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Title: Identification and Expression of the Heat Shock Protein 60 (GroEL) from the Etiologic Agent of epizootic bovine abortion

Hypothesis: The complete HSP60 gene of aoEBA can be elucidated using degenerate primer PCR and genome walking.

Specific Aim 1: Obtain complete sequence of HSP60 (GroEL) gene
Specific Aim 2: Expression of HSP60 gene using Invitrogen Champion pET kit
Specific Aim 3: Purification of the HSP60 using Profinia and His-tag

Results: The complete sequence of the aoEBA HSP60 gene was obtained using the alignment and degenerate protocol described within the proposal. The HSP60 sequence was determined to be approximately 1,600bps. During the walking, I was able to walk into the HSP10 gene as it sits upstream of the HSP60 gene. The complete HSP10 sequence was approximately 270bp and had a 71% homology to Sorangium cellulosum.

Both sequences for HSP60 and HSP10 were successfully amplified from several bovine and murine isolates to confirm the lack of variation within the gene sequence. Once the gene sequences were confirmed, both amplicons were bluntly cloned into the pDrive cloning vector (Qiagen), and resequenced. Specific primers were then designed to clone the amplicons into the expression vector, pET-TOPO100. Once the amplicons were successfully cloned into the expression vector, they were sequenced to ensure that the cloning had occurred in frame and that there were no changes to the sequence during the cloning process. The products were then expressed preliminarily to determine whether they would express and the optimal expression duration. The protein expression was analyzed using a combination of a SDS-page gel stained with a Coomassie-blue-like stain and Western blot with an anti-His antibody. The protein was then purified using a Profinia Native isolation protocol in which the bacteria were lysed using sonication and purified over an IMAC column which selectively binds the histidine tag on the recombinant protein and allows all the other bacterial proteins to wash off. The purification efficiency was assessed using both an SDS-page gel and a western and was determined to be quite efficient in terms of protein purification and recovery. Protein concentrations were quantified using the BCS method with a BSA standard curve.