

# Chemical testing by molecular biological methods

## Progress report

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University of Helsinki, Department of Applied Chemistry and Microbiology

Marko Virta, Principal Investigator  
Johanna Rajasärkkä (née Kovanen), PhD student

### ABSTRACT

This project develops alternative methods for the assessment of Endocrine disruptive activity in chemicals and environment. The tests can be applied in environmental monitoring and chemical testing (such as described in REACH). The test system will be simple: it will require no specific expertise, training or instrumentation. Tests are based on yeast cells that contain a human receptor gene that controls the expression of luciferase gene and the receptor defines the specificity of the strains. The yeast strains developed will be evaluated with industrial waste and chemicals and environmental samples followed with comparison the results with the data available from literature and from collaborators. The evaluation of the strains will be done in cooperation with domestic and foreign partners. Moreover, new partners (especially industrial partners where chemical industry and forest industry are the most obvious candidates) will be identified during the study.

Special emphasis will be put on the robustness and ease of the use of the test system since it should work reliable in different laboratories and also outside of the laboratories. The experience obtained with bacterial test shows that by using microbes it is possible to construct test that is both robust and easy to use.

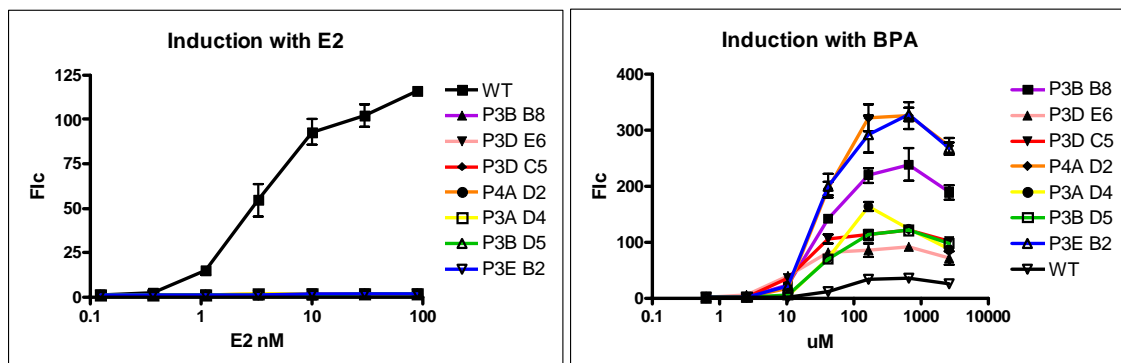
During the project one PhD degrees will be produced. The PhD student is subject to active training by not only the senior group members but also by the foreign and domestic co-operation partners and graduate school networks.

After the project we will have a test system for the assessment of Endocrine disruptive activity. It will have the following features:

- Simple to use (without special training)
- Robust enough (to be able to measure waste waters and waste chemicals)
- Sensitive enough (suitable for pre-screening before more intensive analysis)
- Cost-efficient

## RESULTS SO FAR

The first libraries for the receptor modification has been completed and they have been subjected to flow cytometric analysis. The selection of the libraries by sorting will has been completed and over 30 interesting mutants have been isolated, sequenced and characterised. The method worked extremely well since we found ten mutants that had lost their natural activity (towards 17- $\beta$ -estradiol) and possessed increased activity towards the new target ligand, Bisfenol A(BPA, see figure below).



The miniaturisation of the measuring protocol has been completed and manuscript on that is under writing. The publication of the results is summarised below.

- Number of refereed international publications: 2
- Number of publications in refereed scientific books: 1
- Number of oral presentation in international conferences: 1
- Number of posters in international conferences: 2

The most important papers so far are:

- Michelini, E., Cevenini, L., Mezzanotte, L., Leskinen, P., Virta, M., Karp, M., and Roda, A. (2008). A sensitive recombinant cell based bioluminescent assay for detection of androgen-like compounds. *Nature Protocols* 3(12):1895-1902.
- Leskinen, P., Hilscherova, K., Sidlova, T., Pessala, P., Salo, S., Verta, M., Kiviranta, H. and Virta, M. (2008) Detecting AhR ligands in sediments using bioluminescent reporter yeast. *Biosensors and Bioelectronics* 23: 1850-1855.

## IMPACT OF THE RESEARCH

Since the work has been on-going only for two years, the major societal impact is yet to be established. However, the work and the possible applications have

been discussed with some companies and research institutes (for details contact the PI)

#### PROGRESS AS COMPARED TO THE PLAN.

Generally the work has progressed according to the plan. The construction of fibre-optic biosensors is still lagging behind and it may be possible that it will not happen even during 2010. The project will result only one PhD since the other person working in the project has been post-doc rather than a PhD student.