

Screening Assays In Development

Type of Screen	Laboratory	Specific Assay	Details	Status	Organism
Expression (normal, endogenous gene)	Aurora (Pollok)	Reporter activity, mRNA stability.	Cell-based screen involving fusion protein, drug-inducible, full-length Htt. Looking at level of activity after a defined length of time. Comparing to normal Htt expression.	In progress	Mammalian cells
Expression of mutant Htt	Hughes (with Jim Olsen)	Selective destruction of CAG RNA	Construct pure CAG (40-50 Qs) Potentially target expanded mRNA Axon 1: delete start codon Expressed as 5' UTR	In planning stage	Mammalian cells (?)
Behavioral (not trying to alter expression)	Neri	Neuronal dysfunction	Internal fragment of Htt shorter than exon 1; the first 128 glutamates  Could be secondary, tertiary screen	In progress	<i>C. elegans</i>

<p>Aggregation (suppression of)</p>	<p>Sherman</p>	<p>Cell survival assay</p>	<p>Control: expression of short (25 Q) is high, Expression of expanded polyQ (103 Q) is low (expression of soluble protein in cell) Long is toxic Suppress aggregation: expression goes up</p>	<p>In progress: 10,000 screened, 13 pulled out.  (aggregation in mammalian: 2 strong, 1 weak)  40,000 more to screen.</p>	<p>Yeast</p>
<p>Aggregation</p>	<p>Berthelie</p>	<p>Ability to inhibit polyQ aggregation</p>	<p>Aggregates in polyQ wells Using Sigma IOPAC library Tag??</p>	<p>Tested 80 compounds: 3 hits</p>	<p><i>In vitro</i></p>

<p>Aggregation / toxicity</p>	<p>Krobitsch</p>	<p>Aggregation patterns</p>	<p>Screen for cellular factors</p> <p>Mutant hsp strain: exp PolyQ: toxic, results in slow growth</p> <p>Using Sigma chemical compounds (Iopac not available until March 2001)</p>	<p>See 1</p> <p>aggregate in WT vs. multiple (varying levels) in different mutated strains</p>	<p>Yeast</p>
<p>Aggregation</p>	<p>Housman</p>	<p>Microscopic assay</p>	<p>1. Genzyme PC12 cells</p> <p>2 million compounds. Ecdysone-independent</p> <p>2. Peptides in PC12 cells. Alterations in number of inducers produce different aggregation levels. Different numbers affect the number of glutamines, but seem to change morphology of aggregates.</p> <p>3. Polypeptide assay. Gene suppressors. To determine whether the effect is on aggregation or interaction with</p>	<p>In progress</p>	<p>PC12 cells</p>

			polyglutamine. (Must test whether aggregates are protective or toxic).		
Aggregation	Bamdad	Track incorporation of peptides into aggregates	<p>Gold nanoparticles turn pink when apart, blue if come together</p> <p>His-polyQ peptides, purified aggregates</p> <p>Can do 1,000 a day</p> <p>Extending into whole cell assay, RB1 (Sigma Iopac) cells from Housman's lab</p> <p>Using Congenics, Coelocanth libraries</p>	Some compounds promote aggregation	<i>In vitro</i>

Aggregation	Ingram	<p>1.</p> <p>A. Interaction assay</p> <p>B. Sequence specificity</p> <p>C. Filtration/ aggregation assay</p> <p>D. Measuring amount, rate of entry into cells.</p> <p>2. Interference with aggregation</p>	<p>Two sets of peptides:</p> <p>1.</p> <p>A. Fluorescence tag A(Beta)42 (combinatorial library)</p> <p>B. Cause calcium influx into neuron cells (for alpha-2?)</p> <p>C. Htt Q67-exon 1-6FP</p> <p>D. PC12 cells.</p> <p>2. Peptide arrays, 4-12 ads (Qx, x being any amino acid). Based on QA repeat peptides.</p>	<p>A. Interaction of pentapeptides, D amino acids; 3 of 7 interfere greatly with Beta-sheet formation</p> <p>B. All cytotoxic</p> <p>C. 4 of 7 peptides block aggregation completely, up to 5 micromolar concentration</p> <p>D. In progress?</p> <p>2. Some bind Q67 protein specifically; also promote aggregation,</p>	<i>In vitro</i>
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				result in incorporation into aggregate.	
Aggregation	Schweitzer	Filter-retardation assay	Merek, high-throughput assay	600,000 screened; produced about 150 hits	<i>In vitro</i>

Aggregation	Hughes	Aggregation of fusion of essential protein  (rescue-toxicity assay)	Readout is lack of toxicity  Libraries (possible control hsp) <ol style="list-style-type: none"> <li>1. New chemical entities:</li> <li>2. Lopac – pharmacologically active compounds</li> <li>3. Gullan's FDA 2000</li> </ol>	Decrease aggregation, increase TUB1  Libraries: <ol style="list-style-type: none"> <li>1. 200,000 screened, 2 hits (in retest)</li> <li>2. 200,000 being screened (in progress?)</li> <li>3. 1,000 tested, 3 hits (in retest)</li> </ol> Probably will retest in different yeast strains for enhanced susceptibility to drugs	Yeast
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Aggregation /Toxicity	E. Wanker	Aggregation?	Merck library (FDA- approved drugs?)	600,000 tested, about 150 hits	<i>In vitro</i>
Transcrip- tion	Hughes	Increase transcription	Pho 84 75 CAG 8X decrease in presence of exp Q and reporters (NLS) secondary screen  (not xx enough pheno- typically to use in high-throughput screens)	In progress  Would like to transfer to mammalian cells	Yeast

Conformation	Aurora (Pollak)	Biochemical assay	<p>1. Folding of exp. PolyQ</p> <p>2. xx selective for expansions</p> <p>antibody epitope is surrogate for expanded repeat structures</p> <p>decrease finding to alpha (??)</p>	In progress	<i>In vitro</i> (?)
Conformation	Hughes	Interruption of alpha-Htt interaction	Testing Alpha (?)		Yeast

Conformation	Sanchez	Intramolecular FRET formation?)	Mammalian cell FRET	In progress. If peptide/protein is allowed to molecularize, intramolecular FRET is seen	Mammalian cell
Conformation	Paul Ko Ferrigno	Interruption of peptide aptamers; Htt interaction	Peptides distinguish WT from mutant Aptamers can distinguish point mutations We don't believe aptamers bind to polyQ	Able to detect polyglutamine-polyglutamine interactions; it's a weak, but reproducible effect; not polyglutamine-dependent	Yeast?

Cleavage	Amgen		Caspase-related	No longer testing due to change of management (not lack of success)	?
Nuclear localization	Marci		Transformed murine striatal cell lines	?	Mammalian cells?

Nuclear localization	Ko Ferrigno		Testing importance of nuclear localization (?)	In progress	Mammalian cells
HD-induced toxicity	Sanchez		<p>HELA cell-based expQ</p> <p>Readout:</p> <ul style="list-style-type: none"> <li>-Decreased ATP levels (transient)</li> <li>-FRET aggregation</li> <li>-Caspase activation</li> </ul> <p>Verification: Select against potential caspase inhibitors</p> <p>Toxicity driven by other toxic insults, apoptotic inducers, etc.</p>	<p>2,000 tested from Gullan's Library, 4 hits</p> <p>18,000 from Chembridge</p> <p>13 hits total.</p>	Mammalian cells?

<p>HD-induced toxicity</p>	<p>Schweitzer</p>		<p>Pc12 cells, 10<sup>7</sup> molecules per cell</p> <p>Express Htt<sup>ex</sup> on WT exon-1 of HD</p> <p>Inducible exon1-6FP: 103Q, 25Q</p> <p>Bombyx ecdysone:expQ: cell death within 2 days</p>	<p>In planning; will test compounds</p> <p>90 percent of cells die within 48 hours, rest don't divide anymore in absence of NGF.</p> <p>Detect cell death by observation of cell cultures.</p> <p>-Reason for clear difference unknown</p>	<p>?</p>
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Toxicity	Henderson	(true toxicity assay)	<p>1. 384-well dishes Read survival by high throughput  Count survivors using dye that fluoresces when in cells (number of cells still capable of cleaving ACIMC?)</p> <p>2. Primary neuron cultures are needed to find new neurotrophic factors</p>	?	?
HD-induced toxicity	Ross		<p>Tet-off PC12 (Cloneteck strain)</p> <p>23 Q N63</p> <p>Better for secondary screens in primary striatal cortical rat neurons: transient transfections</p>	<p>50 percent toxicity after 4 days: plus NGF, plus induction (tryptan blue assay)</p> <p>Apparently reproducible toxicity</p> <p>Will use to test compounds</p>	?

