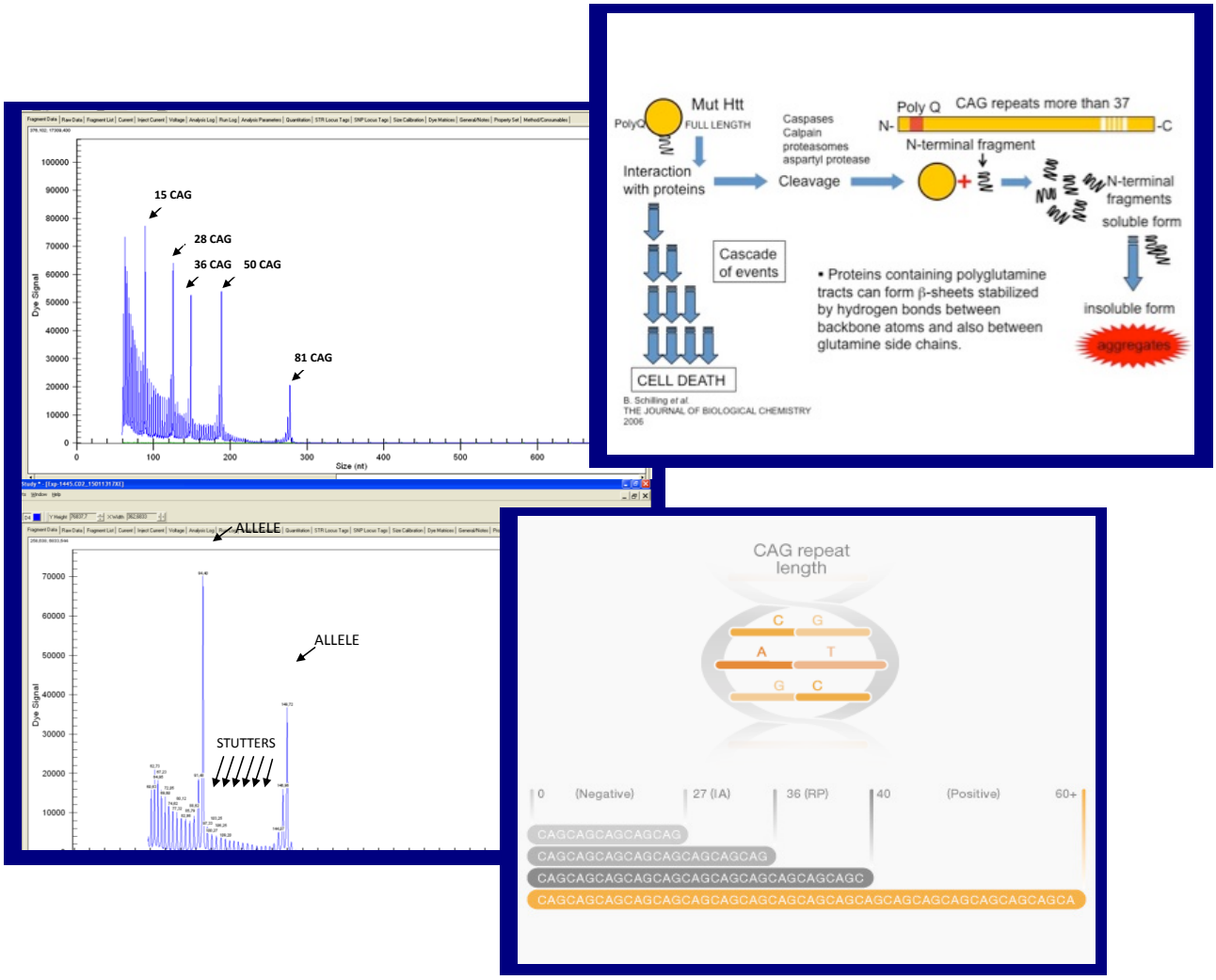
System for genetic testing for Huntington Disease (HD) by triplet-repeat primed PCR (TP-PCR) and fragment sizing through capillary electrophoresis

HUNTINGTON DISEASE KIT 1 – FL

cod. HD.01FL



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Huntington Disease is a progressive neurodegenerative disorder that presents with motor symptomps, cognitive impairment and phychiatric disturbances. The first symptomps usually manifest between 35 and 50 years of age and duration of the disease is between 15 an 20 years. A small number of cases present before the age of 20 (juvenile onset) and about 25% of cases present after 50 years of age.

It is characterized pathologically by loss of specific neuronal populations in many brain regions, although the patology is not limited to neurons. Neuropathological features include selective degeneration of neurons in the caudate and putamen and less severe loss in the cerebral cortex

HD is caused by the expansion of an unstable polymorphic **trinucleotide (CAG)n** repeat in exon 1 of the **HTT gene** (4p16.3), which translates into an extended polyglutamine tract in the protein.

Alleles with **<27 CAG** repeats are classified as **normal**, whereas alleles with **≥36 repeats** are detected in patients. Alleles with **27-35** repeats (called **mutable** or intermediated alleles) are not associated with the disease symptoms but can expand into the affected range upon (predominantly paternal) germline transmission and thus cause HD in offspring.

Repeats of **36-39 CAG** are **incompletely penetrant** and can be found in affecetd individual as well as individuals who show no clinical symptomps. HD appears to be fully penetrant for allele size of **≥ 40** repeats.

Genetic testing for HD is usually requested by a neurologist or a clinical geneticist for a confirmation or exclusion of a clinical diagnosis; if the diagnosis is confirmed by DNA analysis, the patient and family members Should be referred for genetic counseling and a possible offer of a presymptomatic testing.

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How does the kit work?

The ***Huntington Disease kit-FL*** is used for the molecular testing of the Huntington disease. This system amplifies the **HD (CAG)n** repeat region by **TP-PCR** (triplet-repeat primed PCR) using a fluorescently labeled forward primer located upstream of the (CAG)n region and a chimeric reverse primer located partially within the (CAG)n region.

The chimeric reverse primer hybridizes to multiple locations within the (CAG)n repeat region, creating PCR products of varying sizes. Reactions are separated by capillary electrophoresis. TP-PCR provides a characteristic ladder on the fluorescence trace, enabling the rapid identification of a large pathogenic repeats that cannot be amplifies using flanking primers.

The **"HD sequence specific standard"** (provided), that has to be included on each run, allows to detect accurately normal alleles, mutable alleles and HD alleles.

Starting samples: peripheral blood

DNA isolation method: QIAamp DNA blood mini kit, QIAcube, QIAsymphony (Qiagen), High Pure PCR template preparation kit (Roche).

DNA Sequencer: CEQ 8000/8800, GeXP Genetic Analysis System (Beckman Coulter-ABSciex), 310, 3100, 3130, 3730, 3500 Genetic Analyzers (Applied Biosystmes),

Procedure: according to "EMQN/CMGS best practice guidelines for molecular genetic testing of HD" 2013.

Kit contents

Label	Contents
D4/6FAM-HD MASTER MIX	Mix for amplification of trinucleotide (CAG)n repeat of the gene HTT
HD DNA polymerase	DNA Polymerase for difficult amplification
HD sequence SS	HD Specific Size Standard