

Chapter 2

Global RT-PCR and RT-qPCR Analysis of the mRNA Expression of the Human PTPome

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Abstract

Comprehensive comparative gene expression analysis of the tyrosine phosphatase superfamily members (PTPome) under cell- or tissue-specific growth conditions may help to define their individual and specific role in physiology and disease. Semi-quantitative and quantitative PCR are commonly used methods to analyze and measure gene expression. Here, we describe technical aspects of PTPome mRNA expression analysis by semi-quantitative RT-PCR and quantitative RT-PCR (RT-qPCR). We provide a protocol for each method consisting in reverse transcription followed by PCR using a global platform of specific PTP primers. The chapter includes aspects from primer validation to the setup of the PTPome RT-qPCR platform. Examples are given of PTP-profiling gene expression analysis using a human breast cancer cell line upon long-term or short-term treatment with cell signaling-activation agents.

Key words Protein tyrosine phosphatase, Reverse transcription PCR, Real-time quantitative PCR, PTPome

1 Introduction

The global analysis of changes in the expression of well-defined gene or protein families during physiological or pathological conditions provides valuable information to understand the regulation of cell physiology in health and disease. Genome-wide DNA microarray analyses generate reliable comparative global information on gene expression patterns and on major changes in the expression of individual genes. However, the wide scope of genome-wide analysis may hamper the optimization and comparison of the gene expression changes on specific groups of genes. In addition, small changes in gene expression, especially for genes expressed at low levels, may be overlooked due to the limited sensitivity of the DNA microarray assays [1–3]. Reverse transcription quantitative real-time PCR (RT-qPCR) may overcome these problems because of its very high sensitivity and dynamic range and the possibility of individualized choosing and optimization of primers specific for

the genes of interest, including mRNA isoform-specific primers [4, 5]. Semi-quantitative reverse transcription PCR (RT-PCR) provides limited quantitative information, but can be useful to compare the expression of related mRNA variants which can be differentiated by size, or the relative expression of a mRNA of interest versus a reference mRNA, if coupled to agarose gel electrophoresis [6, 7].

The protein tyrosine phosphatase (PTP) superfamily is composed of members that belong to several gene families, which differ in the number and type of catalytic domains, as well as in their catalytic mechanism and overall substrate specificity. As such, an array of enzymes or enzyme-like proteins directly related with Tyr dephosphorylation can be considered as the PTPome. A former version of the human PTPome was defined as formed by about 107 members, and in an extended updated form expands to about 125 members [8, 9]. Many PTPome members harbor different noncatalytic regulatory domains, whereas others are small proteins only composed of the catalytic domain [8, 10–18]. The classical PTP enzymes (class I Cys-based classical PTPs) and the dual-specificity (DSPs) VH1-like PTPs (class I Cys-based DSPs) form the core of the PTPome and account for most of its members. They harbor one or two (classical PTPs), or only one (DUSPs), conserved catalytic PTP domains, and contain the conserved HCxxGxxR catalytic signature motif. The classical PTPs form a homogeneous group of enzymes with substrate specificity mostly restricted towards pTyr residues, whereas the DSPs include several subfamilies with different substrate specificities, including specificity towards pTyr/pSer/pThr, phosphoinositides, phosphorylated carbohydrates, and mRNA. Other PTPome enzymes contain a less-conserved CxxxxxR catalytic motif and employ a Cys-based catalysis to dephosphorylate pTyr/pSer/pThr residues or phosphoinositides, but harbor different classes of catalytic domains [arsenate reductase domain (class II-Cys based); rhodanese domain (class III Cys based)]. Finally, some phosphatases dephosphorylate pTyr using catalytic mechanisms non-Cys based, which belong to independent phosphatase gene families (Asp-based phosphatases, His-based phosphatases) [9]. This is of importance since the catalytic active sites of each of these enzyme families, as well as the physiologic regulation of their activities, display differences, making possible phosphatase family specific drug targeting with experimental or therapeutic purposes.

The PTPome is well suited to perform RT-PCR and RT-qPCR approaches to analyze comparatively the gene expression of their members [19–23]. We describe here methods to perform comparative semi-quantitative (RT-PCR) and quantitative (RT-qPCR) analysis of the gene expression of the human PTPome. Examples are shown using the MCF-7 human breast cancer cell line grown under different experimental conditions.

2 Materials

All solutions are prepared in double-distilled, RNase-free water. Cell culture and transfection procedures require sterile conditions.

2.1 Analysis of Human PTPome mRNA Expression by Semi-quantitative RT-PCR

1. Tissue-cultured cells, or biological samples, as a source of RNA.
2. Specific primer sets for amplification of the PTPs of interest and the reference control genes (*see* **Notes 1** and **2**).
3. RNA isolation kit (*see* **Note 3**).
4. cDNAs of the PTPs of interest, for primer specificity validation.
5. RT and PCR reagents (*see* **Note 4**).
6. Thermocycler.
7. Agarose gel electrophoresis and DNA visualization reagents (*see* **Note 5**).
8. Ultraviolet light detection system.

2.2 Global Analysis of Human PTPome mRNA Expression by Quantitative RT-PCR

1. Tissue-cultured cells, or biological samples, as a source of RNA.
2. Validated primer sets for quantitative RT-PCR (RT-qPCR) (*see* **Note 6**).
3. RT-qPCR reagents (*see* **Notes 4** and **7**).
4. RNA isolation kit (*see* **Note 3**).
5. cDNAs of the PTPs of interest, for primer amplification efficiency validation.
6. Plate setup suitable for loading the qPCR PTPome set.
7. Real-time thermocycler.

3 Methods

The first method (Subheading **3.1**) is aimed to monitor semi-quantitatively (band intensity) and qualitatively (band size) the expression of mRNAs from different related PTPs under different experimental conditions. This approach is useful when the relative size of the amplified bands provides information on the expression of highly related PTP isoforms or variants, which may display different functional properties [7, 24]. In addition, this methodology can provide a good and sensitive overall view of the relative expression of the members of PTP subfamilies (*see* Fig. 1). The second method (Subheading **3.2**) is designed to perform a global and quantitative monitoring of the mRNA expression of the PTPome under different experimental conditions. Our setting uses real-time RT-qPCR methodology scaled to accommodate, in 96-well (96-w) or in 384-well (384-w) plates, primers that amplify individually the members of the PTPome in a single experiment (*see* Fig. 3)

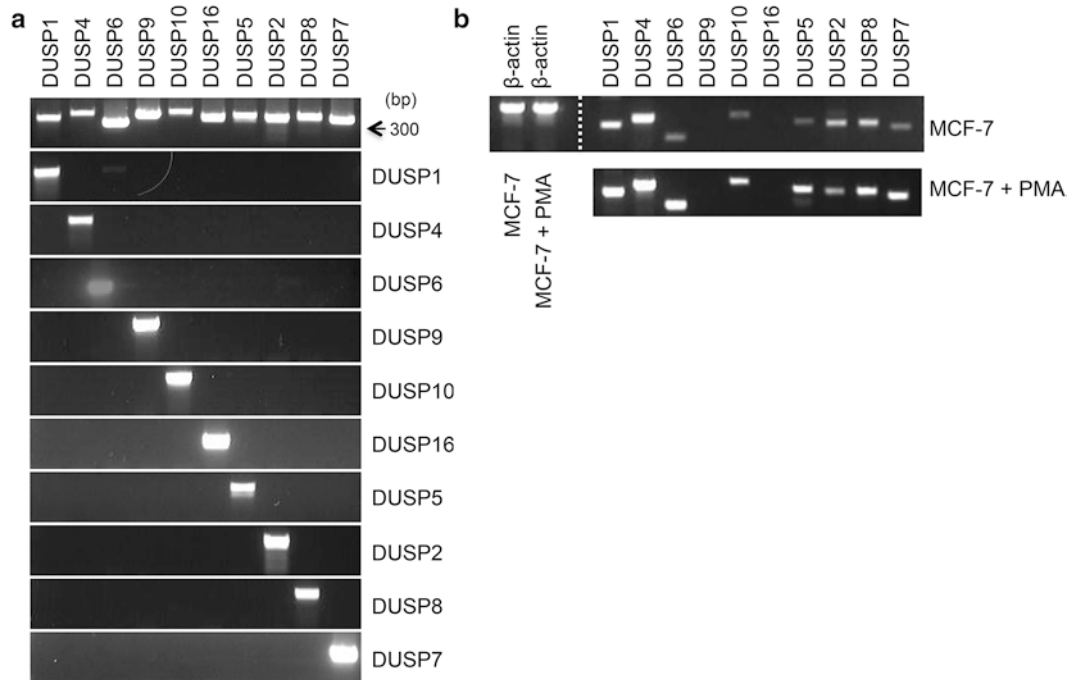


Fig. 1 (a) PCR analysis of oligonucleotide primer sets for the members of the human MKP family of active PTPs. Plasmids that contained cDNAs encoding each MKP were used as the template for each PCR reaction with the respective primer set (*upper row*), or with the primer sets of the ten different MKPs (*lower rows*). As shown, all primer sets amplified the expected DNA fragment from their corresponding cDNA template, and did not amplify DNA fragments from the other members of the MKP family. Thus, the ten primer sets designed are specific for the ten different MKP genes. Samples were resolved on 1 % agarose gels, and the amplified DNA was stained with ethidium bromide. In the *right* of the *upper row*, size in base pairs (bp) is indicated. Note that, when samples are run together, the slight differences in the size of the amplified bands (see Table 1) serve as internal controls. **(b)** Semi-quantitative RT-PCR analysis of the mRNA expression of the human MKP family of active PTPs. Poly(A) RNA obtained from human breast carcinoma MCF-7 cells untreated or treated with PMA (50 ng/ml) for 96 h was subjected to retrotranscription, and the resulting cDNA (50–200 ng/reaction) was processed for PCR using oligonucleotide primers specific for ACTB/ β -actin (as a reference gene) and the ten active members of the MKP family, as in (a). mRNAs from DUSP1, DUSP4, DUSP6, DUSP10, DUSP5, DUSP2, DUSP8, and DUSP7 were detected in non-treated MCF-7 cells, while mRNAs for DUSP9 and DUSP16 were not detected in MCF-7 cells. PMA-treated MCF-7 cells displayed upregulation of DUSP6 and DUSP5 mRNAs, and, to a lower extent, of DUSP10 and DUSP7. These results have been previously published in [21]

**3.1 Analysis
of Human PTPome
mRNA Expression
by Semi-
quantitative RT-PCR**

1. Design specific oligonucleotide primer sets for the group of PTPs of interest, and perform PCR tests for primer specificity and cross-reactivity using as templates plasmids containing the distinct PTP cDNAs (*see Note 8*). An example of primer specificity test, using primers that amplify the members of the human MKP family of active PTPs (Table 1), is shown in Fig. 1a. Each PCR reaction contained 10 ng template, 0.5 mM dNTP mix (2.5 μ l from 10 mM stock [2.5 mM each dNTP]), 0.3 μ M of each primer (1.5 μ l from 10 μ M stock),

Table 1**Oligonucleotide primers specific for the members of the human MKP family of PTPs and human β -actin^a**

Gene/protein	Sense primer (5'–3') Antisense primer (5'–3')	Amplified fragment size (bp)	Localization ^b
DUSP1/MKP1	GTG GGC ACC CTG GAC GCT GCT GAG CCC CAT GGG GGT	426	C-term
DUSP4/MKP2	GAC TGC AGT GTG CTC AAA AGG AAC CGG GGG TGG GAT GGC	483	C-term
DUSP6/MKP3	ATA GAT ACG CTC AGA CCC GTG CTC GCC GCC CGT ATT CTC G	333	C-term
DUSP9/MKP4	GAG GGT CTG GGC CGC TCG CGC CAT GCT GGA GCC GGC	450	C-term
DUSP10/MKP5	GCA CTA TCT AGG CCC GTC C GTT GTA CTC CAT GAA GGG CC	516	C-term
DUSP16/MKP7	CAT GAG ATG ATT GGA ACT CAA A AGG GAC TAG AGT GGA TTT TCC T	426	C-term
DUSP5	TCG CTC GAC GGG CGC CAG CTC ACT CTC AAT CTT CTC TTG T	450	C-term
DUSP2	CTG GAG TGC GCG GCG CTG CAG CGC AGG GGC GGG GG	429	C-term
DUSP8	GGG GAC CGG CTC CCG AG GCT CAT GGG TAG CAG GGC A	438	C-term
DUSP7/MKPX	AAC GCC TTC GAG CAC GGC G GGA CTC CAG CGT ATT GAG TG	408	N-term
ACTB/ β -actin	CCA AGG CCA ACC GCG AGA AGA TGA C AGG GTA CAT GGT GGT GCC GCC AGA C	562	Core

^aPrimers were used for experiments shown in Fig. 1^bThe protein region targeted by each pair of primers is indicated

and 1 U GC-rich DNA Polymerase (0.5 μ l from 2 U/ μ l stock) (*see Note 4*) in a final volume of 50 μ l. PCR conditions were a denaturation step, 95 °C, 5 min, followed by 35 cycles: denaturation, 95 °C, 1 min; annealing, 55 °C, 2 min; extension, 72 °C, 1 min.

2. Isolate total RNA, or poly(A) RNA, from the cells or tissues of interest (*see Note 3*) and measure RNA concentration and purity in a nanodrop spectrophotometer (*see Note 9*).
3. Incubate (12.5 μ l final volume) 1 μ g total RNA, or 10 ng poly(A) RNA (*see Note 3*), and oligo(dT)18 primers (1 μ l from 100 μ M stock) at 70 °C, 10 min to denature RNA secondary structure, and transfer to ice to let the primers to anneal to the RNA.

4. To perform the reverse transcription reaction, add 4 mM dNTP mix (2 μ l from 40 mM stock [10 mM each dNTP]), RNase inhibitor (0.5 μ l, 20 U), reverse transcriptase (RT; 1 μ l from 200 U/ μ l stock), and RT buffer (4 μ l 5 \times), to give a final volume of 20 μ l (*see* **Note 4**). Incubate at 42 °C, 1 h.
5. Optional: Incubate at 70 °C, 10 min, to inactivate the RT enzyme.
6. Measure concentration and purity of cDNA in a nanodrop spectrophotometer (*see* **Note 10**).
7. Perform the PCR reaction using the validated primer sets and 50–200 ng cDNA/reaction, as described in **step 1** (*see* **Note 11**).

An example of the relative expression of the mRNAs from the MKP family of PTPs, in human breast carcinoma MCF-7 cells untreated or treated with phorbol 12-myristate 13-acetate (PMA), is shown in Fig. 1b.

3.2 Global Analysis of Human PTPome mRNA Expression by Quantitative RT-PCR (RT-qPCR)

The high sensitivity of RT-qPCR makes necessary maximal accuracy in the manipulation and processing of the samples, as well as in the design and control of the experiment itself [25].

1. Choose or design the appropriate set of oligonucleotide primers sets for the members of the PTPome and for the reference genes (*see* **Note 6**). It is recommended to test individually the specificity of the primers by checking that a single sharp peak is obtained in the melt curve at the end of the PCR reaction (*see* **Note 12**).
2. Efficiency of the primers: it is recommended (especially when the primers are not prevalidated commercially) to test that the efficiency (E) of the amplification reaction is close to 2.0 (two-fold amplification per cycle = 100% efficiency). This can be calculated from the slope of the quantification cycle (C_q) standard curve, which is made by running PCR reactions using decimal dilutions (e.g. 1:1, 1:10, 1:100) of the cDNA to be analyzed or of a template plasmid (starting with 10 ng) containing the cDNA amplified by the primers, and representing C_q (y) vs. LOG template concentration (x) [$y = \text{slope} \times x + C_{q(x=0)}$; $E = 10^{(-1/\text{slope})}$] (*see* **Note 13**). The Pearson's correlation coefficient (r) of the standard curve should be >0.990 . Examples of C_q standard curves for a set of commercial primers (QIAGEN) for the human MKP family of active PTPs, and the calculation of the efficiency for one of them (DUSP10), are given in Fig. 2.
3. Obtain cDNA by retrotranscription of the RNA of interest, as in Subheading 3.1, to be used as template.
4. Setup of plates: primers for all PTPs and controls are aliquoted at 10 μ M in stock plates (Fig. 3). From the stock plate, a multipipette is used to make 384-w PCR working plates

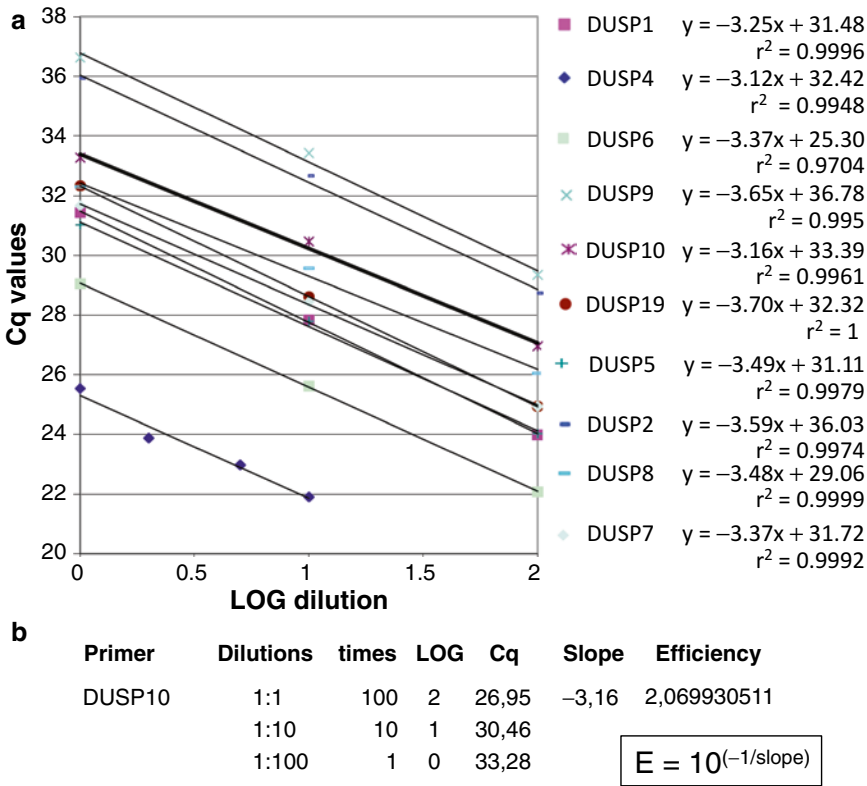


Fig. 2 Validation of qPCR primers for efficiency. (a) Primer efficiency (E) [$E = 10^{(-1/\text{slope})}$] of human MKP primers is calculated from the slope of the plot, generated by running reactions using decimal dilutions (1:1, 1:10, and 1:100) of a template cDNA (starting at 100 ng) from control cells. (b) An example for DUSP10 primers is shown in the *bottom*

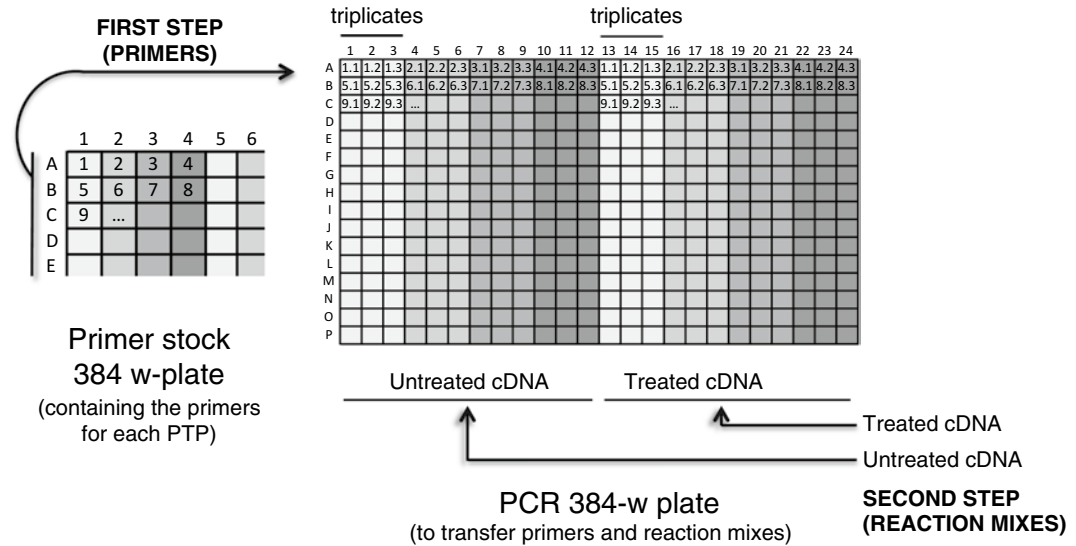


Fig. 3 Setup for the RT-qPCR analysis of the PTPome. A stock plate with primer aliquots for each PTP (numbered as 1, 2, etc., as an example) is used to transfer the primers to the PCR working plate, in triplicate (1.1, 1.2, 1.3, 2.1, 2.2, 2.3, etc., as an example) or in duplicate (not shown), for both untreated and treated conditions. Reaction mix is then added to the working plate and PCR is run

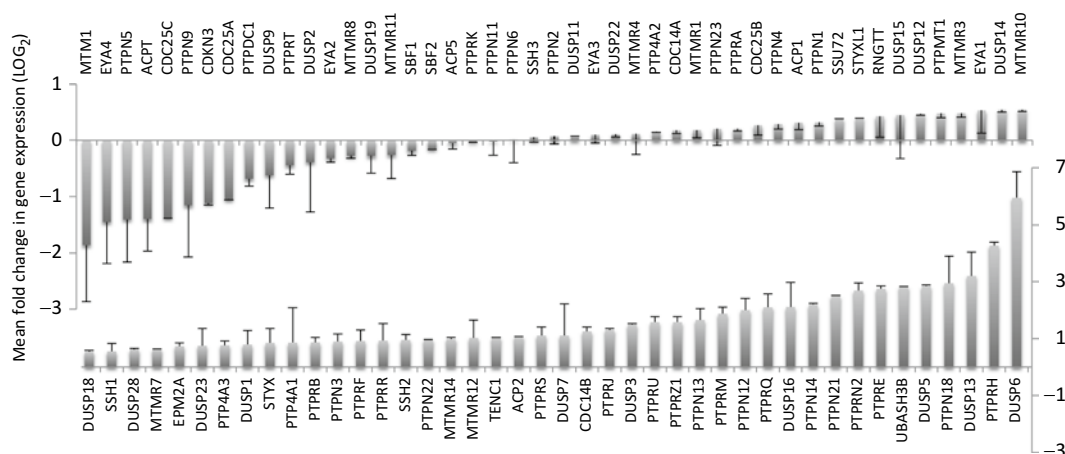


Fig. 4 Example of RT-qPCR expression analysis of the PTPome in MCF-7 cells treated with PMA. MCF-7 cells were left untreated or treated with PMA (50 ng/ml) for 72 h, and total RNA was purified and used for retrotranscription. cDNA was processed for PCR using a set of commercial primers (QuantiTect, QIAGEN). For this example, PCR was performed using technical duplicates. Relative expression values in LOG₂ scale are shown. Only a fraction of the PTPome, ordered from lower to higher fold change, is shown

containing 2 µl of each primer set per well, in duplicate or in triplicate, including in the same plate the experimental conditions to be compared (e.g., “untreated” vs. “treated”) (Fig. 3) (*see Note 14*). All plates are always kept on ice.

5. Reaction mix (20 µl/reaction): 2 µl primers, 5 µl cDNA (50–200 ng) in PCR-grade H₂O, 3 µl PCR-grade H₂O, 10 µl Master Mix (2×) (containing DNA polymerase, dNTPs, Dye, and reaction buffer) (*see Note 7*).
6. Add reaction mix with a multipipette. When pipetting of working plate is finished, cover the plate with sealing foil and keep covered with aluminum foil, on ice, until placed in the thermocycler.
7. Run the reaction (*see Note 15*). An example of the mRNA expression of most of PTPome members from MCF-7 cells untreated or treated with PMA for 72 h, using a set of commercial primers (QuantiTect, QIAGEN), is shown in Fig. 4. PCR conditions were a denaturation step, 95 °C, 10 min, followed by 40 cycles: denaturation, 95 °C, 15 s; annealing, 55 °C, 20 s; extension, 72 °C, 15 s.
8. Analyze the data using the appropriate software. A common way to present the data is in logarithmic scale (LOG₂), where significant changes are usually considered > or equal to 2, or < or equal to -2. For relative changes, fold change can be calculated using the $\Delta\Delta Cq$ equation: $\Delta\Delta Cq = 2^{-[(Cq_{ptp, treat} - Cq_{ref, treat}) - (Cq_{ptp, untreat} - Cq_{ref, untreat})]}$; where the Cq from the reference (ref) genes is subtracted to the Cq of each PTP in both the “untreated” (untreat.) and “treated” (treat.) conditions. For the choosing of reference genes, *see Note 16*.

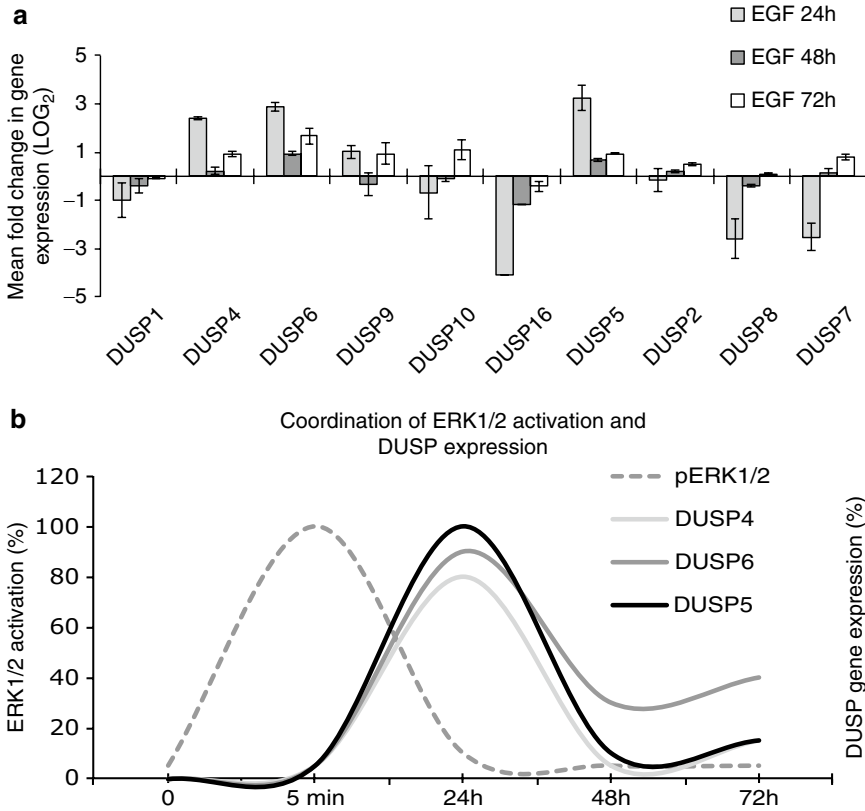


Fig. 5 (a) RT-qPCR analysis of the mRNA expression of the human MKP family of active PTPs from MCF-7 cells treated with EGF. MCF-7 cells were left untreated or treated with EGF (50 ng/ml) 24, 48, and 72 h, and processed for RT-qPCR as in Fig. 4. Mean fold change in gene expression is represented as LOG₂. DUSP4, DUSP6, and DUSP5 were significantly upregulated. **(b)** Example of coordination of ERK1/2 activation and DUSP4, DUSP6, and DUSP5 expression during transient activation of ERK1/2 by EGF stimulation. Data are normalized from (a) and from [21]

As an example, we show the mRNA expression of MKPs from MCF-7 cells stimulated with Epidermal Growth Factor (EGF), an ERK1/2-activating stimulus (Fig. 5). Six MKPs showed significant changes in gene expression after 24 h of EGF treatment, which declined after 48 and 72 h. The highest upregulated MKPs were DUSP4, DUSP6, and DUSP5 (Fig. 5a). In Fig. 5b, a comparative schematic kinetics of ERK1/2 activation, and DUSP4, DUSP6, and DUSP5 mRNA upregulation by EGF, is shown.

4 Notes

1. When comparison between groups of PTPome members is going to be done, the primers should target nonconserved regions, and amplify fragments of similar size (*see* also **Notes 8** and **12**).

2. The reference gene chosen for the experiment shown in Fig. 1b is ACTB/ β -actin. Note that the expression of ACTB/ β -actin mRNA, as that of many other commonly used reference genes, may change under your study conditions [26, 27]. An evaluation of the more appropriate reference genes for each study conditions should be made [28, 29].
3. There are many kits suitable for isolation of total or poly(A) RNA. As a rough estimation, poly(A) RNA constitutes 1 % of total RNA cellular content, which has to be taken in consideration for the amount of RNA used in the RT reaction. In general, total RNA works well for a reliable relative quantification of large sets of different target mRNAs [30]. For the experiments shown here, we have used Illustra QuickPrep *Micro* mRNA Purification Kit and IllustraRNAspin Mini Kit (GE Healthcare Life Sciences).
4. Many retrotranscriptase and DNA-polymerase options are available. For analytical purposes (nonpreparative), any high-amplification efficiency and high GC content tolerant Taq-polymerase is well suited. We commonly use RevertAidTM reverse transcriptase, oligo(dT)18 primers (stock 100 μ M), and RiboLock RNase inhibitor from Fermentas. In the experiments shown in Fig. 1, GC-rich DNA polymerase from Roche was used. dNTPs mixes often come in the Master mix of some commercial kits or can be purchased separately. For RT, we use a commercial dNTP mix (4 \times 10 mM; 40 mM total) from Fermentas. For PCR, we prepare a stock dNTP mix 4 \times 2.5 mM (10 mM total) from the individual dNTPs.
5. When small differences in size (for amplicons between 250 and 750 bp), or when small amplicons have to be compared, 1%–2 % agarose gels are convenient. The gels shown in Fig. 1 were visualized by staining with 0.6 μ g/ml ethidium bromide, but alternative less-toxic DNA stainers, such as GelRedTM or GelGreenTM, are already available.
6. There are a wide variety of programs and online tools for qPCR primer design (see, for instance, <http://molbiol-tools.ca/PCR.htm>). When a relatively large number of different genes are going to be analyzed, as in the case of the PTPome analysis, the use of sets of prevalidated commercial primers from the same source gives the advantage of more homogeneous optimal amplification conditions. In the experiments shown in Figs. 2, 4 and 5, we have used QuantiTect Primer Assays (QIAGEN) primers.
7. In the experiments shown here, we have used SYBR[®] Green-based reagents (Roche Applied Science), but other dyes are also suitable for use.
8. For proper comparison, the DNA amplified fragments should be of similar size, and the pairs of primers should have a similar

T_m . For the experiments shown in Fig. 1, the length of the MKP primers was between 18 and 22 mer, and the T_m (calculated as $(G+C) \times 4 + (A+T) \times 2$) was 62–64.

9. The ratio of absorbance at 260/280 nm for RNA should be around 2.0.
10. The ratio of absorbance at 260/280 nm for cDNA should be around 1.8. Sometimes removal of the template RNA is necessary by treating the RT reaction with RNase H before performing the PCR reaction.
11. In this method, the amplified band is the end product of the PCR reaction. This makes important to work with amounts of RNA and cDNA that allow visualization of differences. The number of PCR cycles for semi-quantitative PCR should be optimized to avoid oversaturation of the PCR reaction product. 30–35 cycles is a good range depending on the relative intensity of the bands of interest. To avoid false negatives and false positives, sometimes it is convenient to separate the reactions in two groups and amplify each group with different number of cycles. Note that in such case, comparisons have to be made within each group of PTPs.
12. A typical run for the melting curve is 95 °C, 15 s; 55 °C, 40 s. An additional control of specificity is to run the PCR product on an agarose gel to check the appropriate size of the amplicon. Note that sometimes amplicons from qPCR are of small size (*see Note 5*).
13. In practical terms, for a standard curve made with decimal dilutions of template, $E=2$ means that 3.32 cycles more are needed to reach the C_q when using ten times less template (slope = -3.32 means 100% efficiency).
14. It is convenient to have the PTPome primers in a 384-w primer stock plate, and make the transferring to the PCR working plate using a 12-channel 384-w multipipette. Alternatively, transferring can be made using an 8-channel 96-w multipipette, but taking six alternate wells each transfer, two transfers per row (an 8-channel 96-w multipipette will dispense samples to alternate wells in a 384-w plate). For two conditions (“untreated” vs. “treated”) and technical triplicates, 48 genes can be analyzed in one 384-w plate. For two conditions (“treated” vs. “untreated”) and technical duplicates, 72 genes can be analyzed in one 384-w plate. It is recommended to use technical triplicates.
15. There are different qPCR devices and detection systems, which have to be compatible. In the experiments shown here, we have used a LightCycler™ 480 thermocycler and the corresponding SYBR™ Green I Master Mix (Roche Applied Science).

16. The reference genes chosen for the experiments shown in Figs. 4 and 5 were HPRT1/hypoxanthine phosphoribosyl-transferase 1, ACTB/ β -actin, and GAPDH/glyceraldehyde-3-phosphate dehydrogenase (*see also Note 2*).

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References

1. Draghici S, Khatri P, Eklund AC, Szallasi Z (2006) Reliability and reproducibility issues in DNA microarray measurements. *Trends Genet* 22(2):101–109. doi:[10.1016/j.tig.2005.12.005](https://doi.org/10.1016/j.tig.2005.12.005)
2. Katagiri F, Glazebrook J (2009) Overview of mRNA expression profiling using DNA microarrays. *Curr Protoc Mol Biol* Chapter 22:Unit 22 24. doi:[10.1002/0471142727.mb2204s85](https://doi.org/10.1002/0471142727.mb2204s85)
3. Mehta JP (2011) Microarray analysis of mRNAs: experimental design and data analysis fundamentals. *Methods Mol Biol* 784:27–40. doi:[10.1007/978-1-61779-289-2_3](https://doi.org/10.1007/978-1-61779-289-2_3)
4. Kubista M, Andrade JM, Bengtsson M, Forootan A, Jonak J, Lind K, Sindelka R, Sjoberg R, Sjogreen B, Strombom L, Stahlberg A, Zoric N (2006) The real-time polymerase chain reaction. *Mol Aspects Med* 27(2–3):95–125. doi:[10.1016/j.mam.2005.12.007](https://doi.org/10.1016/j.mam.2005.12.007)
5. VanGuilder HD, Vrana KE, Freeman WM (2008) Twenty-five years of quantitative PCR for gene expression analysis. *Biotechniques* 44(5):619–626. doi:[10.2144/000112776](https://doi.org/10.2144/000112776)
6. Marone M, Mozzetti S, De Ritis D, Pierelli L, Scambia G (2001) Semiquantitative RT-PCR analysis to assess the expression levels of multiple transcripts from the same sample. *Biol Proced online* 3:19–25. doi:[10.1251/bpo20](https://doi.org/10.1251/bpo20)
7. Pulido R, Krueger NX, Serra-Pages C, Saito H, Streuli M (1995) Molecular characterization of the human transmembrane protein-tyrosine phosphatase delta. Evidence for tissue-specific expression of alternative human transmembrane protein-tyrosine phosphatase delta isoforms. *J Biol Chem* 270(12):6722–6728
8. Alonso A, Sasin J, Bottini N, Friedberg I, Friedberg I, Osterman A, Godzik A, Hunter T, Dixon J, Mustelin T (2004) Protein tyrosine phosphatases in the human genome. *Cell* 117(6):699–711. doi:[10.1016/j.cell.2004.05.018](https://doi.org/10.1016/j.cell.2004.05.018)
9. Alonso A, Pulido R (2015) The extended human PTPome: a growing tyrosine phosphatase family. *FEBS J*. doi:[10.1111/febs.13600](https://doi.org/10.1111/febs.13600)
10. Andersen JN, Jansen PG, Echwald SM, Mortensen OH, Fukada T, Del Vecchio R, Tonks NK, Moller NP (2004) A genomic perspective on protein tyrosine phosphatases: gene structure, pseudogenes, and genetic disease linkage. *FASEB J* 18(1):8–30. doi:[10.1096/fj.02-1212rev](https://doi.org/10.1096/fj.02-1212rev)
11. Bhaduri A, Sowdhamini R (2003) A genome-wide survey of human tyrosine phosphatases. *Protein Eng* 16(12):881–888. doi:[10.1093/protein/gzg144](https://doi.org/10.1093/protein/gzg144)
12. Hatzihristidis T, Liu S, Prysacz L, Hutchins AP, Gabaldon T, Tremblay ML, Miranda-Saavedra D (2014) PTP-central: a comprehensive resource of protein tyrosine phosphatases in eukaryotic genomes. *Methods* 65(2):156–164. doi:[10.1016/j.ymeth.2013.07.031](https://doi.org/10.1016/j.ymeth.2013.07.031)
13. Li X, Wilmanns M, Thornton J, Kohn M (2013) Elucidating human phosphatase-substrate networks. *Sci Signal* 6(275):rs10. doi:[10.1126/scisignal.2003203](https://doi.org/10.1126/scisignal.2003203)
14. Patterson KI, Brummer T, O'Brien PM, Daly RJ (2009) Dual-specificity phosphatases: critical regulators with diverse cellular targets. *Biochem J* 418(3):475–489
15. Sadatomi D, Tanimura S, Ozaki K, Takeda K (2013) Atypical protein phosphatases: emerging players in cellular signaling. *Int J Mol Sci* 14(3):4596–4612. doi:[10.3390/ijms14034596](https://doi.org/10.3390/ijms14034596)
16. Tautz L, Critton DA, Grottegut S (2013) Protein tyrosine phosphatases: structure, function, and implication in human disease.

- Methods Mol Biol 1053:179–221. doi:[10.1007/978-1-62703-562-0_13](https://doi.org/10.1007/978-1-62703-562-0_13)
17. Nunes-Xavier C, Roma-Mateo C, Rios P, Tarrega C, Cejudo-Marin R, Tabernero L, Pulido R (2011) Dual-specificity MAP kinase phosphatases as targets of cancer treatment. *Anticancer Agents Med Chem* 11(1):109–132
 18. Rios P, Nunes-Xavier CE, Tabernero L, Kohn M, Pulido R (2014) Dual-specificity phosphatases as molecular targets for inhibition in human disease. *Antioxid Redox Signal* 20(14):2251–2273. doi:[10.1089/ars.2013.5709](https://doi.org/10.1089/ars.2013.5709)
 19. Arora D, Kothe S, van den Eijnden M, Hooft van Huijsdijnen R, Heidel F, Fischer T, Scholl S, Tolle B, Bohmer SA, Lennartsson J, Isken F, Muller-Tidow C, Bohmer FD (2012) Expression of protein-tyrosine phosphatases in Acute Myeloid Leukemia cells: FLT3 ITD sustains high levels of DUSP6 expression. *Cell Commun Signal* 10(1):19. doi:[10.1186/1478-811X-10-19](https://doi.org/10.1186/1478-811X-10-19)
 20. Nunes-Xavier CE, Elson A, Pulido R (2012) Epidermal growth factor receptor (EGFR)-mediated positive feedback of protein-tyrosine phosphatase epsilon (PTPepsilon) on ERK1/2 and AKT protein pathways is required for survival of human breast cancer cells. *J Biol Chem* 287(5):3433–3444. doi:[10.1074/jbc.M111.293928](https://doi.org/10.1074/jbc.M111.293928)
 21. Nunes-Xavier CE, Tarrega C, Cejudo-Marin R, Frijhoff J, Sandin A, Ostman A, Pulido R (2010) Differential up-regulation of MAP kinase phosphatases MKP3/DUSP6 and DUSP5 by Ets2 and c-Jun converge in the control of the growth arrest versus proliferation response of MCF-7 breast cancer cells to phorbol ester. *J Biol Chem* 285(34):26417–26430. doi:[10.1074/jbc.M110.121830](https://doi.org/10.1074/jbc.M110.121830), M110.121830 [pii]
 22. Pulido R, Serra-Pages C, Tang M, Streuli M (1995) The LAR/PTP delta/PTP sigma sub-family of transmembrane protein-tyrosine-phosphatases: multiple human LAR, PTP delta, and PTP sigma isoforms are expressed in a tissue-specific manner and associate with the LAR-interacting protein LIP.1. *Proc Natl Acad Sci U S A* 92(25):11686–11690
 23. Schmidt F, van den Eijnden M, Pescini Gobert R, Saborio GP, Carboni S, Alliod C, Pouly S, Staugaitis SM, Dutta R, Trapp B, Hooft van Huijsdijnen R (2012) Identification of VHY/Dusp15 as a regulator of oligodendrocyte differentiation through a systematic genomics approach. *PLoS One* 7(7):e40457. doi:[10.1371/journal.pone.0040457](https://doi.org/10.1371/journal.pone.0040457)
 24. Erdmann KS, Kuhlmann J, Lessmann V, Herrmann L, Eulenburg V, Muller O, Heumann R (2000) The Adenomatous Polyposis Coli-protein (APC) interacts with the protein tyrosine phosphatase PTP-BL via an alternatively spliced PDZ domain. *Oncogene* 19(34):3894–3901. doi:[10.1038/sj.onc.1203725](https://doi.org/10.1038/sj.onc.1203725)
 25. Taylor S, Wakem M, Dijkman G, Alsarraj M, Nguyen M (2010) A practical approach to RT-qPCR-Publishing data that conform to the MIQE guidelines. *Methods* 50(4):S1–S5. doi:[10.1016/j.ymeth.2010.01.005](https://doi.org/10.1016/j.ymeth.2010.01.005)
 26. Ruan W, Lai M (2007) Actin, a reliable marker of internal control? *Clin Chim Acta* 385(1–2):1–5. doi:[10.1016/j.cca.2007.07.003](https://doi.org/10.1016/j.cca.2007.07.003)
 27. Schmittgen TD, Zakrajsek BA (2000) Effect of experimental treatment on housekeeping gene expression: validation by real-time, quantitative RT-PCR. *J Biochem Biophys Methods* 46(1–2):69–81
 28. Sturzenbaum SR, Kille P (2001) Control genes in quantitative molecular biological techniques: the variability of invariance. *Comp Biochem Physiol B Biochem Mol Biol* 130(3):281–289
 29. Hellemans J, Vandesompele J (2014) Selection of reliable reference genes for RT-qPCR analysis. *Methods Mol Biol* 1160:19–26. doi:[10.1007/978-1-4939-0733-5_3](https://doi.org/10.1007/978-1-4939-0733-5_3)
 30. Petersen K, Oyan AM, Rostad K, Olsen S, Bo TH, Salvesen HB, Gjertsen BT, Bruserud O, Halvorsen OJ, Akslen LA, Steen VM, Jonassen I, Kalland KH (2007) Comparison of nucleic acid targets prepared from total RNA or poly(A) RNA for DNA oligonucleotide microarray hybridization. *Anal Biochem* 366(1):46–58. doi:[10.1016/j.ab.2007.03.013](https://doi.org/10.1016/j.ab.2007.03.013)

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