# Northwestern Blot of Protein-RNA Interaction from Young Rice Panicles

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**[Abstract]** The northwestern assay is employed to study the interaction between protein and RNA. The RNA binding proteins tend to bind to different kinds of RNA through either known domains or unknown sequences of proteins. Rice LGD1 recombinant protein, a grass-specific novel protein with RNA binding sequences in its C-terminal, was used to probe its function as an RNA binding protein. The LGD1 comprising von Willebrand factor type-A domain (vWA), coiled-coil and nuclear localization signal (NLS) is a class of protein that localizes both in the nucleus and cytoplasm. Although LGD1 does not contain any putative RNA binding domains, we could find high-affinity RNA binding residues at the C-terminus using 'RNABindR' prediction software (Terribilini *et al.*, 2007). The LGD1 recombinant protein, purified from bacteria, somehow forms both dimer and monomer even under denaturing conditions. However, only the dimeric form is able to bind to total and mRNAs. Due to its reproducibility and reliability, we believe that this protocol can be used across different organisms.

## Materials and Reagents

1. Young panicle tissues of wild type (*Oryza sativa* L. *ssp*. Japonica cultivar Tainung67) plants
2. Tissues were collected from young panicles while they were in secondary branch initiation
3. Recombinant GST-LGD1 protein from *E. coli* (BL21)
4. pGEX-4T-1 GST expression vector (GE Healthcare, catalog number: 28-9545-49)
5. Total RNA isolation kit - Trizol (Life Technologies, Invitrogen™, catalog number: 15596-026)
6. mRNA purification kit - Oligotex mRNA Mini Kit (QIAGEN, catalog number: 72022)
7. RNA 5'-end Labeling kit (T4 Polynucleotide Kinase) (Thermo Fisher Scientific, catalog number: EK0031)
8. FastAP Thermosensitive Alkaline Phosphatase (Thermo Fisher Scientific, catalog number: EF0651)
9. [$\gamma$-$^{32}$P]-ATP – for isotope labeling

10. PVDF Immobilon-P membrane (EMD Millipore, catalog number: IPVH15150)
11. G-50 gel filtration dye terminator removal column (Geneaid, Taiwan, catalog number: CG050)
12. Primary anti-GST antibody (Bioman, Taiwan, catalog number: GST001M); HRP-conjugated secondary antibody (Thermo Fisher Scientific, catalog number: SA1-9510)
13. SuperSignal West Pico Chemiluminescence detection system (Thermo Fisher Scientific, catalog number: 34087)
14. Blocking buffer (see Recipes)

## Equipment

1. Gel electrophoresis (Bio-Rad Laboratories, catalog number: 165-8005EDU)
2. SNAP i.d.® 2.0 protein detection system
3. Equipment for phosphor imaging (Bio-Rad Laboratories, catalog number: 170-9460)

## Procedure

1. RNA isolation and end-labeling of total and mRNA
   a. Total RNA was isolated from young panicles of wild type plants with Trizol kit.
   b. ~0.5 g tissue was used to isolate ~500 µg of total RNA and 200 µg total RNA was used to purify ~2 µg mRNA using Oligotex mRNA Mini Kit. Nanodrop was used for final RNA quantitation (~2 µg/µl for total RNA; ~20 ng/µl for mRNA).
   c. 5 µg total RNA and 1 µg mRNA were first treated with FastAP Thermosensitive Alkaline Phosphatase (10 U) at 37 °C for 1 h to remove 5'-phosphate group according to manufacturer's protocol.
   d. The total and mRNAs were end-labeled with [$\gamma$-$^{32}$P]-ATP using 1 µl (10 U) T4 Polynucleotide kinase at 37 °C for 30 min.
   e. Labeled RNAs were separated from unincorporated label by gel filtration on Sephadex G-50 column.
2. Northwestern blot assay
   a. GST-LGD1 fusion protein was extracted from *E. coli* harboring pGEX-4T-1 GST expression vector according to manufacturer's protocol.
   b. The proteins were separated on 12% SDS PAGE under denaturing conditions, and transferred to PVDF Immobilon-P membrane and detected using the SNAP i.d. protein detection system as follows.

c. For the Western blot, the primary anti-GST antibody (1:2,000) and secondary HRP (horseradish peroxidase)-conjugated antibody (1:3,000) were used for the enhanced chemiluminescence (ECL) detection.
d. SuperSignal West Pico Chemiluminescence was employed to detect western signals according to the manufacturer's instructions.
e. For the Northwestern, recombinant proteins separated on 12% SDS PAGE under denaturing conditions were transferred to PVDF Immobilon-P membrane.
f. The proteins were then renatured on the blots overnight at 4 °C in a renaturation buffer containing 0.1 M Tris HCl (pH 7.5) and 0.1 % (v/v) NP-40.
g. Blots were washed 4 times 15 min each in renaturation buffer and incubated 5 min at room temperature in blocking buffer.
h. Blots were hybridized overnight at 4 °C in blocking buffer (without BSA) in the presence of labeled total and mRNAs.
i. Finally, the blots were washed 4 times 5 min each in blocking buffer (without BSA and Triton) and autoradiographed to obtain the signal as seen in Figure 1.

## Representative data

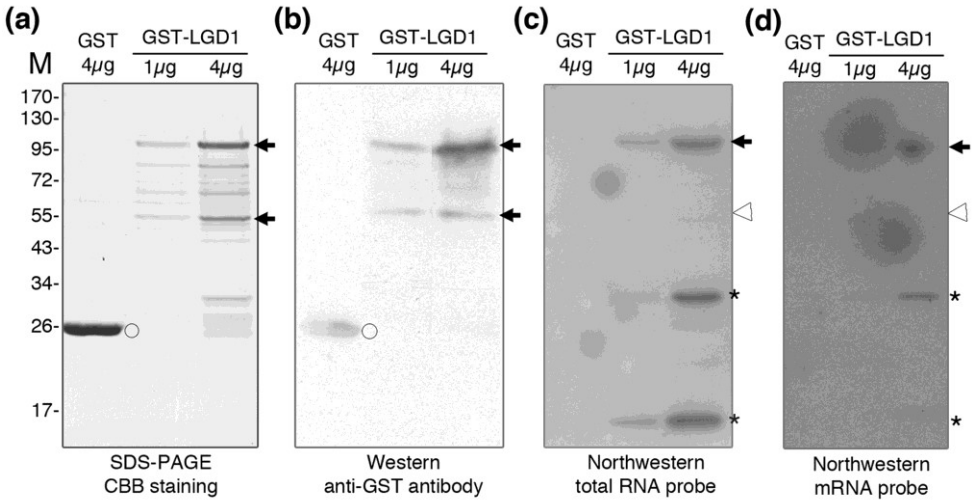


**Figure 1. Northwestern blot analysis of LGD1 recombinant protein.** The recombinant GST-LGD1 was purified from *E. coli* and analyzed through SDS-PAGE, western and northwestern blots. (a) Coomassie Brilliant Blue (CBB) staining shows two major bands (arrows). The size of the lower band is ~56 kD (monomer) and upper band is ~112 kD (homodimer). (b) Anti-GST antibody recognizes the GST-LGD1 fusion protein (arrows) as well as GST (open circle). Northwestern blot shows the total RNA. (c) and mRNA (d) isolated from young panicle tissues bind to upper band (arrows), but not to the lower band (open arrowhead). Asterisks indicate RNA binding to degraded form of LGD1.

## Recipes

1. Blocking buffer
   10 mM Tris HCl (pH 7.5)
   5 mM Mg acetate
   2 mM DTT
   5 % (w/v) BSA
   0.01 % (v/v) Triton X-100

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