

## Pyromark Q96 ID instrument for:

- quantitative analysis of genetic or epigenetic DNA modifications using Pyrosequencing technology

Pyrosequencing™ is a well established technology for DNA analysis. The Neurogenetics Unit has evaluated the Pyrosequencing method and our previous PSQ™ 96 instrument for the analysis of genetic variations [Nordfors et al. 2002. Hum Mut 19(4): 395-401], which showed that the instrument is very robust and has a > 99% accuracy.

The instrument is user-friendly and fast (96 samples are analyzed within 10 min). The Assay Design Software helps you choose your optimal PCR- and pyrosequencing primers.

The core facility will provide start up theoretical and technical assistance for “hands-on” users. Each user reserves a date and time for occupying the instrument by a booking system.

### Description of the method:

The method does not require any gels or dyes. However, one PCR primer needs to be biotinylated for the DNA template amplification.

The Pyrosequencing method is based on sequencing-by-synthesis and on real-time detection of pyrophosphate.

In short, a sequencing primer is hybridized to a single-stranded DNA template, which is then incubated with luciferin as well as with a 4-enzyme mixture of DNA polymerase, ATP sulfurylase, firefly luciferase and the nucleotide degrading enzyme apyrase.

The different nucleotides are added one at a time in a pre-programmed order (the dispensation order) using ink-jet technology. If a nucleotide is complementary to the next nucleotide in the template DNA strand, it will be incorporated by the DNA Polymerase.

For every incorporation event, PPi will be released in proportion to the number of nucleotides that have been incorporated.

The PPi is converted to ATP by the ATP sulfurylase and the ATP in turn will drive the luciferase mediated conversion of luciferin into visible light. Between each cycle, unincorporated nucleotides will be degraded by the apyrase.

The light is detected by a CCD camera and the result is presented as real-time signals in a pyrogram where the peak heights are proportional to the number of nucleotides that have been incorporated.

<i>Price list for genotyping on PSQ™96MA</i>	
“Hands on” service	
<b>Introduction course (users outside CMM)</b>	1 200 SEK/user <sup>1,2</sup>
<b>Introduction course (CMM users)</b>	600 SEK/user <sup>1</sup>

<b>Pyrosequencing of ≤ 96 samples</b>	400 SEK <sup>1,2</sup>
<b>Pyrosequencing of ≤ 96 samples, including reagents</b>	1700 SEK/run* <sup>1,2</sup>

\* including 1xSNP reagent kit , 1 PSQ96 Plate, 0.3 ml streptavidin coated sepharose.

<sup>1</sup> The KI INDI fee will be added to the price for users within KI.

<sup>2</sup> An administrative fee of 30 % will be added for academic users outside KI and 50% for non-academic users. The indicated prices are exclusive of value-added tax.

Please note: Bookings that are missed and that are not cancelled at least 24hrs in advance will be charged by 50% of the running cost.