Technical Demo #4

A Sensitive Method for Monitoring Gene Responses to Environmental Estrogens

E Thomas-Jones, C Morris, G Thomas, J Cryer, I Weeks, S Woodhead & P Kille*

Molecular Light Technology Research Limited, 5 Chiltern Close, Cardiff CF14 5DL
*School of Biosciences, Cardiff University, PO Box 911, Cardiff CF1 3US.

emma.thomas-jones@mltresearch.com, Tel: +44(0)29 2074 7033
Fax: +44(0)29 2074 7118

Environmental pollutants having the capacity to disturb the endocrine system have attracted considerable interest during recent years. Environmental estrogen mimics are thought to cause endocrine disturbances in fish living close to effluent discharges. Expression of the egg proteins vitellogenin (Vtg) and zonal protein 3 (ZP3) in male fish has been proposed as a means of monitoring estrogenic disruption.

We have developed rapid, reliable and sensitive tests for these biomarkers. Our system quantifies specific mRNA transcripts for Vtg and ZP3 through the use of specific oligonucleotide probes, labelled with a chemiluminescent acridinium ester (AE), in a hybridisation protection assay (HPA). In this method, hybridisation protects the AE from hydrolysis such that the magnitude of the signal is a direct function of the concentration of specific mRNA target in the sample. The detection limit of 0.1 fmol of transcript means that only a small sample of RNA is needed (e.g. 10 µg total RNA), therefore avoiding any requirement for target amplification. The time taken from sample collection to end-point measurement is less than 3 hours.

With his technology we can quantify responses in fish exposed to estrogens. Moreover, the technology itself provides a platform for the monitoring of biological responses to environmental pollutants.