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AMPLIFICATION OF hTSH α- AND β-SUBUNIT GENES BY PCR, USING PITUITARY cDNA, FOR RECOMBINANT hTSH PREPARATION.

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Human thyrotropin (hTSH) is a member of a family of pituitary and placental heterodimeric glycoproteins containing a common α- and unique β-subunit, which provides biological specificity of these hormones. The recombinant hTSH (rec hTSH) can fully replace the extracted hormone, as tracer and standard in diagnostic "in vitro" immunoassay systems and stimulate ^{131}I uptake in thyroid carcinoma therapy. In our laboratory, after the unsuccessful screening of classical libraries for the hTSH β-subunit in particular, we isolated and amplified α- and β- hTSH subunits by Polymerase Chain Reaction (PCR), using human pituitary cDNA. Specific primer pairs (0.5 μM) for α- and β- hTSH subunits were designed and amplification was carried out by PCR in 50 μl (30-70 ng cDNA) under specific denaturation, annealing and extension conditions. The PCR amplified genes were subsequently introduced into appropriate expression vectors, in order to transfect Chinese hamster ovary (CHO) cells. The same PCR methodology can be successfully applied to the isolation and amplification of other glycoprotein hormone genes, which are also necessary for the obtainement of diagnostic and medicinal products derived by recombinant DNA technology, such as hFSH, hLH, hPRL, hGH and hCG.

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