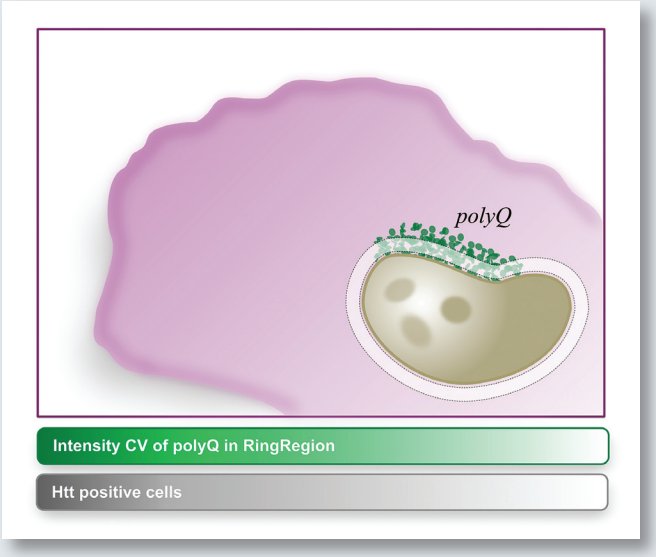

APPLICATION NOTE

Image-based Quantification of the Huntingtin Protein using the Operetta

Key Features

- Automated image acquisition using the Operetta™ High Content Screening system
- Data analysis using the versatile Harmony™ software
- Recognition of the specific huntingtin protein conformation

Perinuclear accumulation of polyQ, Htt positive cells

Background

Huntington's disease (HD) is a dominantly inherited neurodegenerative disease caused by a polyglutamine (polyQ) expansion in the huntingtin (Htt) protein. A widespread phenotype of all neurodegenerative diseases is aggregate formation, and in HD it is the Htt protein which forms inclusion bodies. In recent studies it was suggested that a more soluble form of Htt represents the toxic species of Htt protein [Arrasate *et al.*, 2004] rather than the aggregate form. An automated measurement and analysis of Htt protein in ST Hdh Q111/111 cells offers a promising basis for the development of new therapeutics against the disease.

Here, we present a high throughput High Content Analysis (HCA) assay for the quantification of the specific, more soluble conformation of the Htt protein which is located in the perinuclear region. Using the Harmony software we designed a fast analysis sequence to provide the desired read-out parameters.

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Applications

The ST Hdh Q111/111 cell line from Marcy MacDonald (Massachusetts General Hospital) was derived from knock-in mice expressing an expanded glutamine tract in mutant huntingtin [Fossale *et al*., 2002]. Treatment of the cells with antifungal Hexachlorophene reduces the polyQ aggregates in Htt protein in a dose dependant manner.

Cells were seeded at a density of 4500 cells / well in 384 well Collagen I coated CellCarrier™ plates and incubated for 24 hr at 33 °C and 5% CO_2. A 10-point dilution of Hexachlorophene was then added to the wells and the cells were incubated for a further 18 hr. Subsequently, cells were fixed and immunofluorescently stained with an anti-polyglutamine primary antibody which recognizes a specific Htt conformation [Legleiter *et al*., 2009]. The primary antibody was visualized using an AlexaFluor® 488 labeled secondary antibody and the nuclei were counterstained with DRAQ5™. The resulting immunofluorescence of untreated ST Hdh Q111/111 cells is visible in the perinuclear region. The positive control substance Hexachlorophene reduces this staining pattern (Figure 1).

Images were acquired on the Operetta using the 20X long WD objective. For image analysis we designed a Harmony analysis sequence (Table 1) providing two assay specific read-outs: median CV of perinuclear intensity and polyQ huntingtin positive cells (Figure 2).

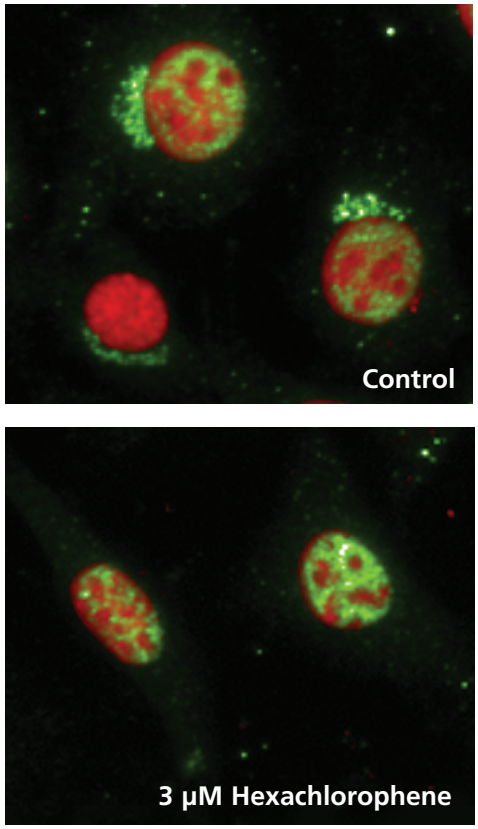

Figure 1. Images of control (upper panel) and Hexachlorophene treated (lower panel) ST Hdh Q111/111 cells. DRAQ5™ stained nuclei are shown in red. The polyQ of the Htt protein was labeled by indirect immunofluorescence with AlexaFluor® 488 and is shown in green. The images show the effect of Hexachlorophene which reduces the perinuclear staining pattern. Images were acquired on the Operetta using the 20X LWD objective.

Harmony Analysis Sequence	
Input image:	AlexaFluor® 488 Htt – marker channel, DRAQ5™ – nuclear stain channel
Find nuclei:	detects nuclei
Select cell region:	defines the cell region based on the nuclear region, a small region of interest (ROI) around the nucleus (= perinuclear region)
Calculate intensity properties:	quantifies the coefficient of variance (CV) in the perinuclear region in the marker channel (= perinuclear intensity CV)
Select population:	applies a threshold for perinuclear intensity CV and defines the resulting population as Htt positive cells
Define results:	number of cells perinuclear intensity CV – median number of Htt positive cells percentage of Htt positive cells

Table 1. Harmony analysis sequence designed to provide read-outs of the median CV of perinuclear intensity and polyQ huntingtin positive cells.

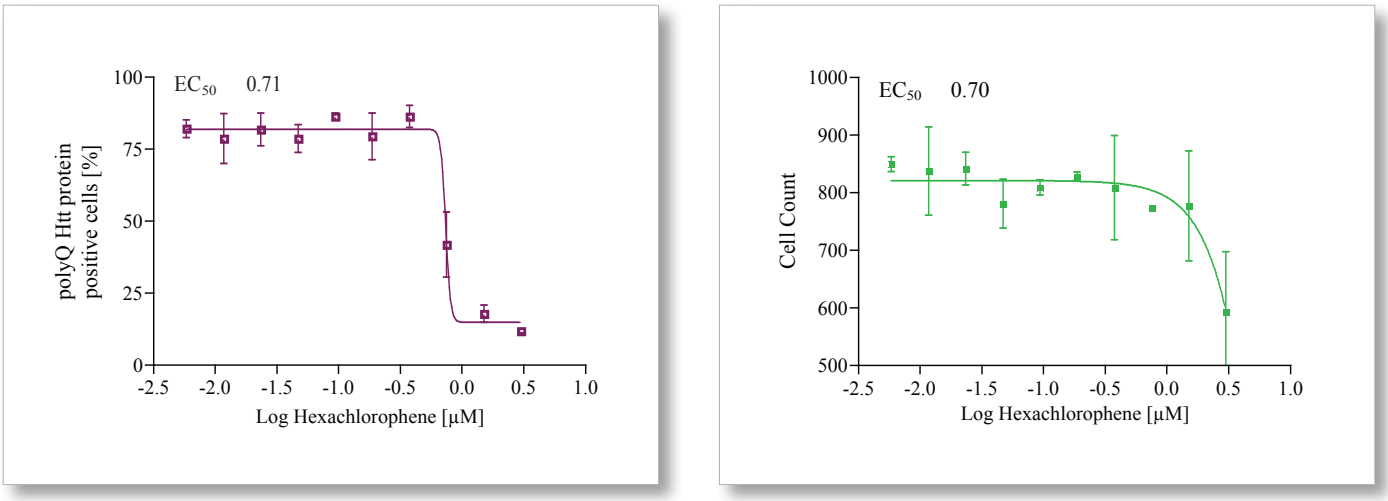

Figure 2. Hexachlorophene generated dose-response curves deduced from the median CV of perinuclear intensity (A) and from polyQ Htt protein classified positive cells (B). N = 2 wells.

Conclusions

The Operetta HCA application presented here is a very fast and efficient way of quantifying huntingtin protein in ST Hdh Q111/111 cells. We developed a customized image analysis sequence providing robust and reliable assay results. The two read-out parameters "mean CV of perinuclear Htt intensity" and "polyQ Htt protein positive cells" resulted in similar EC_{50} values (Figure 2). These data are comparable with Jäger *et al.*, 2009 who analyzed this assay using the Opera™ High Content Screening system and Acapella™ image analysis software.

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Authors

Judith Lucke
Eleni Mumtsidu

PerkinElmer
Cellular Technologies Germany GmbH
Cellular Imaging & Analysis
Hamburg, DE

Stefan Jäger
Evotec AG
Hamburg, DE

For further details please visit www.cellularimaging.com

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

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