

# Mutant and wildtype huntingtin protein quantitation utilizing automated capillary electrophoresis

Nancy Cheng PhD, Life Edit Therapeutics, Inc. Durham, North Carolina USA

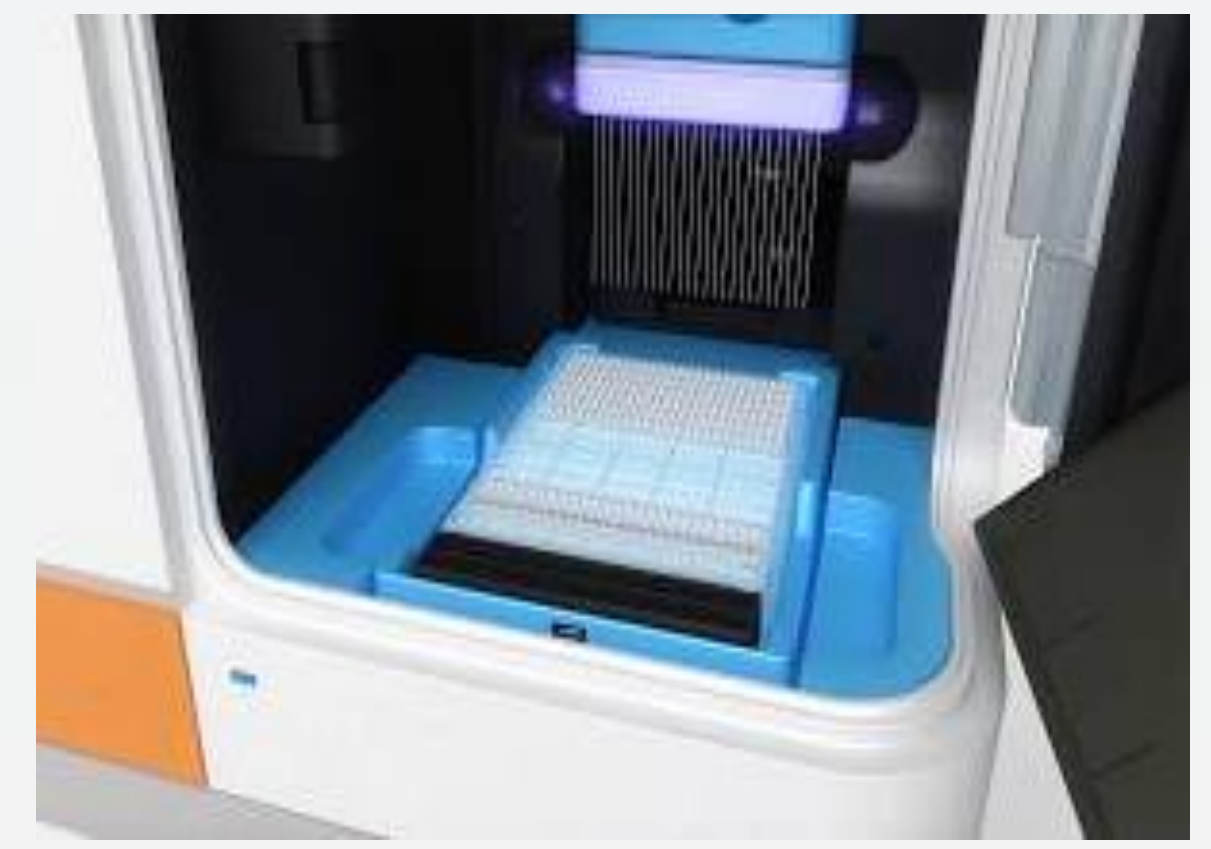
## Life Edit Goals in Huntington's Disease (HD)

<b>Unique and versatile platform of CRISPR-based enzymes</b>	<ul style="list-style-type: none"> <li>Allelic editing of mutHTT protein (&gt;40% reduction)</li> <li>wtHTT protein is untouched</li> </ul>
<b>Assessment of editing systems</b>	<ul style="list-style-type: none"> <li>Differentiate mutHTT and wtHTT protein</li> <li>No bias in quantification of the two alleles</li> </ul>
<b>Assay Development Metrics using full length Q7 and Q73</b>	<ul style="list-style-type: none"> <li>Specificity – measure only the analyte of interest</li> <li>Linearity – readout is proportional to analyte</li> <li>Precision – proximity of measurement results to each other</li> <li>Accuracy – proximity of measurements to the true value (nominal)</li> <li>Lower Limit of Quantification (LLOQ) – Signal/Noise (S/N) ratio is <math>\geq 17</math></li> </ul>

## Instrumentation – Jess Capillary Western

### Robotic Western

- Samples loaded onto plate
- About 25 nL loaded to capillaries coated with dextran matrix
- Electrophoresis
- Proteins covalently linked to the capillaries
- Western procedure
- HRP luminescent substrates for detection

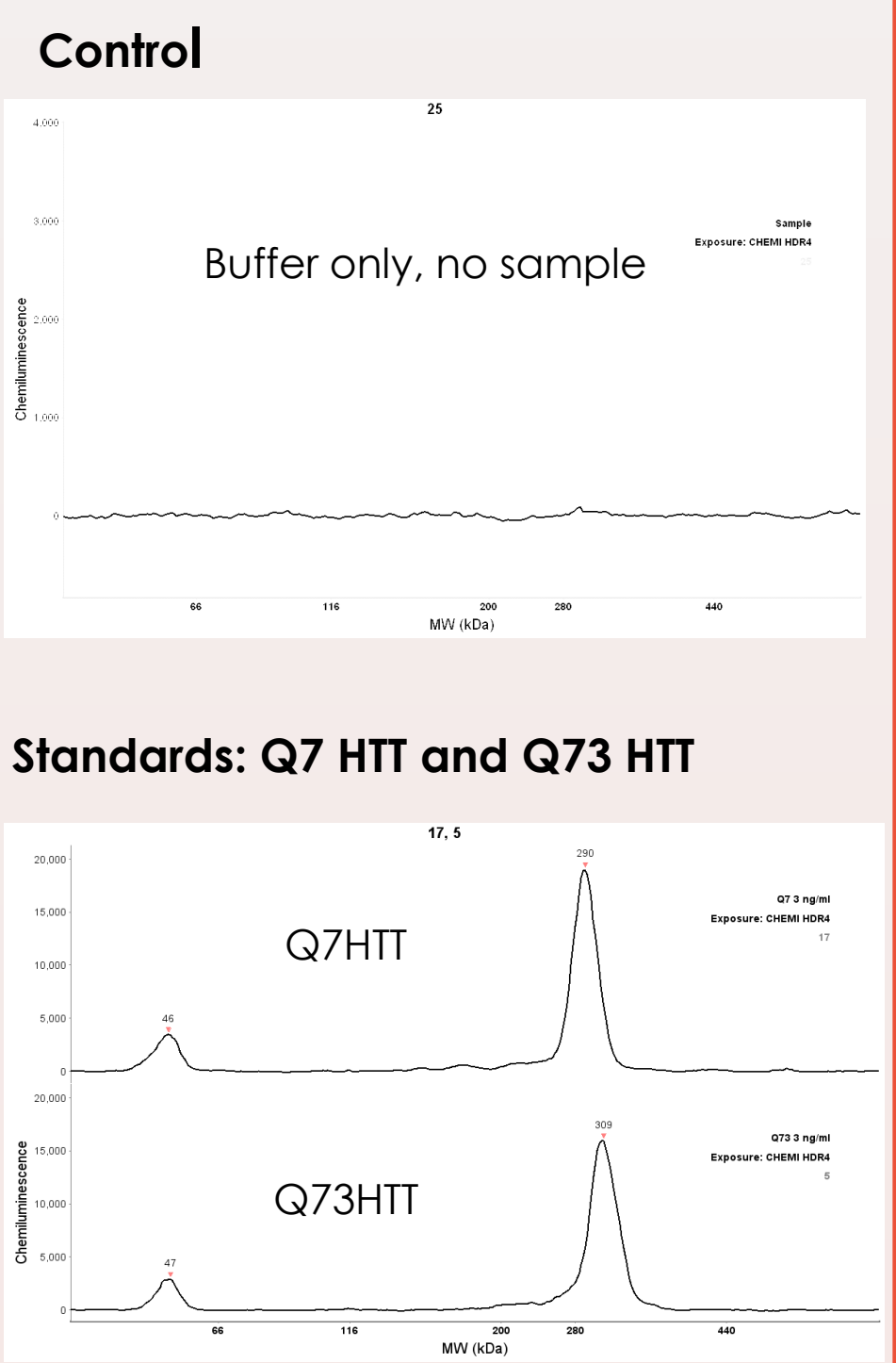


### Advantage

- No blotting, thus no transfer bias
- Direct integration of electropherogram peaks

## Assay Method Development Metrics

### SPECIFICITY

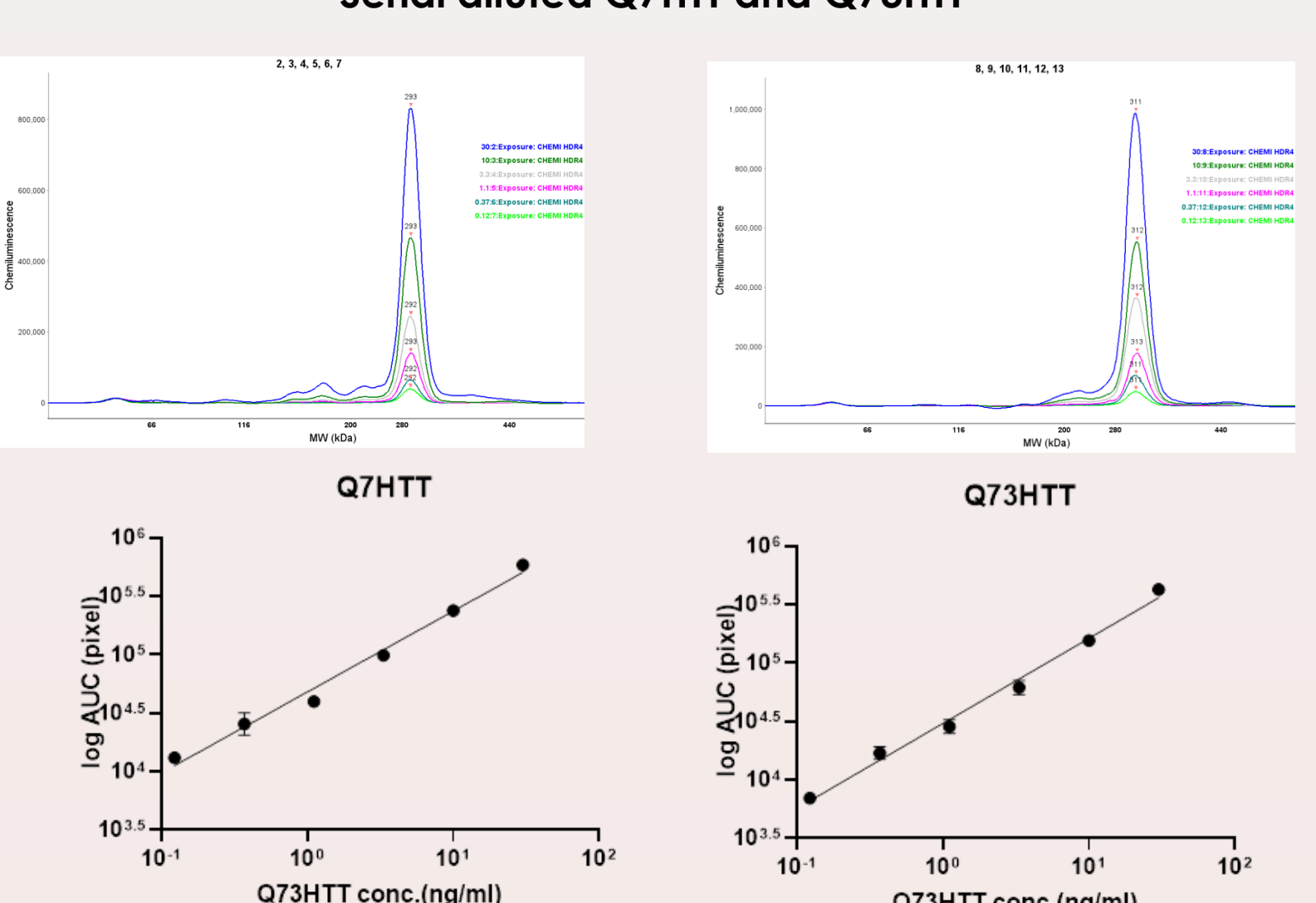


Control: Buffer only, no sample

Standards: Q7 HTT and Q73 HTT

### LINEARITY


Serial diluted Q7HTT and Q73HTT



Preparation #	Preparation Standards (ng/mL)	Calc. HTT (ng/mL)	RLU* (Pixels)	[Back-Calculated] (ng/mL)
Prep 1	30	30.0	442,506	35.4
	10	10.0	161,938	9.21
	3.3	3.33	68,008	2.88
	1.1	1.11	31,354	1.02
	0.37	0.37	15,471	0.396
	0.12	0.12	6,802	0.131
Prep 2	30	30.0	412,811	32.3
	10	10.0	149,353	8.26
	3.3	3.33	55,940	2.22
	1.1	1.11	25,856	0.787
	0.37	0.37	18,439	0.500
	0.12	0.12	7,098	0.139
Intercept		4.48		
Slope		0.73		
R <sup>2</sup>		0.99		


\* Relative Luminescent Unit

### ACCURACY



[Q73] ng/ml	Prep #	[Back-Calc.] ng/ml	[Mean Back-Calc.] (ng/ml)	% Recovery	Mean % Recovery
30	1	35.4	33.86	118%	113%
	2	32.3	±2.23	108%	
10	1	9.21	8.74	92.1%	87.4%
	2	8.26	±0.667	82.6%	
3.3	1	2.88	3.05	87.2%	92.3%
	2	3.22	±0.238	97.4%	
1.1	1	1.02	1.00	92.7%	91.2%
	2	0.987	±0.023	89.8%	
0.37	1	0.396	0.40	107%	108%
	2	0.400	±0.003	108%	
0.12	1	0.131	0.14	110%	116%
	2	0.139	±0.005	116%	

### PRECISION

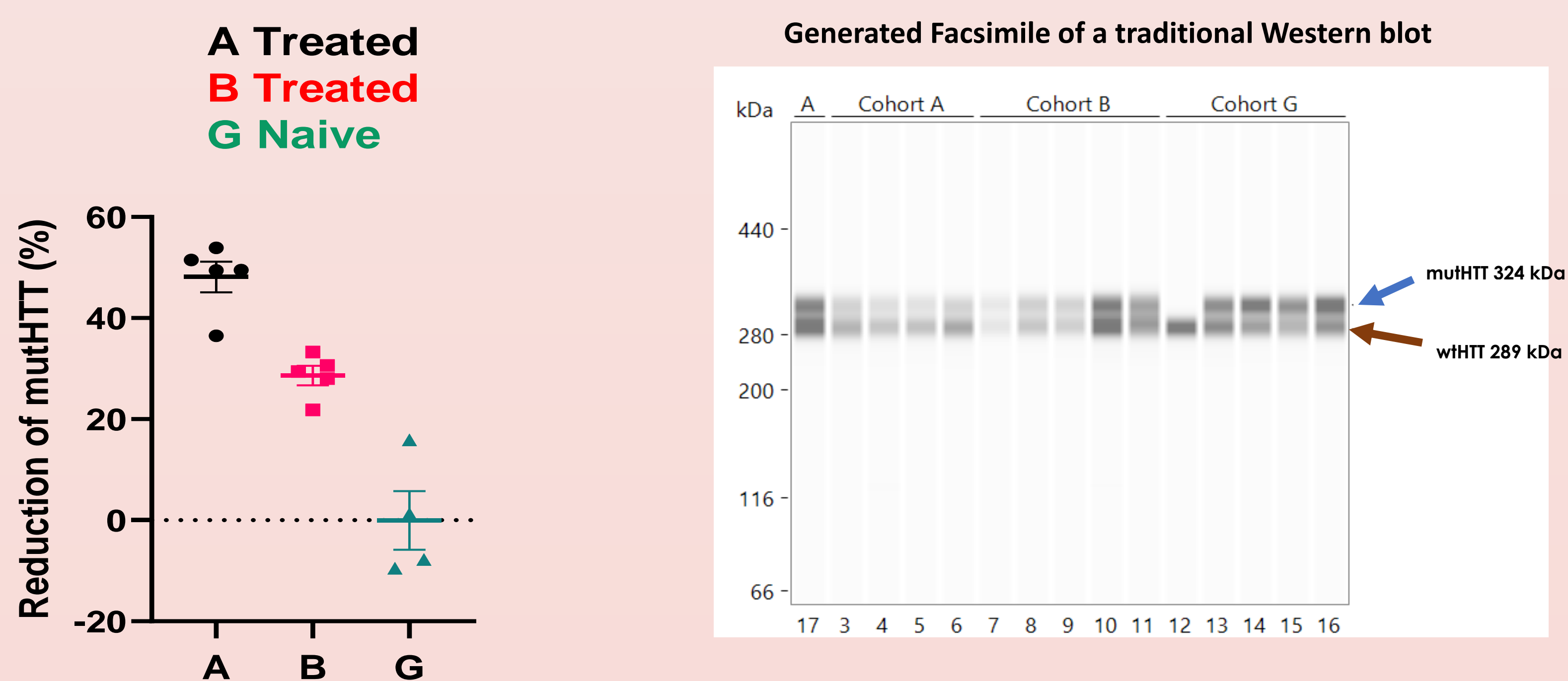


[Q73] ng/ml	Prep #	Apparent MW (kDa)	Mean Apparent MW (kDa)	% MW RSD	Peak Area (pixels)	Mean Peak Area (pixels)	% Peak Area RSD
30	1	311	308	0%	424214	418512	1.93%
	2	311	±0.71		412811	±8060	
10	1	312	308	0%	141938	140444	1.30%
	2	312			139353	±1830	
3.3	1	313	306	0.20%	58008	56474	3.84%
	2	312	±0.71		54940	±2170	
1.1	1	311	296	0%	21354	22105	4.80%
	2	311	±2.12		22856	±1062	
0.37	1	311	289	0%	9471	9955	6.88%
	2	311	±2.12		10439	±684	
0.12	1	311	287	0%	4098	4284	6.13%
	2	311			4469	±262	

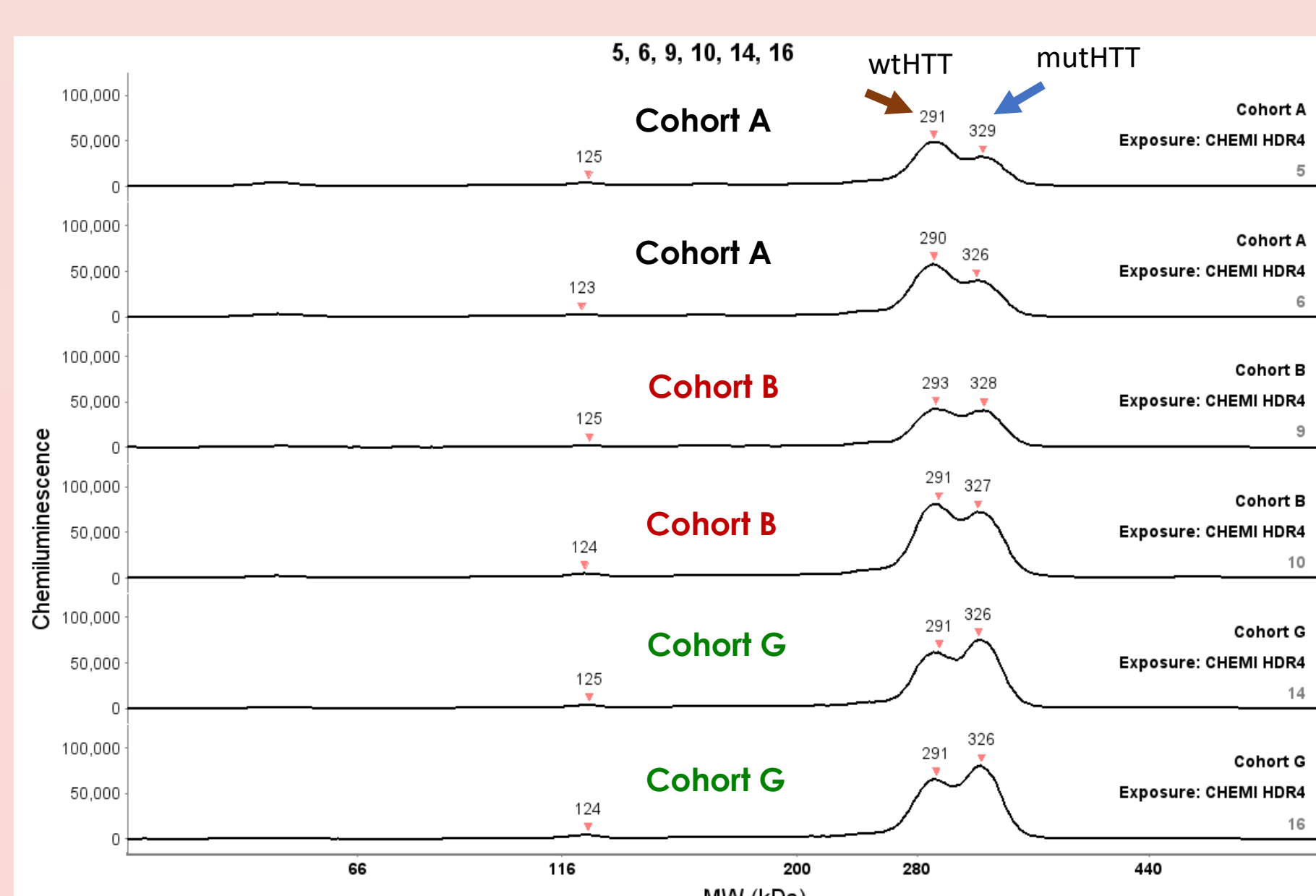
### LLOQ

LLOQ Sample (pg/ml)	[Calc. HTT] (pg/ml)	Mean [Calc. HTT] (pg/ml)	%RSD	% Recovery	Mean % Recovery	S/N ratio
50 #1	56	60.5 ± 9.85.0	16.3%	112%	121%	17
50 #2	71.8			144%		
50 #3	53.7			107%		
25 #1	41	35.3 ± 14.4	40.7%	164%	141%	11
25 #2	46			184%		
25 #3	19			76%		

## In Vivo Editing in BACHD Mice



Electropherogram of the samples



## Conclusions

- A wide range of linearity -50 pg/ml to 10 ng/ml
- Accuracy, comparing theoretical to calculated concentrations of samples, yielded recovery from 87% to 113%
- Precision was evaluated by comparing the %RSD for the average MW and average peak size in pixels; they were 0-0.20% and 1.30-688% respectively
- Back-calculated recoveries were generally >90%
- Sensitive method; LLOQ of 50 pg/ml with S/N of 17
- Successfully used a synthetic CE-Western method to evaluate the % reduction of mutHTT protein in a clinically relevant murine model