

Primer Pairs for Neurotrophic Factor mRNA Analysis

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Promega is introducing a series of Primer Pairs for analyzing the expression of specific human and rat mRNAs by RT-PCR. The first Primer Pair sets being offered are for beta-Actin, BDNF (brain-derived neurotrophic factor), CNTF (ciliary neurotrophic factor) and NGF (nerve growth factor).

INTRODUCTION

Neurotrophic factors are under intense study in basic research for their supportive effects on specific subsets of neurons, and in clinical neurobiology research as potential therapies for human neuropathies stemming from ALS, Alzheimer's and Parkinson's diseases, to name a few. Moreover, the emerging scenario for neurotrophic factors is their important communicative role between the nervous and immune systems. There is great interest in analyzing these proteins at the molecular level, but the availability of research reagents for neurotrophic factors remains limited. The need for molecular tools, therefore, is the limiting step in the pursuit of a better understanding of these factors.

As part of our continuing effort to facilitate life sciences research, Promega is proud to announce the availability of Primer Pairs for analyzing the expression of specific human and rat mRNAs by RT-PCR^(a). Each Primer Pair includes separate 5' - and 3' - oligonucleotide primers (100µM each) ready to add to the reaction mixture. The first Primer Pair sets being offered are for beta-Actin, BDNF (brain-derived neurotrophic factor), CNTF (ciliary neurotrophic factor) and NGF (nerve growth factor). These Primer Pairs were developed and tested to be robust for amplification reaction conditions. Therefore, all Primer Pairs can be used at the same annealing temperature and in the same thermal cycler run.

^(a)The PCR process is covered by patents issued and applicable in certain countries. Promega does not encourage or support the unauthorized or unlicensed use of the PCR process. Use of this product is recommended for persons that either have a license to perform PCR or are not required to obtain a license.

BDNF, NGF AND CNTF NEUROTROPHIC FACTORS

BDNF and NGF are neurotrophins that exhibit pleiotropic effects across different neuronal cell types in the mammalian brain as well as CNS of lower order species. NGF, the classic target-derived neurotrophic factor, acts through two distinct receptors - p75^{NTR} and the TrkA receptor (1). Many neuronal types, especially sympathetic neurons cultured from neonatal animals, require NGF for survival (2). Moreover, NGF 'knockout' mice exhibit a selective loss of neurons in the sympathetic ganglia and dorsal root ganglia (2). Increasingly, NGF is being recognized for its neurocytokine - 'neurokine' - properties. The hematopoietic and immune systems possess NGF-responsive cells, as do cell populations in the brain involved in neuroendocrine functions (3). NGF is produced by mast cells and is increased in these cells during inflammation and autoimmunity (3). BDNF shares some effects with NGF but was first shown to promote the outgrowth of spinal sensory neurons (4). Other neuron types supported by BDNF include neural crest-derived and placode-derived sensory neurons, parasympathetic nodose ganglion neurons, dopaminergic striatal neurons, and cranial and spinal motoneurons (1). CNTF is a neurokine that acts through the CNTF receptor, a receptor tyrosine kinase. CNTF has been implicated in enhancing neuronal survival in the face of programmed cell death during development, in sprouting and synapse stabilization during synapse elimination, and in maintaining synaptic efficacy during functional maturation (1). All three of these neurotrophic factors are of great interest in many disciplines of neurobiology today. A more thorough discussion of these three factors can be found in reference 2.

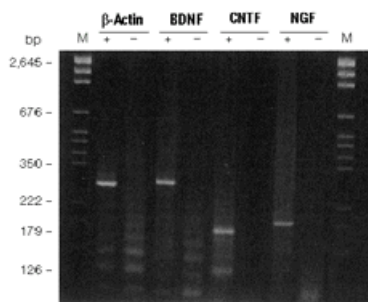


Figure 1. One-step amplification products generated using Primer Pairs for beta-Actin, BDNF, CNTF, NGF and

Promega's Access RT-PCR System^(a). Rat brain total RNA (Clontech) was used as a template in reactions containing 200ng of RNA, Promega's AMV/*Tfl* Reaction Buffer, 1mM MgSO₄, 50pmol of each primer, 0.2mM each dNTP, 5 units AMV Reverse Transcriptase and 5 units *Tfl* DNA Polymerase in a 50µl reaction volume. The RNA was reverse transcribed (48°C for 45 minutes), the AMV-RT inactivated (94°C for 2 minutes), and the cDNA immediately amplified using an amplification profile consisting of 40 cycles of denaturation (94°C for 30 seconds), annealing (70°C for 1 minute) and extension (72°C for 2 minutes). After a final extension step (68°C for 7 minutes) the reactions were stored at 4°C. Fragments were resolved on a 3.0% NuSieve[®] (FMC) agarose gel stained with ethidium bromide. Markers are pGEM^{®(b)} DNA Markers (Cat.# G1741); lanes with (+) are positive test reactions and lanes with () are negative control (no template) reactions.

^(b)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.

Table 1. Product Size, Species Use and Amplification Methods for the Primer Pairs.

Primer Pair	Expected Product Size	Two-Step RT-PCR		One-Step RT-PCR		Reference
		Rat RNA	Human RNA	Rat RNA	Human RNA	
beta-Actin **	285bp	*	*	*	*	5
BDNF	296bp	*	*	*	*	6
CNTF	168bp	*	*	*	n.r.	6
NGF	189bp	*	*	*	*	6

** Also has been shown to work with mouse RNA.
 * = positive for expected size; n.r. = not recommended

AMPLIFICATION USING PRIMER PAIRS

All of the Promega Primer Pair sequences are derived from the scientific literature with modifications allowing for uniform amplification conditions among the entire set of primers. To ensure the specificity of the Primer Pairs, amplified products from each set have been confirmed by sequencing. For added flexibility, each primer is provided in a separate vial with sufficient material for twenty PCR amplifications. [Table 1](#) contains additional information on PCR product sizes, tested species cross-reactivity and recommended amplification methods.

The beta-Actin Primer Pair serves as a robust positive control for the amplification reaction with virtually any tissue sample. Since beta-actin is ubiquitous, this Primer Pair can be used as a positive control for reaction conditions and as an internal reference of message quantity. In addition, the 5' and 3' primers span an intron (except BDNF Primer Pair) and, therefore, can be used to distinguish amplification of cDNA versus genomic DNA. This feature is extremely useful and can serve as a control when amplifying other messages of intronless genes.

[Figures 1](#) and [2](#) illustrate the product bands typically obtained using the Primer Pairs and rat brain total RNA in standard amplification reactions. In [Figure 1](#), Promega's Access RT-PCR System^(a) (Cat.# A1260) was used following recommended instructions and an amplification annealing temperature of 70°C. In [Figure 2](#), the Primer Pairs were used in a traditional two-step RT-PCR amplification with an amplification annealing temperature of 65°C. When amplifying a low-copy message or when working with limiting amounts of RNA as in neurotrophin research, Promega's *TaqBead*TM Hot Start Polymerase (Cat.# M5661) can further enhance the signal. As seen in [Figure 3](#), a sharper PCR product band is generated from rat brain total RNA using *TaqBead*TM Hot Start Polymerase compared to cold start amplification. There also is a decrease in primer-dimer formation, made possible by the combined aspects of barrier and polymerase inactivation techniques (7,8). No observable differences result when amplifying message for beta-actin ([Figure 3](#)), a representative high-copy message product.

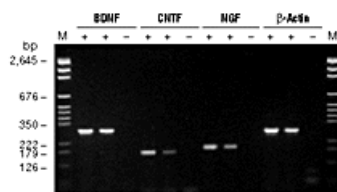


Figure 2. Two-step amplification products generated using Primer Pairs for BDNF, CNTF, NGF, beta-Actin and Promega's Reverse Transcription System^(c). Rat brain total RNA (1 µg) was used as template; the annealing temperature during the amplification reaction was 65°C and the amplification reaction was as recommended for the Primer Pairs. Fragments were resolved on a 3.0% NuSieve[®] agarose gel stained with ethidium bromide. Markers and lane identifications are as in [Figure 1](#).

^(c)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 comprise 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 President and Fellows of Harvard College and exclusively licensed to Promega Corporation.

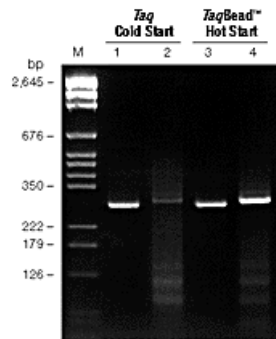


Figure 3. Hot start amplification performed with *TaqBead*TM Hot Start Polymerase^(a) reduces the yield of nonspecific amplification products. Complementary DNA was generated by reverse transcribing rat brain total RNA using Promega's Reverse Transcription System (Cat.# A3500). PCR products generated by using the Primer Pair for beta-actin (285bp) in lanes 1 and 3, and BDNF (296bp) in lanes 2 and 4, were amplified using either Promega's *Taq* DNA Polymerase^(a) (Cat.# M1661, Storage Buffer B) or *TaqBead*TM Hot Start Polymerase in Promega Reaction Buffer supplemented with 1.5mM MgCl₂. Both sets of reactions were assembled on ice and placed in a thermal cycler. The temperature in the thermal cycler was ramped to 94°C as rapidly as possible and the samples were denatured at 94°C for 2 minutes prior to 40 cycled reactions of 94°C for 30 seconds, 70°C for 1 minute and 72°C for 2 minutes. PCR products were extended by a final 68°C, 7-minute incubation and were analyzed on a 3.0% NuSieve[®] agarose gel stained with ethidium bromide. Markers are as in [Figure 1](#); lanes 1 and 2, PCR products amplified with *Taq* DNA Polymerase; lanes 3 and 4, PCR products amplified with *TaqBead*TM Hot Start Polymerase.

SUMMARY

The Primer Pairs have been tested using our Reverse Transcription System (Cat.# A3500) for two-step RT-PCR and with Promega's Access RT-PCR System, featuring easy, single-tube amplification for one-step RT-PCR amplifications. In addition, using *TaqBead*TM Hot Start Polymerase for hot start PCR exhibits increased yield of product and decreased yield of primer-dimers with low-copy messages. Regardless of the amplification technique used, Promega's Primer Pairs provide handy, optimized primers for analyzing the expression of specific mRNAs by RT-PCR. Primer Pairs for BDNF, CNTF and NGF are the first three oligonucleotide primers of many in development for research into neurotrophic factors.

Editor's note: For more information on Promega products for neurobiology research, please request your free copy of Neural Notes. Additional Primer Pairs for other neuro-related factors are currently in development. For information, please contact Pam Guthmiller, Neuroscience Product Manager, Promega Corporation, 2800 Woods Hollow Road, Madison, WI 53711-5399 USA. E-mail: pguthmil@promega.com; phone: 608-277-2620.

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Ordering Information

Product	Size	Cat.#
beta-Actin Primer Pair	20 reactions	G5740
BDNF Primer Pair	20 reactions	G5750
CNTF Primer Pair	20 reactions	G5770
NGF Primer Pair	20 reactions	G5780

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

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