TnT® Rabbit Reticulocyte Lysate Systems — Easy Protein Expression

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Abstract

The TnT® Coupled Reticulocyte Lysate Systems offer a one-tube, coupled transcription/translation approach to protein expression. These systems reduce the time required to obtain in vitro translation results by incorporating transcription directly in the translation mix. There are several factors to be considered when choosing a TnT® System. These include the starting template, the type of protein to be translated, the preferred detection method, the desired reaction format and the intended downstream applications. Here we discuss how to decide which TnT® System is best for you.

TnT® Systems are robust systems for the in vitro expression of protein in a simple, one-step procedure.

Q There are so many different TnT® systems! Which one should I choose?

There are several different factors to consider when choosing which TnT® system to use. The main factors are the ease of reaction setup, type of template used, type of label used, and the desired reaction format.

Reaction Setup: The main difference between the TnT® Quick Coupled Transcription/Translation Systems[a–e] and TnT® Coupled Systems[a,b,d,e] is the way the reactions are assembled. The TnT® Coupled Systems include all the reaction components as separate items, and the reaction is set up by adding together the lysate, polymerase, template, reaction buffer, amino acid mixture and label. The TnT® Quick Coupled Systems, the TnT® T7 Quick for PCR DNA[b,c,e] and the Gold TnT® Express 96 Transcription/Translation Systems[a,b,c,e], all use a reaction master mix that contains the lysate, amino acid mixture, reaction buffer, and polymerase. This allows rapid and convenient reaction setup by adding only the template DNA and an appropriate label to the master mix.

Template: Both the TnT® Coupled and TnT® Quick Coupled Systems are designed for use with plasmid templates containing a cDNA sequence cloned downstream of either a T7 or SP6 promoter. The TnT® Coupled Systems have a T3 promoter-based system available as well. Plasmid templates used in the TnT® Systems can be either circular or linear, although using the SP6 or T3 systems with a linear template will significantly reduce translation efficiency and is not recommended. The TnT® T7 Quick for PCR DNA is specifically optimized for linear PCR[f]-generated templates containing the T7 promoter. The TnT® T7 Quick Coupled System can also use linear templates generated by plasmid digestion or PCR, although some optimization of the salt concentrations is typically required. The PCR Enhancer solution included in the TnT® Quick Coupled System is designed to aid in this optimization.

Label: Many researchers label in vitro translation products with [^{35}S]-methionine. If the protein of interest only contains a few methionine residues, however, it may be necessary to label with an alternative radioactive amino acid or with a non-radioactive labeling system. If there are sufficient cysteine residues in the protein, or if both methionine and cysteine will be used together to label the protein, then the appropriate amino acid mixture can be included in the TnT® Coupled reaction. The TnT® Coupled Systems contain amino acid mixtures lacking either methionine, cysteine or leucine. Amino Acid Mixture Minus Methionine and Cysteine is available separately (Cat.# L5511). Using alternative radioactively labeled amino acids in the TnT® Quick Coupled Systems is not recommended, since the master mix contains all of the amino acids except methionine, the labeling efficiency with other amino acids will be significantly reduced.

The Transcend™ Non-Radioactive Translation Detection Systems (Cat.# L5070) and the FluoroTect™ Green$_{Lys}$ in vitro Translation Labeling System[g] (Cat.# L5001) can be used with any of the TnT® Coupled or Quick Coupled Systems. These systems use a pre-charged lysine-tRNA, which is incorporated into the translated protein. The Transcend™ System incorporates a biotinylated lysine, which can be detected by

Table 1. Comparison of TnT® Systems.

TnT® System	Template	Promoter	Radioactive Label	Reaction Format
TnT® Coupled Transcription/Translation System	circular plasmid, linear[1] DNA	T7, SP6, T3	Met, Cys, Leu	single tube
TnT® Quick Coupled Transcription/Translation System	PCR product, circular plasmid, linear[1] DNA	T7, SP6	Met	single tube
TnT® Quick for PCR DNA	PCR product, linear DNA	T7	Met	single tube
Gold TnT® Express 96 Transcription/Translation System	PCR product, circular plasmid, linear[1] DNA	T7, SP6	Met	96-well plate

[1] Linear templates are not recommended when using SP6 or T3 promoters.

blotting and probing with streptavidin. The FluoroTect™ Reagent incorporates a fluorescently labeled lysine, which can be detected directly from the gel.

Reaction Format: The TnT® Coupled and Quick Coupled reactions are single-tube reactions, which typically have a final volume of 25 or 50µl. For applications that require multiple transcription/translation reactions for a single experiment, the Gold TnT® T7 and SP6 Express Systems provide 96-well plates containing pre-aliquoted reaction master mix. This format allows for high-throughput transcription/translation assays such as translation inhibitor screening (1), in vitro expression cloning (2) or for screening of interacting proteins (3).

Q What kinds of applications have been performed using the TnT® Systems?

TnT®-translated proteins can be used in many types of functional protein studies. TnT® Transcription/Translation reactions have traditionally been used to confirm open reading frames, study protein mutations and make proteins in vitro for protein-DNA binding studies, protein activity assays, or protein-protein interaction studies. Recently, TnT®-expressed proteins have also been used in assays to confirm yeast two-hybrid interactions, perform in vitro expression cloning (IVEC) and make protein substrates for enzyme activity or protein modification assays. For an in-depth list of recent citations using the TnT® Systems in various applications, please visit: **www.promega.com/citations/**

Q What are some important considerations for optimizing TnT® reactions?

There are a number of factors that can affect the efficiency of transcription/translation in the TnT® Systems. Some of these are template amount, purity and type, and the size and type of the protein to be translated. A more complete discussion of translation optimization for the TnT® Systems can be found in reference 4.

Template: For plasmid templates, we recommend using between 0.2–2µg of high-quality plasmid DNA. Typically, 1µg of template should give a good level of protein expression. Using more than 2µg of DNA in a reaction will not increase the protein yields but may actually decrease yields in some cases.

For most of the TnT® Systems, it is recommended to use supercoiled plasmid template, although the T7-based TnT® Systems can also be used with linear templates containing the T7 promoter or templates generated by PCR.

It is possible to generate T7-containing templates using PCR by adding the T7 promoter sequence to the upstream primer. The primer should contain at least 5–7 bases upstream of the T7 site, then a 2–5bp spacer, an optimal Kozak sequence and finally around 20 bases of sequence that is complementary to the target gene. More information on primer design for the T7 Quick for PCR DNA system can be found in reference 5.

Two template elements that are very helpful for increasing the efficiency of in vitro translation are an optimal Kozak sequence and a synthetic poly(A) tail of at least 30 nucleotides. Neither of these elements is required for translation using the TnT® Systems, but each can help improve the efficiency of translation. The Kozak sequence (6) serves to position the ribosome at the initiating AUG codon of the translated RNA, and a poly(A) tail will help to stabilize the RNA transcript within the lysate. Another important consideration is limiting the length of untranslated sequence between the transcription start site and the translation start site—a long untranslated region of RNA can form secondary structures, which may inhibit translation. In addition, there may be additional AUG sequences present in the untranslated region that could be recognized as a translation start site and cause incorrect products.

A common template problem is inhibitor carryover from plasmid purification or linear template preparation. These can include alcohol or residual salt. One method for testing if a template contains an inhibitor is to do a "poison" experiment using equal amounts of the Control DNA supplied with the TnT® Systems and the potentially contaminated experimental template in a TnT® reaction. The amount of luciferase expressed from the Control DNA in the mixed reaction is measured and compared to a reaction using the Control DNA alone. If the expression of the Control DNA is affected by the presence of the experimental template, further template purification may be necessary.

Protein Size: The TnT® Transcription/Translation Systems work best for proteins between 15 and 100kDa. The positive control protein used in the TnT® Systems is luciferase, which is 61kDa in size. Proteins smaller than 15kDa may be targeted for degradation by a ubiquitin-dependent pathway present in the rabbit reticulocyte lysate and are not recommended for translation with the reticulocyte-based translation systems. We recommend the TnT® Coupled Wheat Germ Extract Systems for proteins smaller than 15kDa.

Proteins larger than 100kDa can be difficult to translate in the TnT® Systems as well. However, several papers have demonstrated production of proteins of 167kDa (7), 180kDa (8), and as large as 200kDa (9). For translation of very large proteins, reducing the reaction temperature from 30 to 25°C can increase the yield of full-length protein. Adding protease inhibitors to the reaction can also increase the amount of full-length product by preventing degradation of the proteins after synthesis. Protease inhibitors should be in an aqueous- rather than alcohol-based solution.

Protein Type: Knowing the type of the protein to be translated is important. Membrane proteins in particular can be quite difficult to translate in the TnT® Systems without additional reagents. Addition of either Canine Microsomal Membranes[e] (Cat.# Y4041) or 0.1% final concentration of octaethylene glycol mono n-dodecycl ether (Nikkol; 10) can help to improve the translation of transmembrane proteins by providing an environment that mimics the cellular structures necessary for translation of membrane proteins.

In some cases, the original gene sequence of the protein to be translated may use codons that are not abundant in mammalian systems. This can be seen in proteins from plant, bacterial, parasite or other nonmammalian genomes. When translating proteins of nonmammalian origin, it can be helpful to test several different expression systems to choose the optimal translation system. The TnT® T7 Sample System[a–e] (Cat.# L5900) contains samples of 4 different TnT® Systems, including two different reticulocyte lysate systems (the TnT® Quick Coupled System and TnT® Quick for PCR DNA), the TnT® Coupled Wheat Germ Extract System[a,b,d,e] and the *E. coli* T7 S30 Extract System for Circular DNA[h,i]. For some sequences, the nonmammalian systems may allow more efficient translation than the reticulocyte lysate systems.

References

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Protocols

- *TnT® Quick Coupled Transcription/Translation System Technical Manual* #TM045, Promega Corporation. (www.promega.com/tbs/tm045/tm045.html)
- *TnT® Coupled Reticulocyte Lysate Systems* #TB126, Promega Corporation. (www.promega.com/tbs/tb126/tb126.html)
- *Gold TnT® Express 96 Systems Technical Manual* #TM054, Promega Corporation. (www.promega.com/tbs/tm054/tm054.html)
- *TnT® Coupled Wheat Germ Extract Systems Technical Bulletin* #TB165, Promega Corporation. (www.promega.com/tbs/tb165/tb165.html)
- *TnT®* T7 *Quick for PCR DNA Technical Manual* #TM235, Promega Corporation. (www.promega.com/tbs/tm235/tm235.html)
- E. coli *T7 S30 Extract System for Circular DNA Technical Bulletin* #TB219, Promega Corporation. (www.promega.com/tbs/tb219/tb219.html)

[a] U.S. Pat. Nos. 5,283,179, 5,641,641, 5,650,289 and 5,814,471, Australian Pat. No. 649289 and other patents and patents pending.

[b] U.S. Pat. Nos. 5,324,637 and 5,492,817, Australian Pat. No. 660329 and other patents.

[c] U.S. Pat. No. 5,552,302, Australian Pat. No. 646803 and other patents.

[d] The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673. A license (from Promega for research reagent products and from The Regents of the University of California for all other fields) is needed for any commercial sale of nucleic acid contained within or derived from this product.

[e] U.S. Pat. Nos. 4,966,964, 5,019,556 and 5,266,687, and Australian Pat. Nos. 616881 and 641261 and other pending and issued patents, which claim vectors encoding a portion of human placental ribonuclease inhibitor, are exclusively licensed to Promega Corporation.

[f] 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.

*In the U.S., effective March 29, 2005, U.S. Pat. Nos. 4,683,195, 4,965,188 and 4,683,202 will expire. In Europe, effective March 28, 2006, European Pat. Nos. 201,184 and 200,362 will expire.

[g] FluoroTect™ $Green_{Lys}$ incorporates dye conjugates made with the BODIPY®-FL fluorescent reactive dyes, which are licensed from Molecular Probes, Inc., under U.S. Pat. Nos. 4,774,339, 5,274,113 and 5,433,896 for IVE analysis for research use only including GPR and ASR applications and Fluorotag® technology and under U.S. Pat. No. 6,306,628. BODIPY is a registered trademark of Molecular Probes, Inc., and Fluorotag is a registered trademark of AmberGen, Inc.

[h] Licensed under U.S. Pat. No. 5,252,466 and Australian Pat. No. 647025.

[i] For research purposes only. Not for diagnostic or therapeutic use. For nonresearch uses of the portion of the vector encoding the biotinylation sequence, please contact Promega Corporation for licensing information.

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