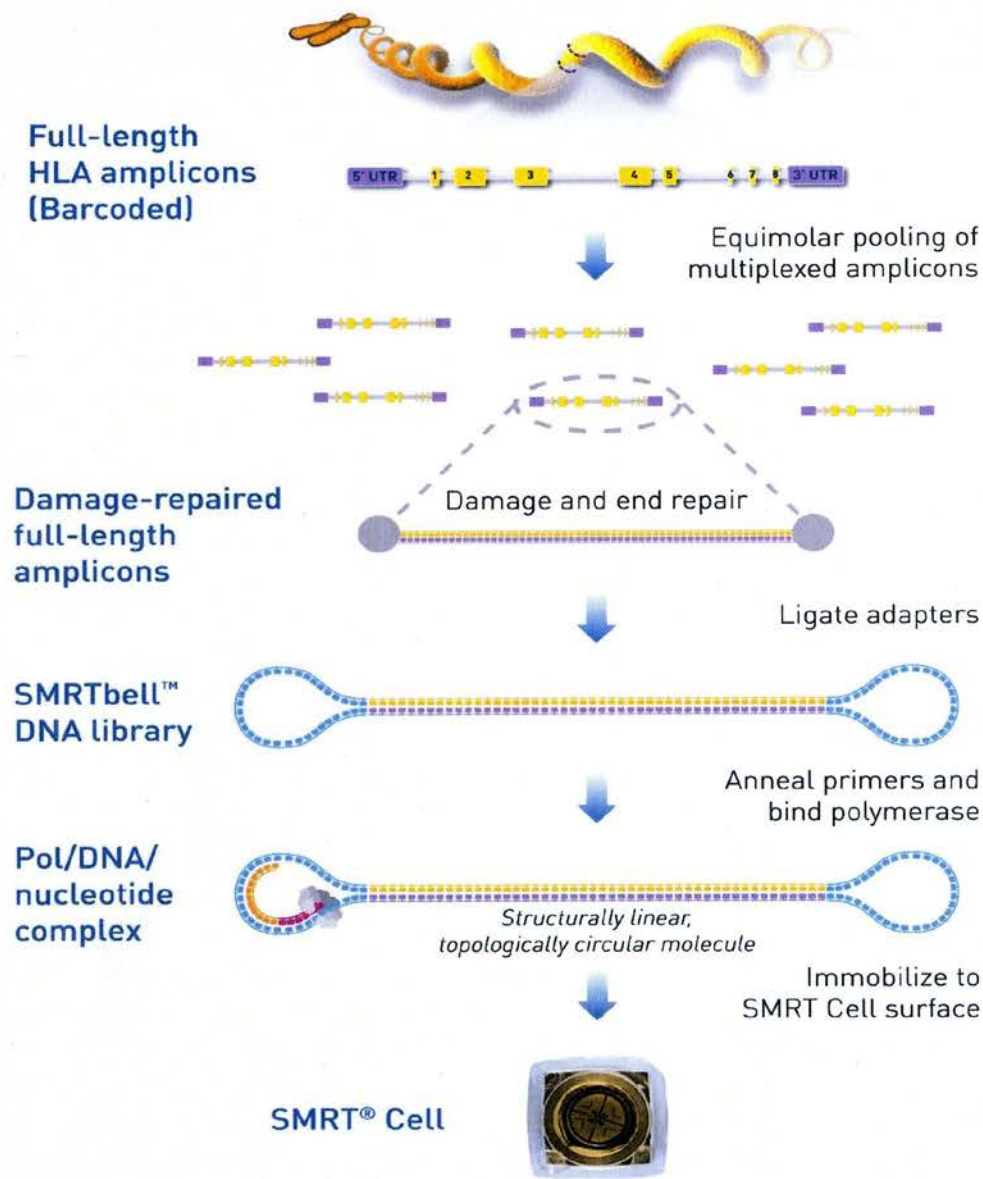
# Gene GAA for Pompe



# Gene ABCD1 for X-ALD



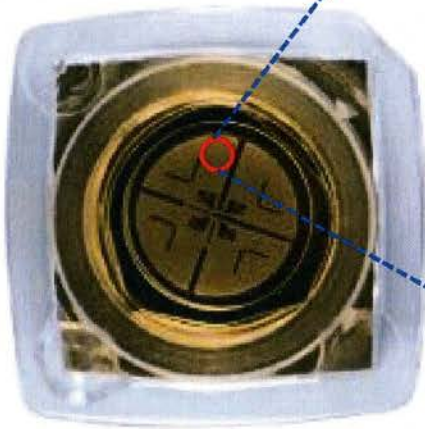
# SMRTbell™ Template Preparation for Amplicon Sequencing



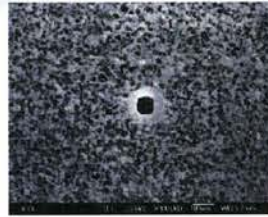
## Assay design flexibility

- Universal library preparation method for any insert size
- Set of 384 barcodes available for multiplexing
- Barcode tags can be added to the primers for scale up
- Samples can be pooled and run across one or multiple SMRT® Cells

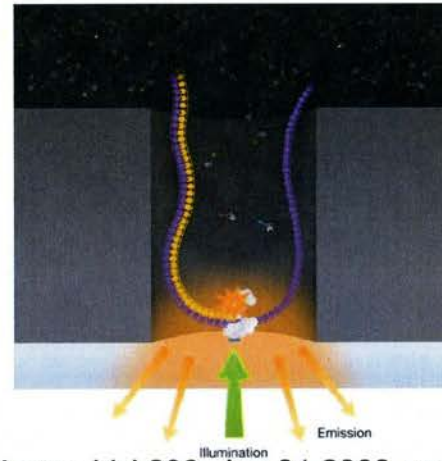
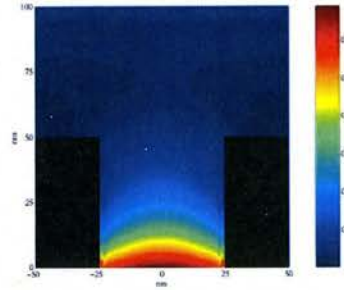
# Zero Mode Waveguides



SMRT cell



Confinement to  $20 \times 10^{-21}$  liters



Science, Vol 299, Jan 31 2003, pp682-686  
J. Appl. Phys. 103, 034301 (2008)



# Processive Synthesis with Phospholinked Nucleotides



**Step 1:** Phospholinked nucleotides are introduced into the zero-mode waveguide (ZMW)

**Step 2:** The nucleotide is held in the detection volume for tens of milliseconds, fluoresces when excited by light. The captured light is converted into a base call with associated quality metrics

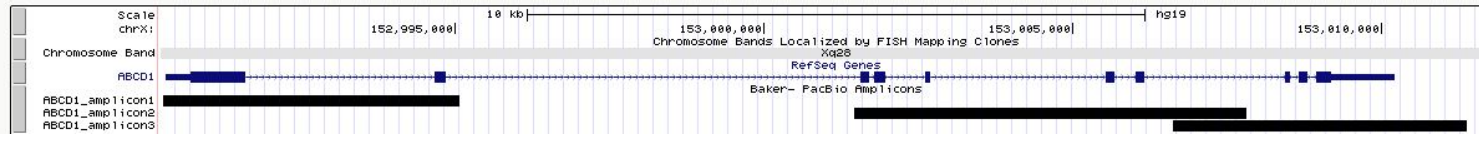
**Step 3:** The polymerase incorporates the nucleotide, releasing the attached dye molecule

**Step 4-5:** The process repeats



# UCSC Genome Browser on Human Feb. 2009 (GRCh37/hg19) Assembly

move <<< << < > >> >>> zoom in 1.5x 3x 10x base zoom out 1.5x 3x 10x 100x  
 chrX:152,990,243-153,012,113 21,871 bp. enter position, gene symbol, HGVS or search terms go



Details: X: 22,639-22,837 (198 bp @ 1002x)





# Funding Support

- **Pompe NBS Pilot**

HHSN275201400D10C

- **PNRC**

The development of this presentation was supported by Cooperative Agreement #UG9MC30369 from the Health Resources and Services Administration (HRSA). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of HRSA.



# Acknowledgments

- **Wisconsin State Laboratory of Hygiene**

- Sean Mochal, BS
- Sam Dawe, PhD

- **University of Wisconsin Biotechnology Center**

- Leah Frater-Rubsam, PhD
- James Speers, BS
- Amanda Maegli, MS
- Robyn Roberts
- Jason Walker, BS
- Mark Berres, PhD