us toward design of a flagellin based adjuvant. These truncated forms can be capable of inducing immune responses and also they can be easily cloned and expressed in the form of a fusion protein.
Keywords: *In Silico*, fliC gene, Enteroaggregative *Escherichia Coli*

279. Fragment F(ab')$_2$ Produced by Digestion of Rabbit Immunoglobin G with Pepsin
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Background: Immunoglobulin (Ig) fragments can be advantageous for a number of experimental methods. F(ab')$_2$ is a bivalent antibody fragment which is currently used for both diagnosis and treatment. F(ab')$_2$ fragments are smaller than whole Igs but maintain antigen binding function. The smaller size results in better tissue penetration and less steric hindrance leading to more sensitive antigen detection. IgG are a class of large proteins of approximately 150 kDa, made of two identical heavy chains (50 kDa) and two identical light chains (25 kDa). Each IgG has two antigen binding sites that can bind a single antigen molecule independently. Fragments of IgG molecules composed of only antigen binding portions can be obtained through enzymatic degradation. The enzyme pepsin cleaves the Fc portion of an IgG into small subfragments leaving a F(ab')$_2$ fragment with two antigen binding sites connected by disulfide bonds. In this study we used whole proteins of *Mycobacterium Avium Paratuberculosis* (causative agent of Johne's disease) as a antigens to produce polyclonal antibodies and produced F(ab')$_2$ through pepsin digestion of rabbit IgG. Materials and Methods: Polyclonal antibodies prepared by immunization of New Zealand white rabbit against *Mycobacterium Avium Paratuberculosis* (MAP) proteins. Rabbit was bleeded and serum was sedimented and Immunoglobulins were obtained. IgG was purified by ion exchange chromatography (DEAE-celloluse) and IgG solution were digested by pepsin for isolated the fragment F(ab')$_2$. Results: Purification was carried out by ion exchange chromatography on DEAE-celloluse that we collected seven fractions composed of IgG and result to one peak. SDS-PAGE of Digested IgG by pepsin gave a single band. Conclusion: In conclusion, we produced a rabbit anti-MAP polyclonal antibodies by immunization and The F(ab')$_2$ fragments are produced by digestion of the whole antibodies with pepsin. We checked the separated F(ab')$_2$ fragments by SDS-PAGE and we obtained high purity of F(ab')$_2$ fragments.
Keywords:Fragment F(ab')$_2$, Rabbit Immunoglobin G, Pepsin

280. Selection of Recombinant Antibodies to Human Epidermal Growth Factor Receptor 4 (HER4)
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Background: The human epidermal growth factor receptor (HER) family comprises four homologous members: EGFR (ErbB1), ErbB2 (HER2), ErbB3 (HER3) and ErbB4 (HER4). Alterations and disruptions in the function of the HER-kinase axis can lead to malignancy. Among HER family members, EGFR and HER2 are the most studied. However, data provide evidence for the significance of HER3 and HER4 alterations in cancers especially breast carcinogenesis. The heterodimerization of HER2 with HER3 and HER4 lead to tumor cell proliferation. Recombinat single chain antibodies (scFv) have been introduced as the most desire agent for cancer immunotherapy due to their human origin, fast penetrating and high affinity properties. In this study scFv to HER4 antigen was selected. Materials and Methods: A phage antibody display library of scFv was panned against immunodominant epitope of HER4. The peptide was coated in Nunc tube, after adding the phage antibody (10^9 PFU/ml), elution was done using log phase Ecoli TG1. The clones were PCR amplified and DNA fingerprinted to select the specific clones against the epitope. Results: Results represented 2 predominant patterns with frequencies 25% and 30%. The other patterns showed frequencies 5%. Conclusion: Recombinant antibodies are the most ideal form of cancer immunotherapy. Trastuzumab, a humanized monoclonal antibody, targets cancer cells that overexpress HER2. Since it is not a full humanized antibody, it produces HAMA (human anti-mouse antibody response). Human scFvs not only are able to overcome this problem but also are able to penetrate to tumor tissue effectively. We have already selected scFvs against HER2 and HER3 epitopes and showed their inhibitory effects on breast cancer cell lines. In order to inhibit the HER2 heterodimerization more effectively, in this study anti-HER4 scFvs were selected. Panning results demonstrated 2 specific clones against HER4 epitope. Further investigations are needed to show the effects of the selected single chain antibody.
Keywords: Recombinant Antibodies, HER4

281. Expression of Camelid-derived Heavy Chain Antibody (Nanobody) against ureC Subunit of Urease from *H.pylori* in pichia pastoris
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Background: The methylotrophic yeast *Pichia pastoris* has become a cost affecting system for production of a variety of proteins particularly those require specific folding such as antibody fragments. The increasing popularity of this expression system is due to several factors such as: the capacity to perform many eukaryotic post-translational modifications, such as glycosylation, disulfide bond formation and proteolytic processing. In the present work we have cloned the gene coding for the camelid- derived heavy-chain antibody (VHH) against ureC subunit of urease from H.pylori in the pPink-HC vector and expressed in yeast *Pichia pastoris*.Since this protein is already expressed in E.coli, therefore our aim is to compare the function of the protein expressed in two different systems .Any change in their function will be subject to their folding. Materials and Methods: The VHH gene fragment was subcloned into the pPink-HC vector. The construct was transferred to *E. coli* top10. After multiplication and purification, the pPink-HC vector was linearzed by cutting at a unique site in order to promote integration into the pichia pastoris genome. The vector was transferred in to competent cells. The cells were spread on MD agar selection plates and white colonies were selected as positive clones and further confirmed with PCR. The colonies were expressed in the BMGY and BMMY mediums.The results of the expression was analysed on SDS PAGE. Results: The expression of a 17-kDa protein was observed in the sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). Conclusion :The affinity of the antibody to its specific antigen will be investigated after purification.
Keywords: Nanobody, ureC, Urease, *H.pylori*

282. Replicating Nonviral Minicircles as a Novel Tool in Gene Therapy: Construction and Transfection Efficiency Evaluation
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Background: Current delivery methods used in gene therapy and vaccination can be subdivided into viral vectors with higher cell transduction efficiency, and nonviral methods with less toxicity without DNA insert size limitations. Bacterial backbone sequences are dispensable for gene transfer application which reduce the efficiency of gene expression. Therefore, an important goal in nonviral vector development is to produce supercoiled pDNA lacking bacterial backbone. Thus, producing episomal minicircle vectors, devoid bacterial backbone was developed. These extra-chromosomal DNA vectors showed persistent and high level of transgene expression. New generation of these vehicles carrying Scaffold/matrix attachment region (S/MAR) elements causes long-term expression of transgene in the absence of selection. Presence of S/MAR elements in minicircle DNA can exploit the cellular replication machinery for episomal replication. Construction of replicating non-viral minicircle can be achieved by site specific recombination in parental plasmid between two copies of recombinase ΦC31 recognition sites in bacterial cells. This study was designed for constructing an efficient vehicle containing S/MAR elements creating minicircle DNAs in purpose of