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## Cell line development platform optimization for monoclonal antibody production in CHO-DG44

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The Vaccine Production Program Laboratory at the National Institutes of Health translates research products from the Vaccine Research Center into material for proof-of-concept clinical trials. To meet production demands of >4 g/L for monoclonal antibodies, an efficient and reliable process for stable cell line generation is required. Evaluation of the early steps of the process used for VRC CHO-DG44 cells has been undertaken with a focus on transfection and MTX amplification. Transfection efficiencies and cell viabilities of >90% have been achieved through optimization of Amaxa™Nucleofector™ and MaxCyte® STX™ transfection and post-transfection recovery conditions. Additionally, replacing the ActiCHO production medium used for post-transfection culture with HyClone™ CDM4CHO™ medium was found to increase MTX selection sensitivity and improve titers in shake flask batch cultures by 3- to 6-fold. Finally, a monoclonal antibody-producing clone was subjected to varying concentrations of MTX (from 30-300 nM). Cells from wells with the highest titer after 1-2 weeks in 24-well plates were pooled and expanded. In un-optimized shake flask fed-batch runs, product titers for cells grown in 100 or 300 nM MTX were increased by approximately 1.5- to 2-fold compared to those in 30 nM MTX (from 0.7 g/L to 1.2-1.4 g/L). Together, these modifications to the existing process are critical to ensure that subsequent clone selection will provide stable, high-producing cell lines.

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## Bacterial consortium; A porous bio-carrier for enhancement of biodegradation of crude oil contaminated soil

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*Ex-situ* experiment was conducted for the bioremediation dealt with the soil contamination through wild indigenous strains of *Bacilli* and *Cocci*. Removal of aromatic and aliphatic hydrocarbons was observed and the soil reclamation was achieved during the study. An energy source optimization and enhancement and cell building blocks were multiple objectives achieved during the course of study at laboratory scale. Isolation, screening, characterization and identification indigenous microorganisms and their application for bioremediation is presented in this piece of work. The organisms were isolated from a crude oil contaminated soil from the Lower Indus Basin fields of Oil and Gas Development Company Limited (OGDCL), Pakistan. The species showed their exponential growth in crude oil as carbon source and were found as dominanthydrocarbon degraders. Six isolates were finally selected found effective and applied to the contaminated soil for 30 days bio-augmentation in aerobic environmental conditions with time interval of 3 days to each sample. *Bacillus sphaericus* (2), *Bacillus megaterium* (A4) and *Micrococcus luteus* (3d) proved their efficiency as 97, 90 and 84 % respectively.

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