Poster 5.0.9

Bacterial artificial chromosome versus conventional vector based protein expression in CHO cells: a comparison study

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High level expression of proteins from mammalian cells is important for basic research questions and biopharmaceutical production. The most common way to establish a stable recombinant mammalian cell line expressing a gene of interest (GOI) is delivery of plasmid containing the expression cassette including selection marker by transfection methods. Thereby the GOI is randomly integrated into the genome of the host cell. Thus high level expression of the GOI is affected by the genomic environment and gene copy number (GCN). To overcome this positioning effect elements providing an open chromatin region like S/MAR sequences or bacterial artificial chromosomes (BACs) may help. Due to their size (~300 kb) and sequence specificity BACs are considered as open chromatin region. In this study we defined two different anti-HIV1 single-chain antibody fusion proteins as model proteins and expressed them from conventional plasmids and the BAC derived expression system. The homodimers differ only in their originally human variable region to provide independent examples.

The four producer clones were selected regarding specific growth (μ) and specific productivity (qp). Afterwards critical parameters influencing the protein expression, maturation and secretion were analyzed. The evaluation of gene copy number, amount of specific mRNA and intracellular protein content give information about the bottle necks of the recombinant protein secretion pathway.

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Poster 5.0.10

The GlycoPhage display system and its applications

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We have recently extended the power of phage display method for the production and selective enrichment of phages that display asparagine-linked (*N*-linked) glycoproteins [1]. *N*-linked protein glycosylation is an essential and conserved post-translational modification of diverse proteins in eukaryotic organisms, and was found to occur also in the bacterium *Campylobacter jejuni*. The glycosylation machinery that is encoded by the protein glycosylation locus (pgl) in *C. jejuni* has been functionally transferred into *Escherichia coli* [2], enabling the production of several *N*-glycosylated proteins in this well-known bacterial system. We have stably assembled *N*-linked glycoproteins on filamentous phage particles (GlycoPhage) simply by infecting the glycosylation competent *E. coli* with M13 phage displaying an acceptor protein. The developed technique can be used for several purposes. For instance, similar to the phage display system, it provides a genotype-to-phenotype link between the phage-associated glycoepitope and the phagemid-encoded genes, enabling to screen glycoprotein libraries using lectin chromatography [1]. Second, phage-patterned microarray, now a standard art in proteomics, is being extended to include GlycoPhage particles to serve in glycomics. Finally, since bacteriophages have already proven to be useful as recognition elements in biosensors, glyco-phage based biosensors can detect unique glycan chains, which are the biomarkers of severe metabolic diseases and also the signature element of most bacterial and viral pathogens.

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Poster 5.0.11

MEMS biosensor for blood plasma viscosity measurements

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MEMS (micro-electro-mechanical systems) cantilevers made of electroplated nickel are used for detection of viscosity of fluids. Microfabricated cantilevers are immersed in the liquid to be analyzed while continuously monitoring their resonance frequency. Detection of viscosity is based on measurement of change in resonant frequency of microcantilevers. Initially microcantilevers are resonated in a liquid of known viscosity. Then, the measurement chamber is filled with the liquid of interest. The differential frequency measurement is used to determine the viscosity of the second liquid.

The cantilever structures are forced to vibrate at their resonance frequency by magnetic actuation. Their response is measured by optical interference using a photodiode, amplifier and frequency counter. Glycerol/water solutions at different concentrations were tested for initial experiments. Measurement time is approximately 10 s using less than 1 ml of solution. The normal range of blood viscosity is 1.20–1.85 cP which is elevated in symptoms of hyperviscosity. In this study, viscosities in the range of 1–4 cP were

measured with the microfabricated chips with a sensitivity of 0.01 cP. In follow-up studies, blood plasma samples taken from patients will be tested. This system is promising for applications such as diagnostics and prognostic medicine.

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Keywords: MEMs; Microcantilevers; Biosensor; Viscosity; Blood plasma.

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Poster 5.0.12

On-line model-based optimization and control of fed-batch processes using Matlab code, OPC server, SCADA, and PLC

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In fed-batch processes, substrate feeding profiles are usually calculated before the cultivation by means of mathematical models or according to other a priori information. Due to random variations and disturbances in real processes, it is hard to ensure good repeatability. According to the proposed approach, the problem can be solved by correcting the control strategy by means of an on-line model-based optimization procedure each time the new data (glucose/saccharose and biomass measurements) is received. The first phase of the procedure involves re-identification of the model parameters based on the new data, and the second one involves the on-line re-optimization of the feeding profiles based on the mathematical model identified in the first phase. After correction, the optimized feeding profile should be transferred to the process control system (PLC) and executed. Technically, the proposed approach cannot be implemented directly using only standard process controllers (PLC) and process visualization systems (SCADA) due to the limited mathematical tools available in these systems. Hence, a program code for Matlab software was developed, which establishes connection with SCADA over the OPC server. The code also involves mathematical model of the process and optimization routine. The algorithm compares the measured process data with the prediction of the model, and, in case of significant deviations, the optimization routine is performed, and a new feeding profile is calculated. In such a way, a fed-batch cultivation process is always carried out according to the on-line re-optimized control strategy. The proposed approach improves the repeatability of the fed-batch process.

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Poster 5.0.13

Mass spectrometry facility in METU-Central Laboratory, Molecular Biology – Biotechnology R&D Center

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Middle East Technical University Central Laboratory is a scientific research, training and education center, featuring state-of-the-art equipments for material characterization and molecular biology and biotechnology. Molecular Biology-Biotechnology R&D Center is a part of METU Central Laboratory and aims to be a reference laboratory/crossing point for the advanced projects in Turkey promoting the industry-university-government interactions. Mass Specrometry Unit in METU MBB Center was newly established and equipped with AGILENT 6460 QQQ LCMSMS, AGILENT 7100 Capillar Electophorase and AGILENT NanoLC-ChipMS. LCMSMS system has multimode ESI/APCI ionisation system and access for APPI, Nanosprey, MALDI and Chip. The unit has a pesticide database for scanning 284 different compounds, a forensic and toxicology database for 187 compounds in 20 different main groups and METLIN database which is Personal Compound Database and Library. It includes masses, chemical formulas, and structures for over 25,000 endogenous and exogenous metabolites, lipids, and di- and tri-peptides. Capillar electrophorese can be coupled to mass spectrometry and used in different applications ranging from sugar analyses to the double-single DNA strand analyses and peptide profiling. In NanoLC-Chip MS system, the all autosampler, pre-column and analyses column and pump systems are fixed in a small size polymer chip decreasing 50% fitting connections. Nano level analyses and sample volume decresing to 0.01 μL helps for the analyses of the metabolites and drugs in original physiological level. Biomarker analyses, lipidomics, protein phosphorylation analyses and proteomics are the main application areas for NanoLC-ChipMS.

Keywords: LCMSMS; CE; CE/MS; NanoLC-ChipMS

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Poster 5.0.14

Optimization of production conditions for *Trichoderma* sp. P7 as a biocontrol agent in liquid culture

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Trichoderma spp. have been widely used as antagonistic fungal agents against several pests as well as plant growth enhancers. Mycoparasitism, spatial and nutrient competition, antibiosis by enzymes and secondary metabolites, and induction of plant defence system are typical biocontrol actions of these fungi.

Trichoderma spp. can be efficiently used as spores (especially, conidia), which are more tolerant to adverse environmental