# MOLECULAR CLONING, EXPRESSION, AND PURIFICATION OF RECOMBINANT HUMAN PPARγ IN *Escherichia Coli*

Dalila Balderas-Rentería[1], Sonia Montero-Molina[1], Jorge Solís-Estrada[1], Verónica Almaguer-Cantú[2], Katiushka Arévalo-Niño[2], Xristo Zárate-Kalfopulos[1], *Isaías Balderas-Rentería[1].
Universidad Autónoma de Nuevo León. [1]Facultad de Ciencias Químicas, Laboratorio de Farmacología Molecular [2]Facultad de Ciencias Biológicas, Instituto de Biotecnología. Ciudad Universitaria, San Nicolás de los Garza, N.L. CP 66455. isaias.balderasrn@uanl.edu.mx

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**Introduction.** In the presence of an increasing prevalence of obesity, diabetes, and cardiovascular diseases, the nuclear receptor peroxisome proliferator-activated receptor gamma (PPARγ) has emerged as a transcriptional regulator of metabolism whose activity can be modulated by direct binding of small molecules (1). PPARγ ligands are still clinically effective antidiabetic drugs, although side effects limit their use (2). Therefore, to investigate thoroughly the molecular interactions and the relationships between structure, function, and pharmacological properties, it would be desirable the use of full-length PPAR gamma protein. Nonetheless, considerable quantities of active protein are not able to be obtained from the original sources. In this study, a new strategy for human PPAR gamma (hPPARγ) expression in bacteria was developed using a fusion protein (CusF3H+) for purification with Immobilized Metal Affinity Chromatography (IMAC).

**Methodology.** Molecular cloning of the complete human PPAR gamma gene was performed using a pET30(a+) plasmid, reengineered by Zarate et al (3), using the CusF3H+ fusion peptide to improve protein export to the extracelular space in *Escherichia coli*.
Rosetta strain of Escherichia coli was used for the transformation and expression. Different IPTG concentrations as well as different incubation temperatures were used to perform the production of the protein.
Protein expression was analyzed by SDS-PAGE and Purification was carried out by Immobilized Metal Affinity Chromatography (IMAC) using the Äkta Primer Plus FPLC system (GE Healthcare, USA).
Elution was carried out through a 20% to 100% imidazole gradient with an elution buffer (50 mM Tris-HCl, 500 mM NaCl, 250 mM Imidazole, pH 8.0). Fractions with increased absorbance were analyzed by SDS-PAGE 10% to determine their purity.

**Results.** hPPARγ was mainly expressed in soluble fraction at a low temperature (25ºC) and 0.1 mM isopropyl-β-D-thiogalactopyranoside (IPTG). Protein identity was successfully confirmed by Western blot assay (figure 1). Our results indicated the viability of using this approach to produce hPPARγ in *E. coli* Rosetta (DE3), which could be a useful methodology for the study of this protein in *in vitro* assays.

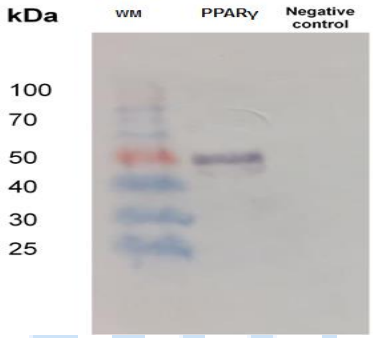


**Figure 1**. Western blot analysis. Lane 1: prestained molecular weight marker. Lane 2: hPPARγ protein (54 kDa). Lane 3: complete protein fraction of E. coli Rosetta (DE3) pLysS, negative control.

**Conclusions.** In this study, a new strategy for bacterial human PPAR expression was developed using a fusion protein (CusF3H+) for purification with IMAC. hPPARγ was mainly expressed in soluble fraction at low temperature (25ºC), and protein identity was confirmed by Western blot assay. Our results shown the viability of using this strategy to produce human PPAR gamma in *E. coli* Rosetta (DE3).

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