

B10

Title: Experimental Design of Subcloning and Identification of the IL10 Gene to Determine Its Role as a Disease Marker of Human Heart Failure

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Background

Human heart failure (HF) has many causes, a change in RNA expression profile of a group of genes (genetic polymorphisms) may contribute to its pathogenesis. The IL10 gene expresses anti-inflammatory properties, regulates cytokine expression and other immune responses. A genetic polymorphism of IL10 is a suspected marker of HF.

Goals

To design an experimental protocol to test the hypothesis that a gene with varied expression in HF patients may act as a disease marker for HF.

Methods

RNA sequencing data from clinical samples was analyzed by NCBI BLAST genome analysis software for candidate genes. Websites (Addgene and Thermo Fisher Scientific) assisted with molecular protocols for RNA extraction and Reverse Transcription. The Primer3web software from University of Tartu Estonian Biocentre helped design appropriate PCR primers, NEBcutter software (New England Biolabs) added restriction enzyme sequences compatible with pcDNA 3.1/myc-His(-) A expression vector. The Invitrogen manual titled “pcDNA 3.1/myc-His(-) A, B, and C” defined the protocol to add the myc-epitope and polyhistidine identification tag to the cloning vector. Finally, the post-PCR protocols including bacterial transformation, transfection, and Western blotting were detailed.

Results

A working experimental design to test the aforementioned hypothesis was established, to find HF disease related genes/markers. Potential function of candidate gene markers can be evaluated by cell culture transfection and gene expression pattern analysis to determine if the selected gene is a real disease marker.

Conclusions

A protocol to test if varied expression pattern of IL10/any other gene implied by clinical RNA sequencing data, can cause HF was successfully designed.