

# PZ-TR: a novel human luciferase reporter cell line for assessment of thyroid receptor transcriptional activity

Peter Illes\*, Zdenek Dvorak



Department of Cell Biology and Genetics, Faculty of Science, Palacký University in Olomouc, Slechtitelu 27, 783 71, Olomouc;

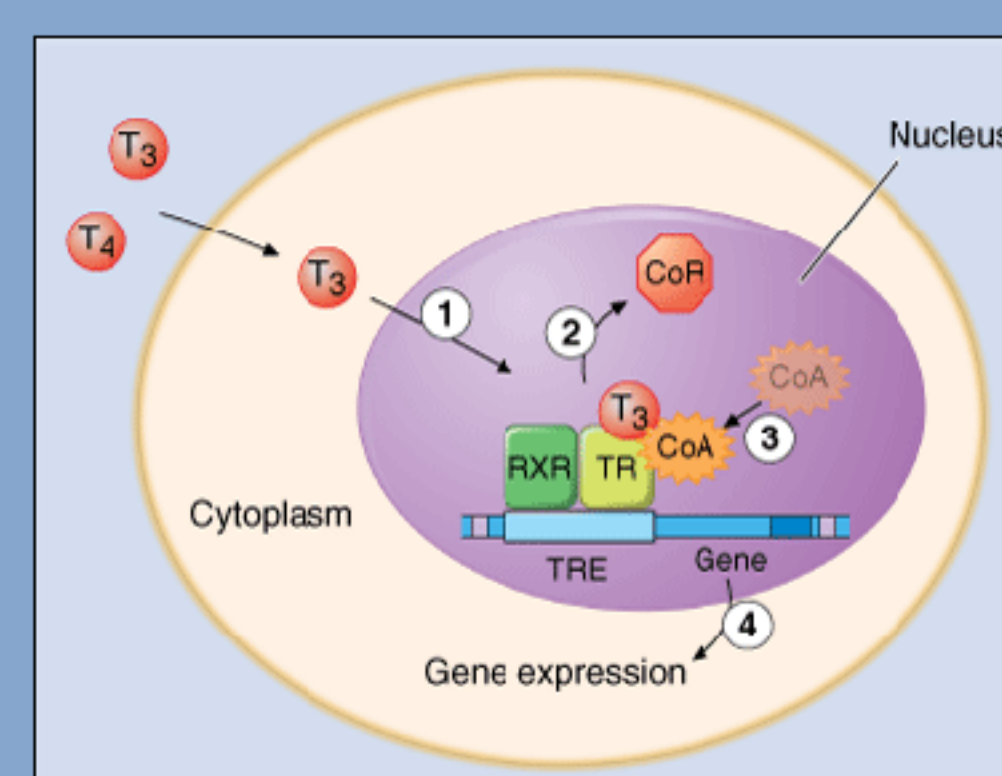
\*peter.illes@upol.cz

## ABSTRACT

Regulation of gene expression mediated by thyroid hormones (THs) plays an essential role in variety of physiological processes. It is known that large group of natural and synthetic compounds, generally termed endocrine disruptors, can interfere with endocrine system and thus disrupt homeostasis of hormonal regulation processes in human organism. In order to screen for substances with potential disrupting effects on TH pathway, we developed and characterized human luciferase reporter gene cell line for assessment of thyroid receptor (TR) transcriptional activity – PZ-TR. PZ-TR cell line was derived from human hepatocellular carcinoma cells HepG2, which were stably transfected with reporter plasmid containing two copies of tandem thyroid response elements (TREs) upstream of reporter gene for luciferase. Dose-response analyses showed that triiodothyronine (T3), natural ligand of TR, induced luciferase activity in PZ-TR cells in dose-dependent manner and sensitivity of luciferase assays allowed detection of T3 in nanomolar range of concentrations. Maximum fold induction of luciferase activity ranged from 2.5 x to almost 3 x after 24 h of exposure to T3. We did not observe unspecific induction of luciferase activity by other steroid hormones and VDR ligands, only exception was partial increase of luciferase activity after treatment of PZ-TR cells with retinoic acids. Cryopreservation of PZ-TR cells did not influence their functionality, similarly responsiveness to T3 and cell morphology maintained unaffected even after long-term cultivation. Novel PZ-TR cell line was used for evaluation of effects of organic tin compounds on transcriptional activity of TR. We found that both, tributyltin derivatives and triphenyltin derivatives induced luciferase activity in PZ-TR cells. These findings indicate that organic tin compounds have potential to interfere with TR-mediated regulation of gene expression and thus influence physiological activity of THs.

## INTRODUCTION

Thyroid hormones (THs), triiodothyronine and thyroxine (T3 and T4), regulate gene expression by binding to high-affinity thyroid hormone receptors (TRs) and play a crucial role in growth, development and energy homeostasis. TRs belong to the subfamily of nuclear receptors that recognize specific response elements (TREs) in promoter regions of TH-regulated genes and activate or repress transcription in response to hormone. It is known that large number of exogenous ligands, including natural and synthetic compounds, drugs and environmental pollutants, may disrupt thyroid hormone mode of action and thus affect normal function of endocrine system. Therefore, the development of stable reporter system for assessment of TR transcriptional activity allowing high-throughput screening of effects of various compounds on TR-dependent regulation of gene expression, would be of great benefit.



**Figure 1.** Mechanism of thyroid hormone action. Thyroid hormone receptor (TR) and retinoid X receptor (RXR) form heterodimer that bind specifically to thyroid response element (TRE) in the promoter region of target genes. In the absence of hormone, TR binds co-repressor (CoR) proteins that silence gene expression. After the T3 enters the nucleus (1), T3 binding dissociates CoR from TR (2), co-activators (CoA) are recruited to the T3-bound receptor (3) and gene expression is altered (4).

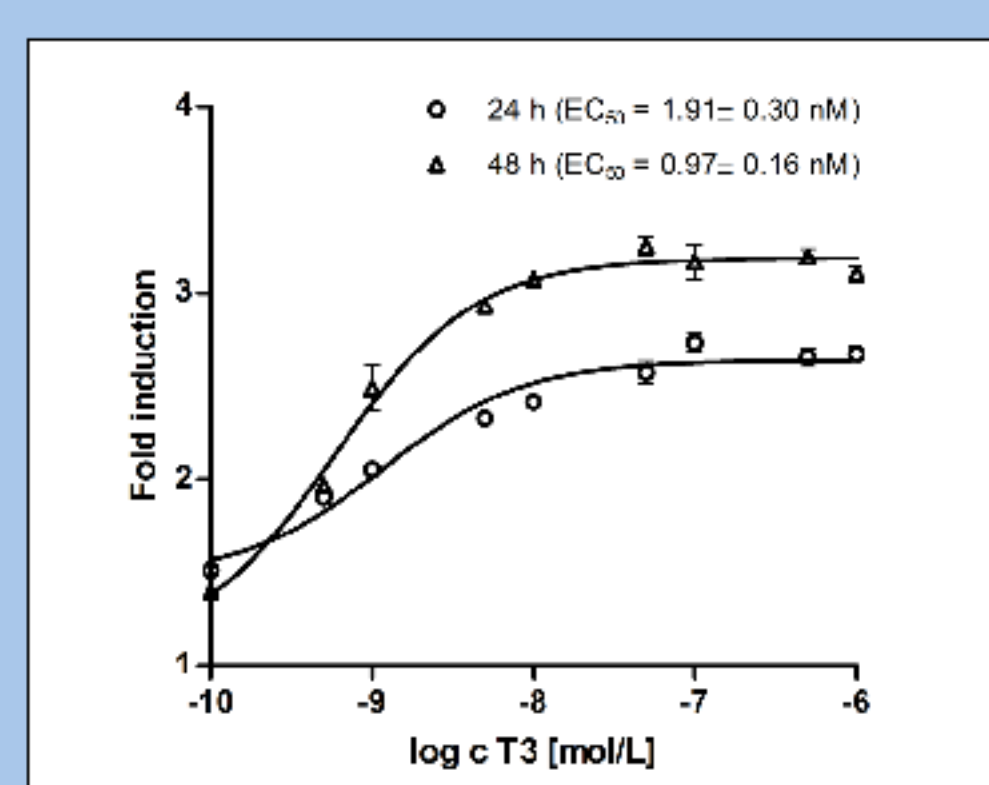
Fauci AS, Kasper DL, Braunwald E, Hauser SL, Longo DL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine*, 17<sup>th</sup> Edition (<http://www.accessmedicine.com>)

## OBJECTIVES

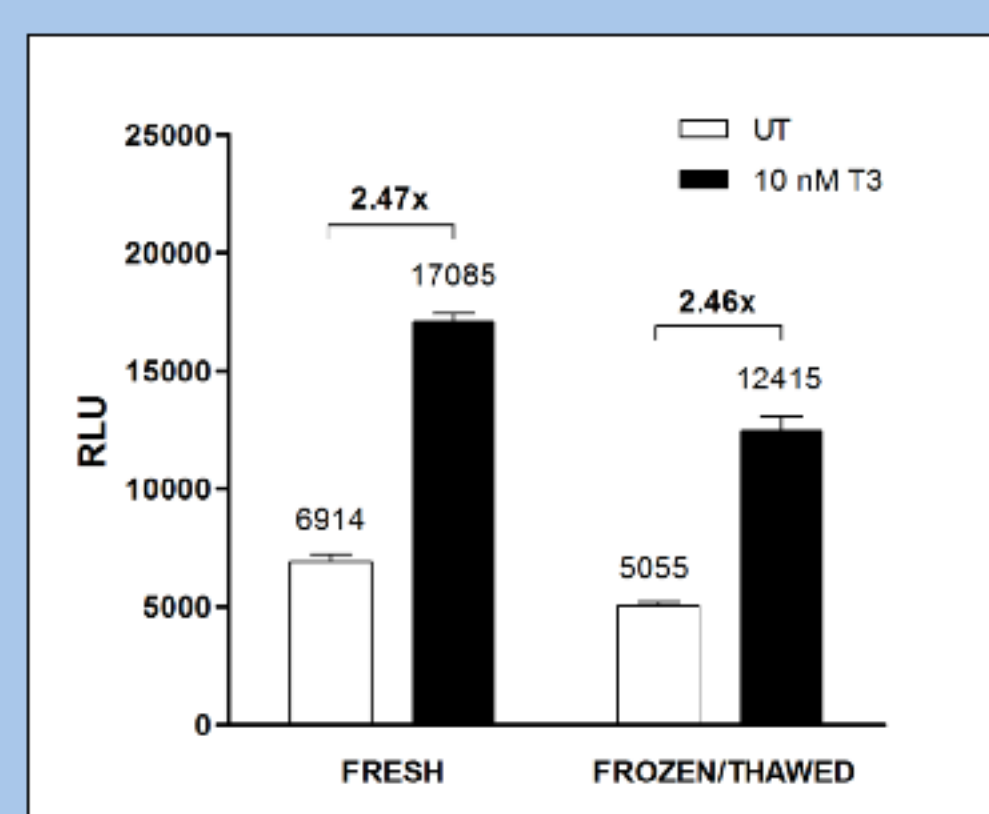
In our recent work we focused on construction of stably transfected human luciferase reporter cell line for assessment of TR transcriptional activity. The main aim of our experiments was to develop a reliable tool allowing cheap, high-throughput screening for substances with potential disrupting effects on thyroid hormonal pathway.

## RESULTS

Novel reporter cell line for assessment of thyroid receptor transcriptional activity PZ-TR was derived from human hepatocellular carcinoma cells HepG2 that were stably transfected with reporter plasmid containing two copies of tandem thyroid response elements (TREs) upstream of reporter gene for luciferase. Dose-response analyses showed that triiodothyronine (T3) induced luciferase activity in PZ-TR cells in dose-dependent manner and sensitivity of luciferase assays allowed detection of T3 in nanomolar range of concentrations. Maximum fold induction of luciferase activity ranged from 2.5 x after 24 h to almost 3.5 x after 48 h of exposure to T3. Unspecific induction of luciferase activity by other steroid hormones and VDR ligands was not observed, only exception represented partial increase of luciferase activity after treatment of PZ-TR cells with retinoic acids. Cryopreservation of PZ-TR cells did not influence their functionality, similarly responsiveness to T3 and cell morphology maintained unaffected even after long-term cultivation. PZ-TR cell line was used for evaluation of effects of organotin compounds on transcriptional activity of TR. We found that both tested groups, tributyltin derivatives and triphenyltin derivatives induced luciferase activity in PZ-TR cells. Parallel experiments revealed that organotin compounds are able to enhance effect of T3, especially after treatment with triphenyltin derivatives.



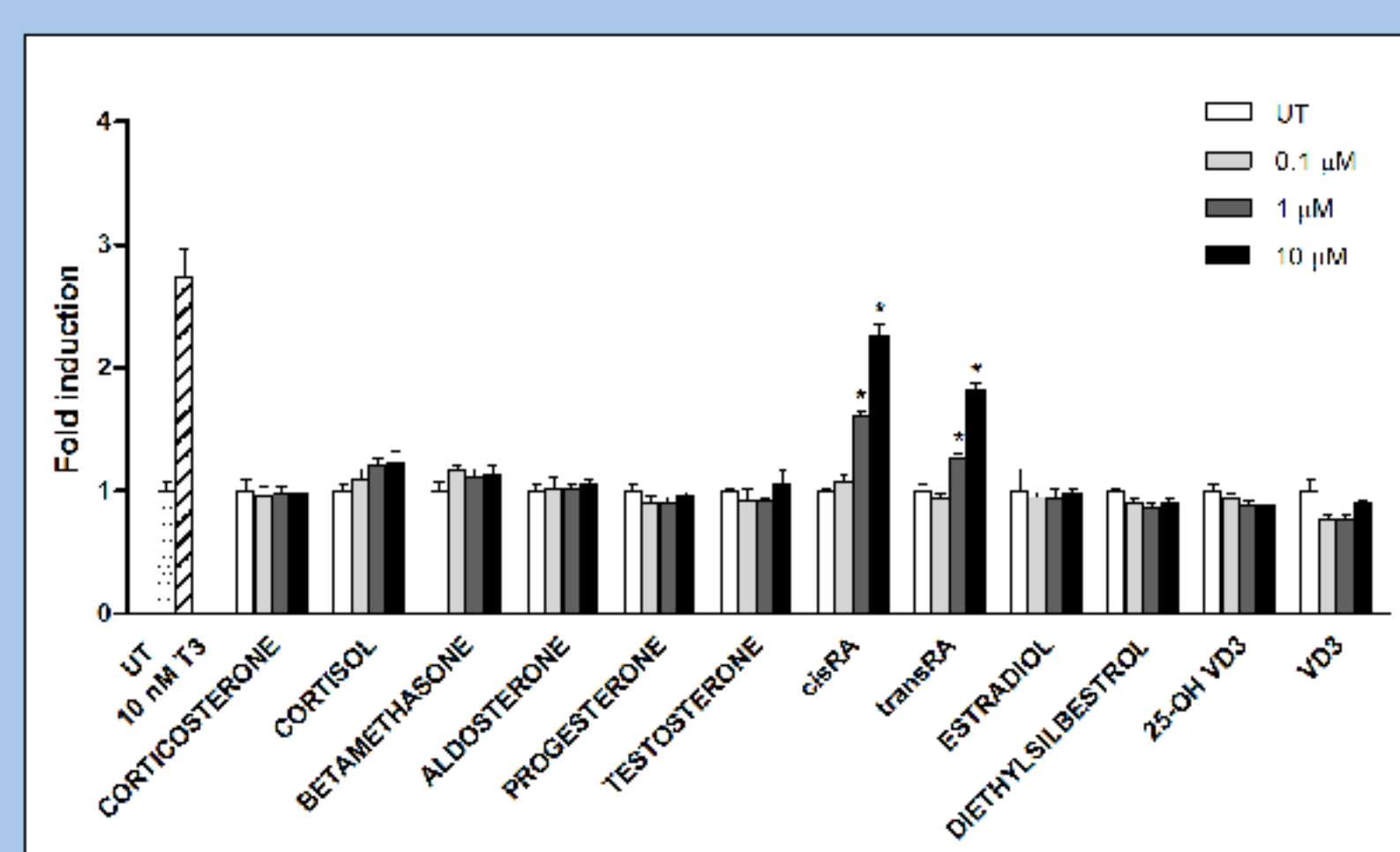
**Figure 2.** Dose-dependent response of PZ-TR cell line to T3. Cells were treated with range of T3 concentrations (0.1 – 1000 nM) for 24 and 48 h.



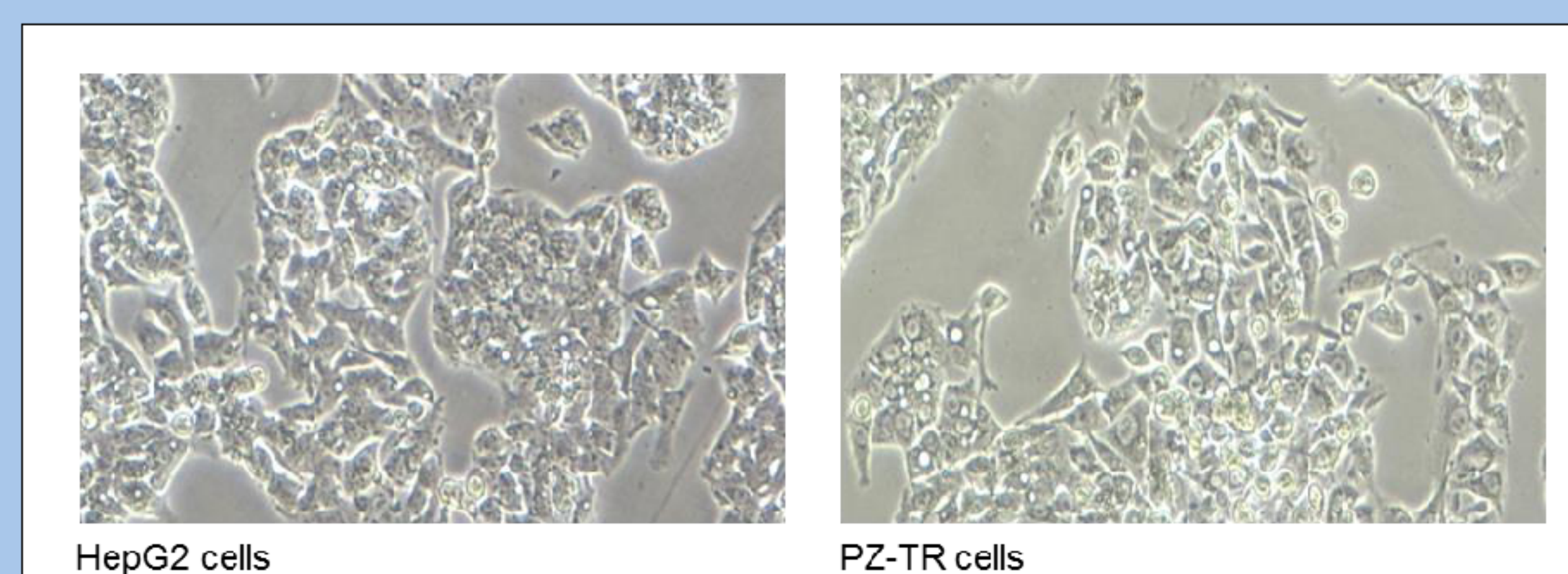
**Figure 3.** Effect of cryopreservation on functionality of PZ-TR cell line. Experiments were performed on fresh cells and cells after freeze-thaw cycle treated with vehicle (UT; 0.1% DMSO V/V) and 10 nM T3.

Passage number	Days in culture	RLU ± SD	Fold
0	4	12415 ± 1109	2,46
3	16	9205 ± 68	2,57
6	29	11344 ± 954	2,73
9	41	8944 ± 520	2,42
12	54	8954 ± 276	2,45

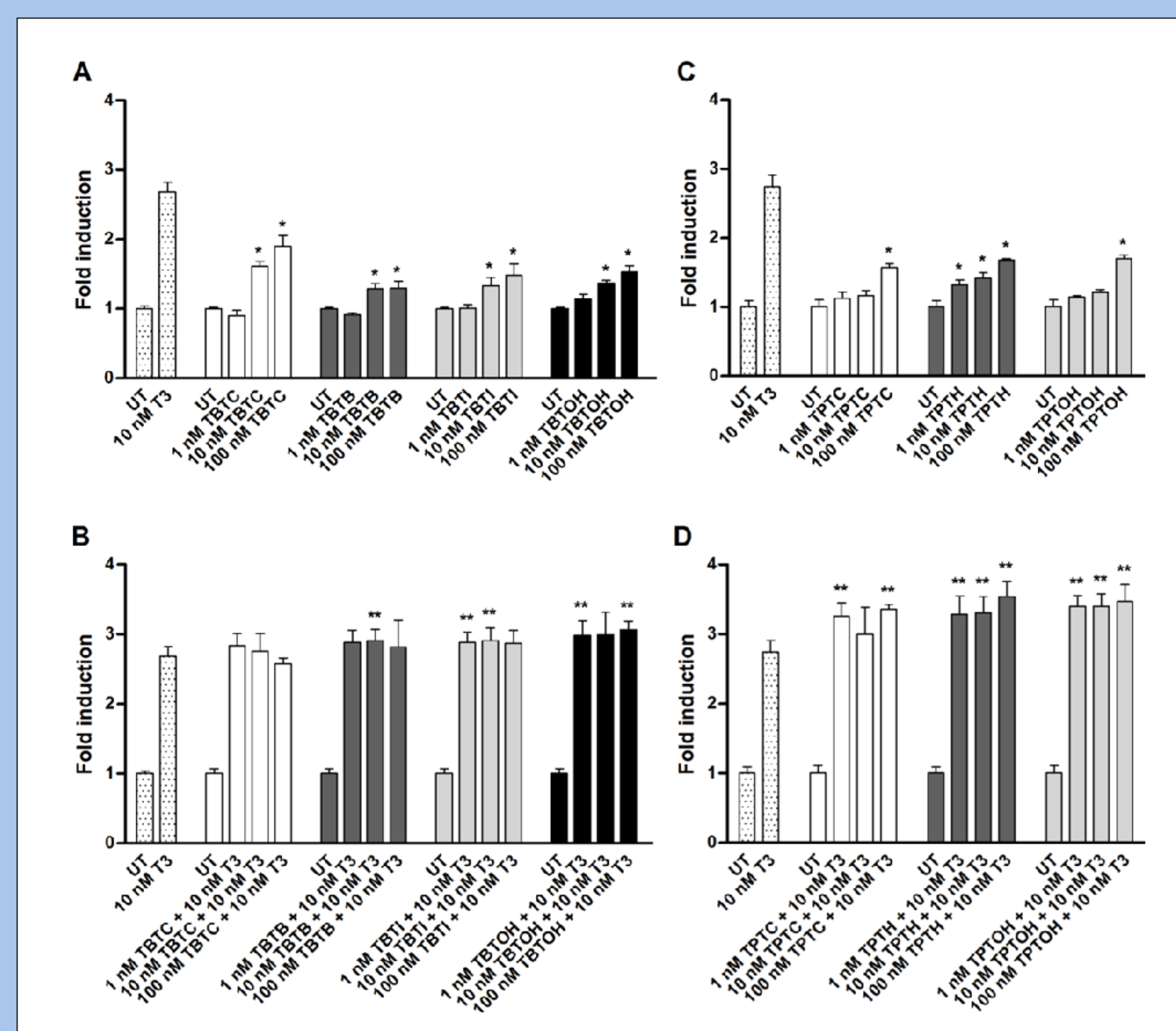
**Table 1.** Maintenance of luciferase inducibility by 10 nM T3 after long-term cultivation.



**Figure 4.** Response of PZ-TR cell line to hormonal treatments. Cells were treated with vehicle (UT; 0.1% DMSO V/V) and different hormones in concentrations of 0.1 mM, 1 mM and 10 mM for 24 h. \* - values are significantly different from values of vehicle-treated cells ( $p < 0.05$ ) as determined by Student's t-test.



**Figure 5.** Morphology of HepG2 and PZ-TR cell lines. Phase contrast micrographs of parent HepG2 cells in 8th passage and PZ-TR reporter cells in 10th passage.



**Figure 6.** Effect of organotin compounds on transcriptional activity of TRE in PZ-TR reporter cell line. Cells were treated with vehicle (UT; 0.1% DMSO or/and ethanol V/V), tributyltin derivatives (TBTC – tributyltin chloride, TBTB – tributyltin bromide, TBTI – tributyltin iodide, TBTOH – tributyltin hydroxide) (A) and triphenyltin derivatives (TPTC – triphenyltin chloride, TPTH – triphenyltin hydride, TPTOH – triphenyltin hydroxide) (C) in concentrations of 1 nM, 10 nM and 100 nM alone and in combination with 10 nM T3 (B, D). \* - values are significantly different from values of vehicle-treated cells ( $p < 0.05$ ) as determined by Student's t-test. \*\* - values are significantly different from values of 10 nM T3-treated cells ( $p < 0.05$ ) as determined by Student's t-test.

## CONCLUSIONS

- we developed and characterized novel stably transfected gene reporter cell line PZ-TR – high sensitivity, specificity and through-put
- exclusively human system derived from human HepG2 cells, endogenously expressing both isoforms of TR
- no need for additional co-transfection with TR expression vector - preservation of stoichiometric ratio between the TR receptor and other transcriptional regulators
- organotin compounds have potential to interfere with TR-mediated regulation of gene expression and thus influence physiological activity of thyroid hormone

- PZ-TR cell line – potential tool for pre-clinical testing of drugs, or other natural and synthetic thyroid-disrupting compounds

## ACKNOWLEDGEMENT

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