



Primer Pairs for Mediator and Receptor mRNA Analysis

By Mary Haak-Frendscho, Lisa Guyer, Randy Hoffman and Maoxiao Yu
Promega Corporation

Corresponding author: e-mail to mhaakfre@promega.com

Promega is expanding the number of Primer Pairs^(a) for analyzing the expression of murine and human mRNAs by RT-PCR. New Primer Pair sets being offered are for gp130 (the signaling domain for the IL-6 family of receptors), TrkA (nerve growth factor receptor), TrkC (neurotrophin-3 receptor) and NT-4/5 (neurotrophin-4/5).

INTRODUCTION

RT-PCR is a powerful technique for analyzing the expression of mRNA that is particularly useful when limiting amounts of sample are available for study. As part of our continuing effort to facilitate life sciences research, we have developed and tested a series of robust oligonucleotide primer sequences. New additions to our growing list of Primer Pairs include gp130, TrkA, TrkC and NT-4/5.

EXPRESSION OF mRNA

Neuronal mediators are under intense investigation due to their supportive effects in development and maintenance of cells comprising the nervous system. The discovery of many of these factors and receptors in the past decade has sparked an explosion of research aimed at gaining a better understanding of the nervous system, as well as interactions of the nervous system with the immune and endocrine systems. Similarly, applied research directed toward developing more effective therapeutics for neuronal diseases has flourished as more tools become available.

[Table 1](#) lists some features and species applications of the new Primer Pairs available from Promega for use in PCR and RT-PCR.

Primer Pair	Expected Product Size	Rat RNA	Mouse RNA	Human RNA	Suggested Annealing Temperature
gp130	473bp	*	*	*	55-60°C
NT-4/5	248bp	*	+/	n.r.	55-60°C
TrkA	690bp	*	n.r.	+/	60°C
TrkC	1,002bp	*	+/	*	60°C

*, recommended; +/, works but may need optimization of reaction conditions; n.r., not recommended.

TrkA, TrkC and gp130 all are tyrosine kinase receptors found on a variety of cell types. gp130 is a transmembrane glycoprotein and acts as the signaling chain for the entire IL-6 receptor family ([Table 2](#)). Depending upon the cognate receptor(s) associated with gp130, this receptor protein is important in many diverse biologic activities in the nervous system, hematopoiesis, the immune system, bone remodeling, acute phase reactions and reproduction (reviewed in reference 1). TrkA and TrkC are part of the Trk family of tyrosine protein kinase receptors responsible for mediating the trophic effects of nerve growth factor (NGF) and NT-3, respectively. These receptors are expressed predominantly in the nervous system, where they play critical roles in regulating the growth, survival and differentiation of cells (reviewed in references 2 and 3).

Table 2. Interleukin 6 Cytokine Family Members.

Interleukin 6 (IL-6)	Leukemia Inhibitory Factor (LIF)
Interleukin 11 (IL-11)	Ciliary Neurotrophic Factor (CNTF)
Oncostatin-M (OSM)	Cardiotrophin-1 (CT-1)

Neurotrophin-4/5 is a recently characterized member of the NGF family of neurotrophins, which are essential for the survival and differentiation of vertebrate neurons. Although not essential for survival based on gene knockout studies (4), NT-4/5 has been most widely studied for its selective effects on motoneuron rescue (5).

AMPLIFICATION USING PRIMERS PAIRS

Sequences for the Promega Primer Pairs are derived from the scientific literature and allow for more uniform amplification conditions across the entire series of primers. To ensure the specificity of the Primer Pairs, amplified products from each pair have been confirmed by sequencing. For added flexibility, 5'- and 3'-primers are provided in separate vials sufficient for twenty PCR amplifications.

[Figure 1](#) illustrates typical product bands obtained using the Primer Pairs and two-step RT-PCR. Complementary DNA was generated from rat brain total RNA using Promega's Reverse Transcription System^(b) (Cat. #A3500) and then amplified in a standard amplification reaction using the annealing temperatures listed in [Table 1](#).

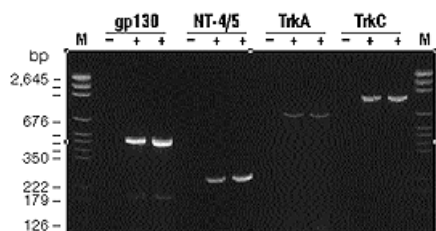


Figure 1. Two-step RT-PCR products generated using Promega's Reverse Transcription System^(b) (Cat.# A3500) and new Primer Pairs for gp130, NT-4/5, TrkA and TrkC. Rat brain total RNA (1µg; Clontech) was used as template, then was reverse transcribed with AMV Reverse Transcriptase and amplified with *Taq* DNA Polymerase^(a). Amplification conditions were as recommended for the Primer Pairs with annealing temperatures of 55°C for gp130 and NT-4/5 and 60°C for TrkA and TrkC. Fragments were resolved on a 3.0% NuSieve[®] agarose (FMC Corporation) gel stained with ethidium bromide. Markers are pGEM[®] DNA Markers^(c) (Cat.# G1741); lanes with (+) are positive test reactions and lanes with (-) are negative control (no template) reactions.

SUMMARY

The Primer Pairs have been tested using our Reverse Transcription System for two-step RT-PCR and with Promega's Access RT-PCR System, which is a simplified, single-tube RT-PCR procedure. Both systems work with the Primer Pairs to provide a convenient, optimized procedure for analyzing the expression of specific mRNAs by RT-PCR.

REFERENCES

1. Taga, T. and Kishimoto, T. (1997) *Annu. Rev. Immunol.* **15**, 797.
2. Barbacid, M. (1994) *J. Neurobiol.* **25**, 1386.
3. Ip, N.Y. and Yancopoulos, G.D. (1996) *Annu. Rev. Neurosci.* **19**, 491.
4. Ibanez, C.F. (1996) *Neurochem. Res.* **21**, 787.
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Ordering Information

Product	Size	Cat.#
gp130 Primer Pair	20 reactions	G4570
TrkA Primer Pair	20 reactions	G4900
TrkC Primer Pair	20 reactions	G4540
NT-4/5 Primer Pair	20 reactions	G4960

Each Primer Pair is sufficient for 20 x 50µl amplification reactions.

Related Products

Product	Size	Cat.#
Reverse Transcription System	100 reactions	A3500
Access RT-PCR System	100 reactions	A1250
	500 reactions	A1280
Access RT-PCR Introductory System	20 reactions	A1260
Taq DNA Polymerase	100u	M1661
Beta-Actin Primer Pair	20 reactions	G5740
BDNF Primer Pair	20 reactions	G5750
CNTF Primer Pair	20 reactions	G5770
ChAT Primer Pair	20 reactions	G5760
NGF Primer Pair	20 reactions	G5780
NT-3 Primer Pair	20 reactions	G6801
p75 Primer Pair	20 reactions	G6861
TrkB Primer Pair	20 reactions	G5790

Each Primer Pair is sufficient for 20 x 50µl amplification reactions.

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^(b)U.S. Pat. No. 5,552,302 has been issued to Promega Corporation for the methods and compositions for production of human recombinant placental ribonuclease inhibitor (PRI). Inhibitors of Angiogenin, which comprises a segment of human PRI, is the subject of U.S. Pat. Nos. 4,966,964, 5,019,556 and 5,266,687 assigned to the President and Fellows of Harvard College and exclusively licensed to Promega Corporation.

^(c)U.S. Pat. No. 4,766,072 has been issued to Promega Corporation for transcription vectors having two different bacteriophage RNA polymerase promoter sequences separated by a series of unique restriction sites into which foreign DNA can be inserted.

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