Eukaryotic Cell-free Protein Expression

- ✔ Express protein in one hour
- ✔ Obtain soluble, functional protein
- ✔ Use protein directly after expression

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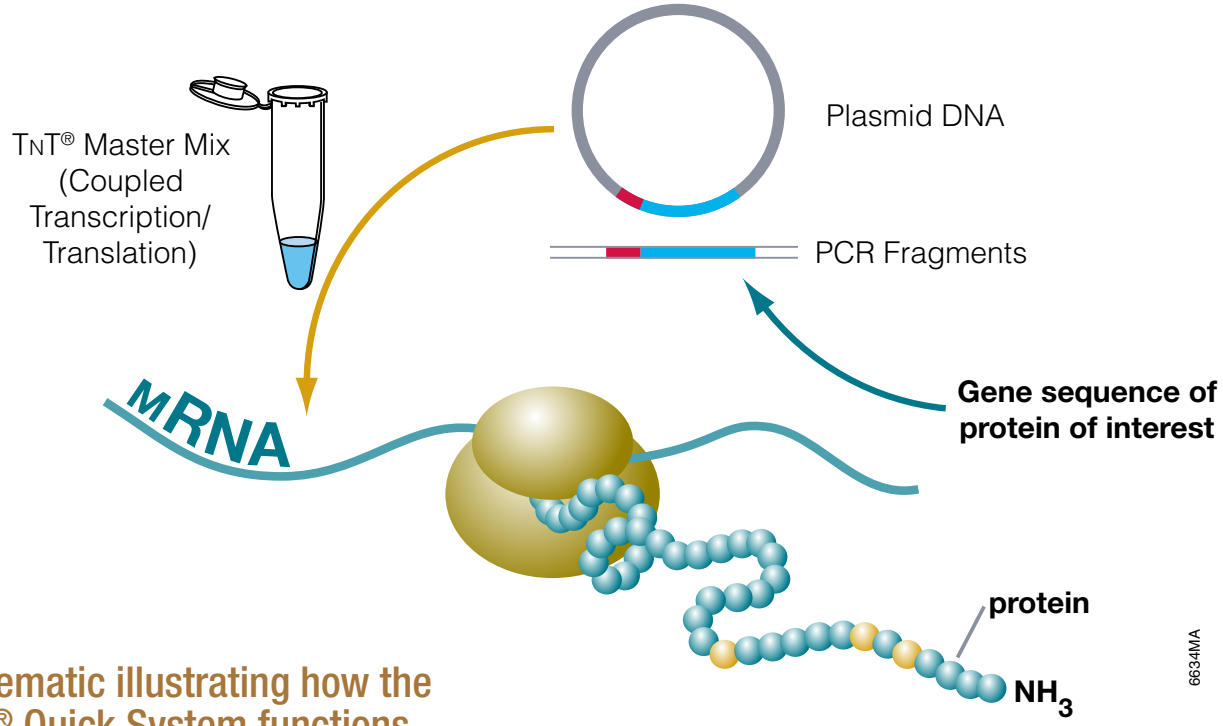
Eukaryotic cell-free protein expression

The TnT® Systems are convenient single-tube, coupled transcription/translation reactions for eukaryotic cell-free protein expression. To use these systems, 0.2–2.0µg of circular plasmid DNA containing a T7, T3 or SP6 promoter, or a PCR-generated fragment containing a T7 promoter, is added to an aliquot of the TnT Quick Master Mix and incubated in a 50µl reaction volume for 60 minutes at 30°C. The reaction produces sufficient quantities of protein that can be used directly for a variety of applications including protein:protein and protein:nucleic acid interactions.



Schematic illustrating how the TnT® Quick System functions.

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Time comparison of the TnT® Quick System and *E. coli* for the expression of recombinant proteins

TnT® Quick System

Add DNA directly to TnT® Master Mix, express protein

1 Hour

Use directly for application

Total: 1 Hour

E. coli expression

Transform and overnight growth

DAY 1

Induction and protein expression

DAY 2-3

Purification/dialysis

DAY 4

Use in application

DAY 5

Total: 4-5 DAYS

6992MA

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Features and benefits

- ✔ **Obtain data faster.** Functional protein is expressed in only one hour, not days as with cell-based expression systems.
- ✔ **Multiple applications with one system.** Use expressed protein for the characterization of protein:protein interaction, protein:nucleic acid interaction, protein modification and more.
- ✔ **Consistent, reliable results.** This mammalian-based system expresses soluble, functional proteins that are post-translationally modified, unlike *E. coli*-based systems.
- ✔ **Fewer steps.** Expressed proteins can be used directly after expression; no requirement for additional purification.
- ✔ **Flexible systems available.** TnT Systems for linear, circular or PCR templates are available.

Visit www.promega.com/selectors/tnt
to select the right system for your needs.

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Applications

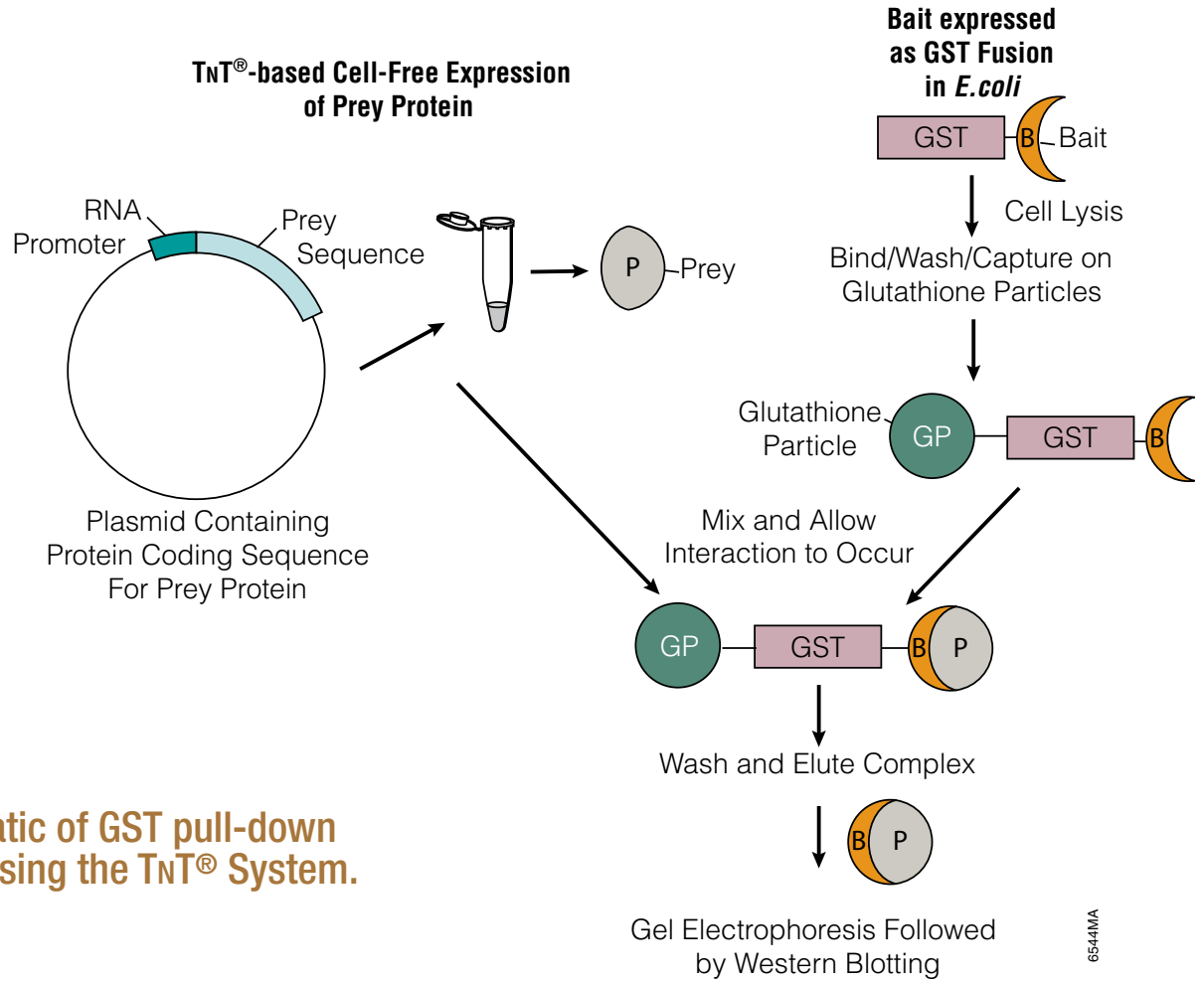
Cell-free expression offers a viable alternative to several applications which have traditionally utilized cell-based expression.

For the characterization of protein interactions, cell-free expression circumvents the requirement to generate large amounts of difficult-to-express proteins. It also enables the use of expressed protein without the time-consuming and technology-challenging purification steps.

For the analysis of protein modifications, cell-free expression can be completed in hours as compared to days for mammalian cell culture. Cell-free expression is an open system allowing the convenient addition of auxiliary components such as modified tRNAs for labeling, accessory proteins and membranes/detergents.

GST Pull-Downs

The TnT® cell-free expression systems are excellent for secondary characterization of protein interaction data generated using yeast two-hybrid or other methods. GST pull-downs is ideal for rapid analysis of mutations and domain mapping of the respective protein partners.



Schematic of GST pull-down assay using the TnT® System.

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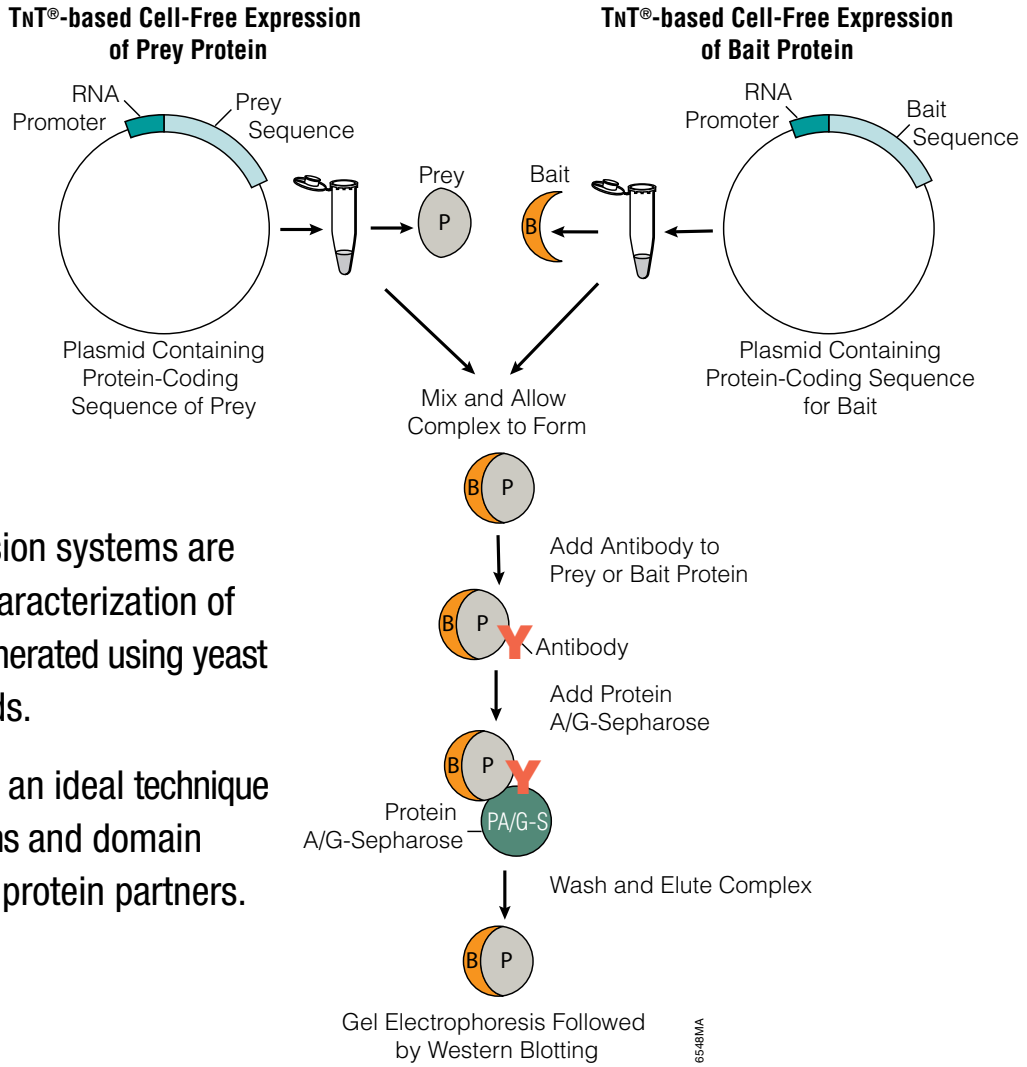
Co-Immunoprecipitation

Schematic of co-immunoprecipitation experiments using the TnT® System.



The TnT® cell-free expression systems are excellent for secondary characterization of protein interaction data generated using yeast two-hybrid or other methods.

Co-immunoprecipitation is an ideal technique for rapid analysis of mutations and domain mapping of the respective protein partners.

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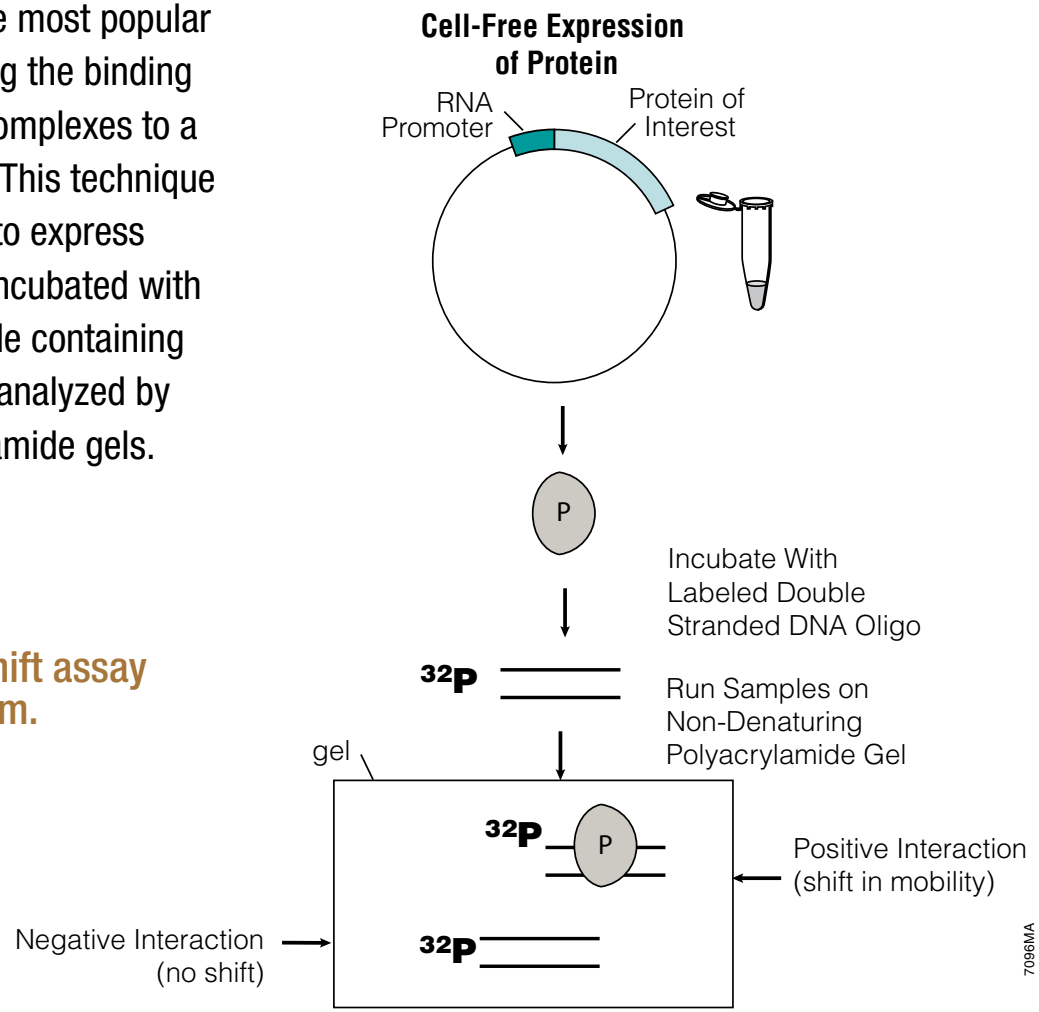
Protein:nucleic acid interactions

Gel shifts are one of the most popular procedures for detecting the binding of proteins or protein complexes to a nucleic acid sequence. This technique uses the TnT® System to express proteins that are then incubated with a labeled oligonucleotide containing a target sequence and analyzed by migration on polyacrylamide gels.



Schematic of a gel shift assay using the TnT® System.

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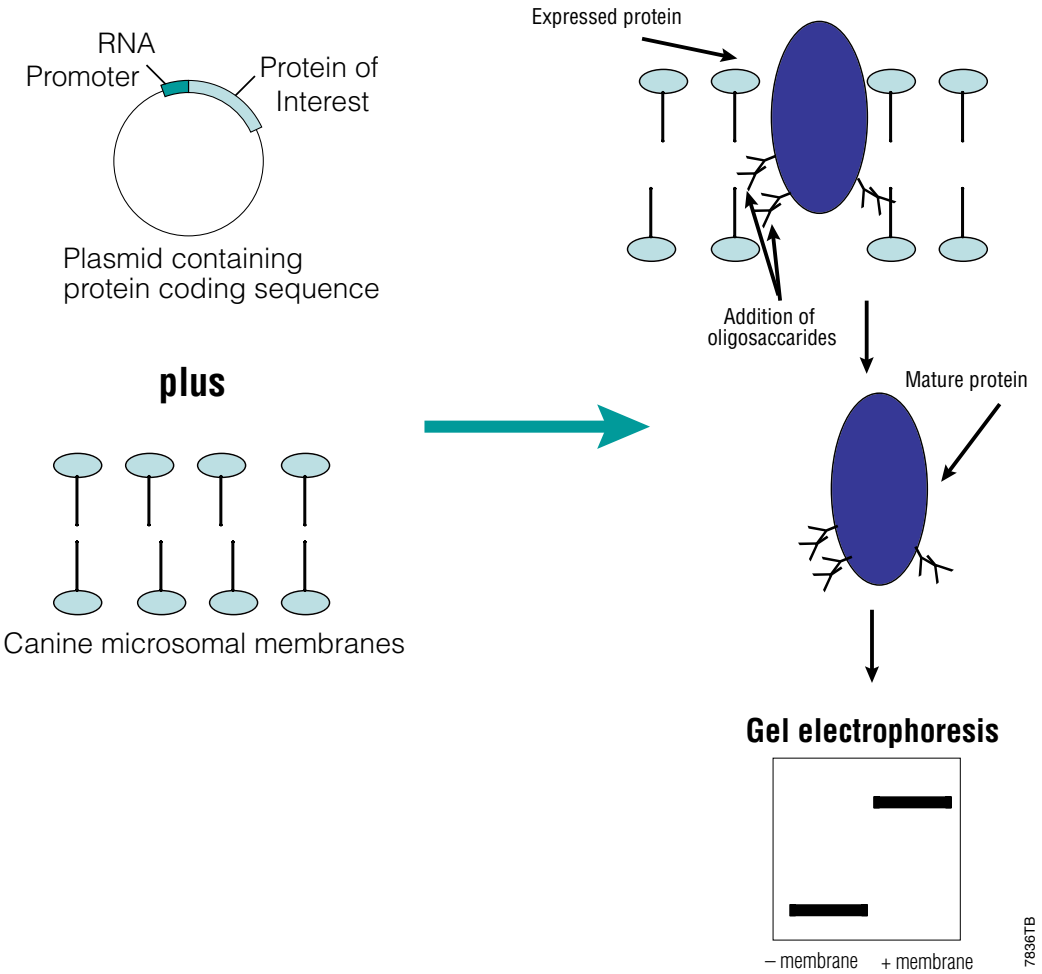
N-linked Glycosylation

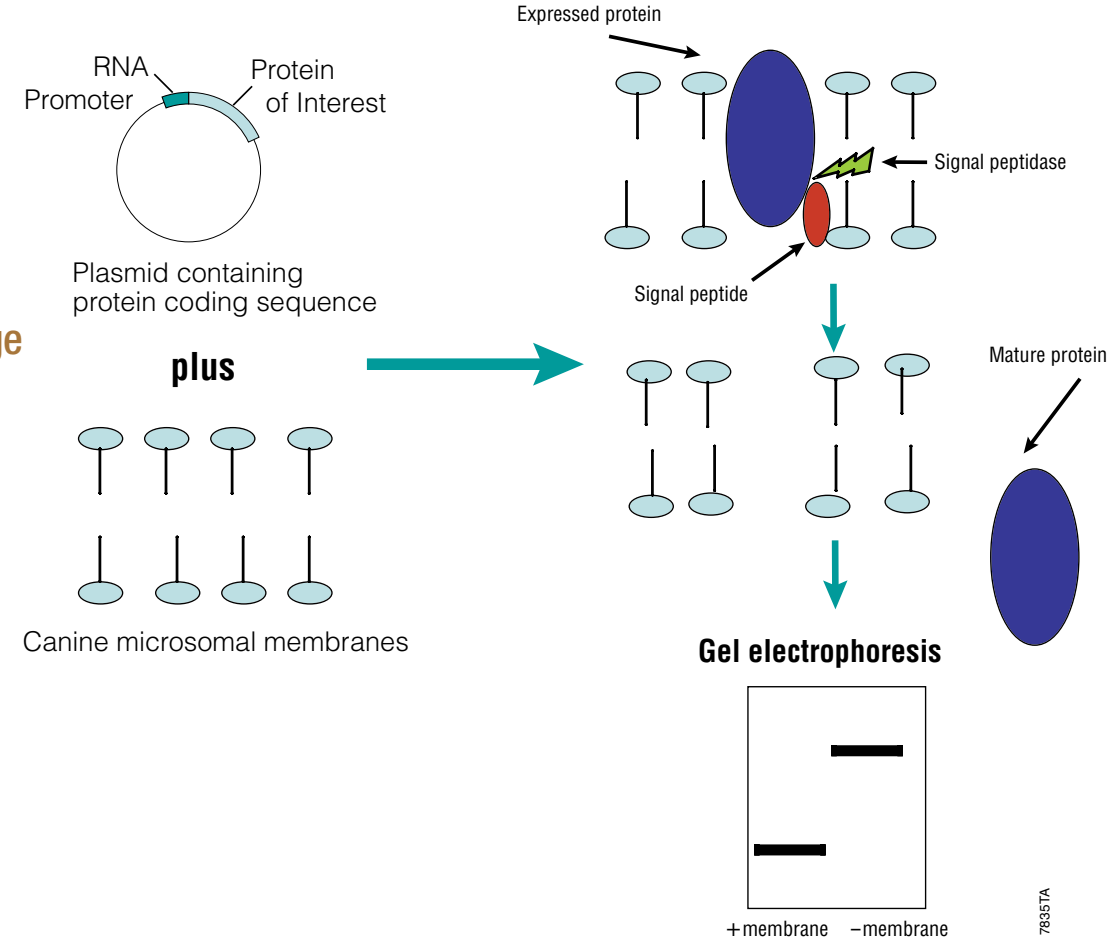
N-linked glycosylation is the addition of oligosaccarides to the NH_4 group of asparagine in the lumen of rough endoplasmic reticulum. The process modulates the structure and function of membrane and secreted proteins. It can be detected when expressing proteins in the presence of canine microsomal membranes and analyzed by a shift in migration on polyacrylamide gels.



Schematic of a glycosylation assay using the TnT® System.

Signal Peptide Cleavage

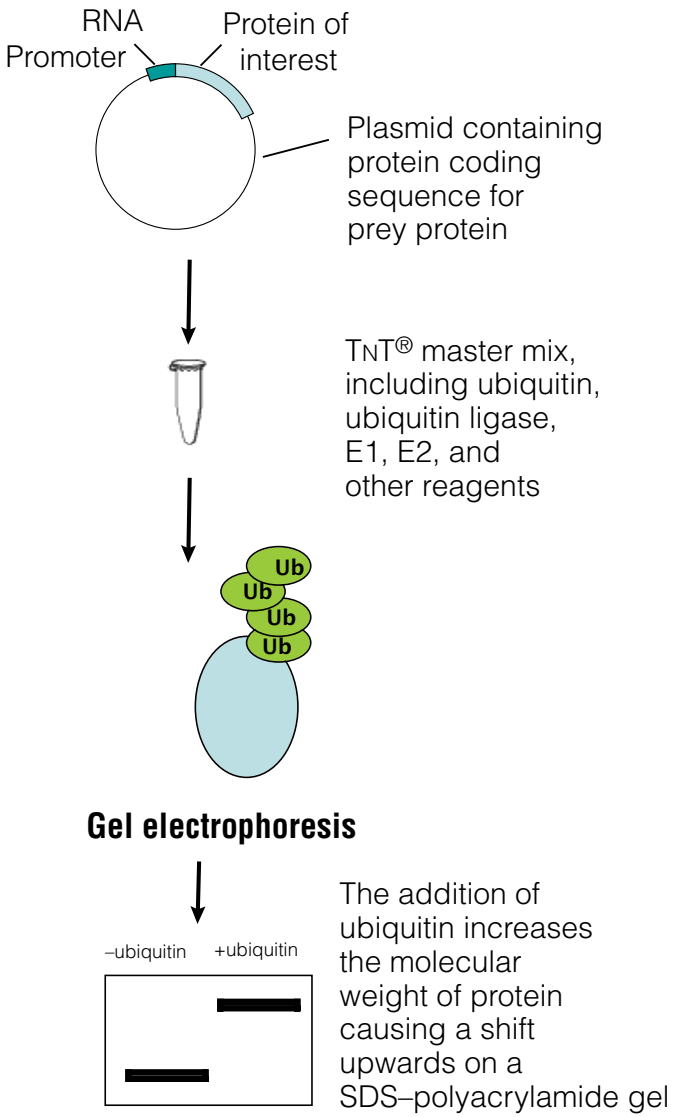
Proteins destined for secretion, membrane insertion or inclusion into the lumen of certain cellular organelles contain a characteristic signal sequence at their N-terminus. Upon insertion into the rough endoplasmic reticulum, the signal sequence is removed by signal peptidase. Signal peptide cleavage has been observed when expressing proteins in the presence of canine microsomal membranes and can be detected by a shift in migration on polyacrylamide gels.



Schematic of a peptide cleavage assay using the TnT® System.

Ubiquitination

Ubiquitination refers to the post-translational modification of a protein by the covalent attachment of one or more ubiquitin monomers. The most prominent function of ubiquitin is labeling proteins for proteasomal degradation. Besides this function, ubiquitination also controls the stability, function, and intracellular localization of a wide variety of proteins. Proteins that have been modified can be analyzed by a shift in migration on polyacrylamide gels.



Schematic representation of a ubiquitination assay using the TnT® System.

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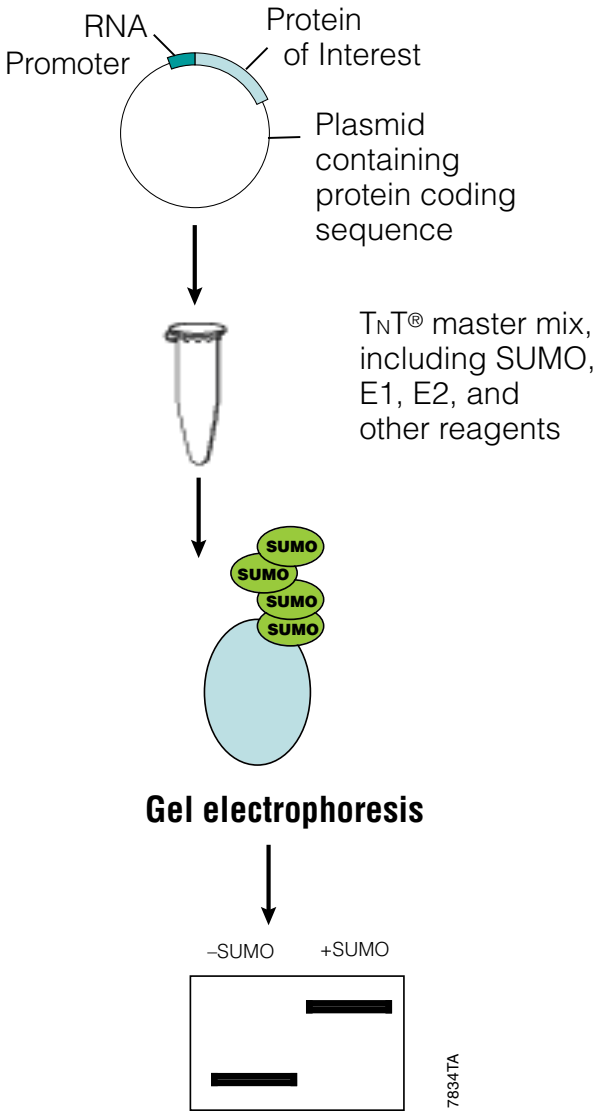
SUMOylation

Small **U**biquitin-related **Mo**difier or SUMO proteins are a family of small proteins that are attached to or detached from other proteins in cells to modify their function.

SUMOylation is a post-translational modification involved in various cellular processes, such as nuclear-cytosolic transport, transcriptional regulation, apoptosis, protein stability, stress response, and progression through the cell cycle. Proteins that have been modified can be analyzed by a shift in migration on polyacrylamide gels.



Schematic of a SUMOylation assay using the TNT® System.

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Click here for additional cell-free expression products

Ordering Information

Product	Size	Catalog Number
TnT® T7 Quick Coupled Transcription/Translation System*	40 reactions	L1170
	5 reactions	L1171
TnT® SP6 Quick Coupled Transcription/Translation System*	40 reactions	L2080
	5 reactions	L2081
TnT® SP6 High-Yield Wheat Germ Protein Expression System	40 reactions	L3260
	10 reactions	L3261
TnT® T7 Quick for PCR DNA*	40 reactions	L5540
TnT® T7 Coupled Reticulocyte Lysate System*	40 reactions	L4610
Accessory Products		
FluoroTect™ $Green_{Lys}$ in vitro Translation Labeling System*	40 reactions	L5001
Transcend™ Colorimetric Non-Radioactive Translation Detection System*	30 reactions	L5070
Transcend™ Chemiluminescent Non-Radioactive Translation Detection System*	30 reactions	L5080
Transcend™ Biotinylated tRNA*	30µl	L5061
Canine Pancreatic Microsomal Membranes	50µl	Y4041
MagneGST™ Pull-down System	80 reactions	V8870

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