Coomassie Brilliant Blue (CBB) staining for Rubisco is an appropriate loading control for western blots from plant material

Erin K. Zess and Sophien Kamoun

The Sainsbury Lab, University of East Anglia, Norwich Research Park, Norwich, United Kingdom

Background – Having an adequate loading control for a western blot is essential for the interpretation of the results. There are two common loading control methods for western blots of proteins from plant material: (i) using specific antibodies to detect for a reference protein, such as actin, tubulin, or GAPDH (Li et al. 2011); and (ii) treating the membrane with Ponceau or Coomassie stains to assay the levels of a constitutively expressed protein, such as Rubisco (Zhang et al. 2017; Lim et al. 2018; Zhuo et al. 2014). Comparative studies in the mammalian biology field have determined that these loading control methods—antibody detection versus staining—are roughly equivalent in their linearity (Romero-Calvo et al. 2010; Wilender and Ekblad, 2011), and thus serve as comparable quality controls. In the plant biology field, it is sometimes debated as to whether staining for Rubisco is an appropriate loading control, due to the high abundance of this protein in the cell.

Results – We undertook an experiment to determine whether the range of detection of staining for Rubisco is similar to that of antibody-based detection of a reference protein. We loaded total protein extract from *Nicotiana benthamiana* leaves transiently expressing GFP into a gel at a range of effective sample volumes, and the resulting western blot was treated with anti-GFP antibodies as well as stained with Coomassie Brilliant Blue (CBB) (Fig. 1a). Quantification of the GFP bands in the western blot and the Rubisco bands in the CBB stained membrane indicated that these detection methods have similar linear correlations between the loading volumes of total protein extract and the detectable band intensities (Fig. 1b). In addition, quantification of a random protein of lower abundance in the CBB stained membrane also showed similar linearity (Fig. 1b).

Conclusions – These results indicate that CBB staining for Rubisco can be an appropriate loading control for western blots from plant material. This representative experiment is consistent with results from other western blot experiments that we routinely perform in our laboratory.

Materials and methods – Four-week old *Nicotiana benthamiana* leaves were agroinfiltrated with a construct expressing GFP. Leaves were harvested 3 days after infiltration, ground to powder in liquid nitrogen, and proteins were extracted in GTEN buffer (10% glycerol, 25 mM Tris pH 7.5, 1 mM EDTA, 150 mM NaCl) containing 2% w/v PVPP, 10 mM DTT, 1X protease inhibitor cocktail (Sigma), 0.1% Tween 20 (Sigma). The total protein extract was loaded into a 4-20%TGX mini-protean gel (Bio-Rad) at a range of effective sample volumes (20 µl, 15 µl, 10 µl, and 5 µl). After separation, proteins were transferred onto a polyvinylidene diflouride membrane using the Trans-Blot turbo transfer system (Bio-Rad). The membrane was immunoblotted with HRP-conjugated anti-GFP monoclonal antibody (Santa Cruz Biotechnology). GFP was detected by exposing the blot to ECL chemiluminescent substrate and the membrane was imaged with an ImageQuant LAS 4000 luminescent imager (GE Healthcare Life Sciences). The same blot was stained with Coomassie Brilliant Blue (SimplyBlue™ SafeStain, Invitrogen), and an image of the stained membrane was captured using a digital scanner. ImageJ software was used to process the western blot and CBB stained membrane images to return arbitrary values corresponding to the signal strength of the key bands in each image: GFP in the western blot (Fig. 2), Rubisco in the CBB stained membrane (Fig. 3), and an unknown band at the bottom in the CBB stained membrane (Fig. 4). The resulting arbitrary values were expressed as percentages of the respective maximums and plotted for each lane as a function of the effective sample loading volume.

References

Li X., Bai H., Wang X., Li L., Cao Y., Wei J., Liu Y., Liu L., Gong X., Wu L., Liu S., Liu G. 2011. Identification and validation of rice reference proteins for western blotting. J. Exp. Bot. 62:4763-72.

Lim G.H., Hoey T., Zhu S., Clavel M., Yu K., Navarre D., Kachroo A., Deragon J.M., Kachroo P. 2018. COP1, a negative regulator of photomorphogenesis, positively regulates plant disease resistance via double-stranded RNA binding proteins. PLOS pathog. 14:e1006894.

Romero-Calvo I., Ocón B., Martínex-Moya P., Suárez M.D., Zarzuelo A., Martínez-Augustin O., Sánchez de Medina F. 2010. Reversible Ponceau staining as a loading control alternative to actin in western blots. Anal. Biochem. 401:318-320.

Welinder C. and Ekblad L. 2011. Coomassie staining as loading control in western blot analysis. J. Proteome Res. 10:1416-9.

Yi G., Gopinath K., Kao C. 2007. Selective Repression of translation by the brome mosaic virus 1a RNA replication protein. J. Virol. 81:1601-9.

Zhang Z., Hu F., Sung M.W., Shu C., Castillo-González C., Koiwa H., Tang G., Dickman M., Li P., Zhang X. 2017. RISC-interacting clearing 3'-5' exoribonucleases (RICEs) degrade uridylated cleavage fragments to maintain functional RISC in *Arabidopsis thaliana*. eLIFE. 6:e24466.

Figures

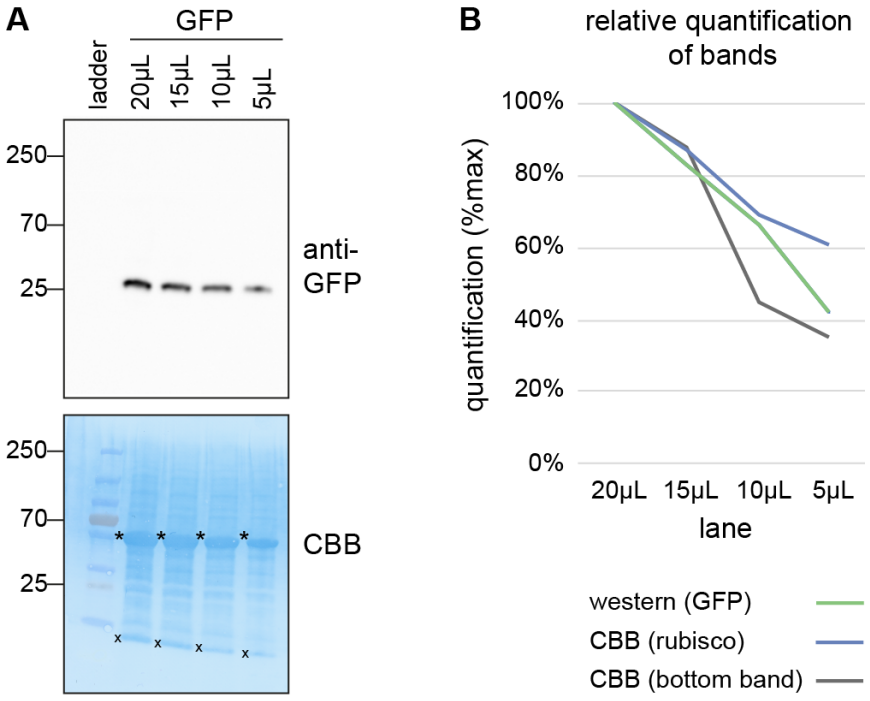

Figure 1. **Coomassie Brilliant Blue (CBB) staining for Rubisco is an appropriate loading control for western blots from plant material.** A) Western blot and Coomassie Brilliant Blue (CBB) stain of a sample dilution series. Stars indicate the bands corresponding to Rubisco in the CBB loading control and x's indicate the bottom bands also used in quantification. B) Comparative quantification of the relative intensity of bands of interest from panel (A) using ImageJ software.

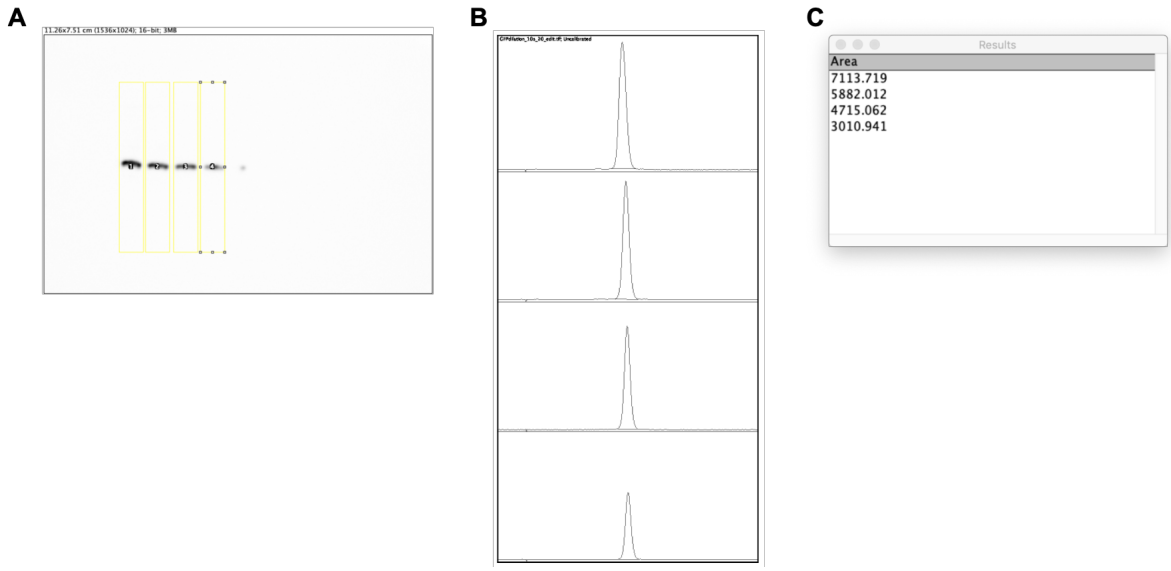

Figure 2. **Quantification of western blot GFP bands using ImageJ.** (A) Lane selection. (B) Plot of lanes, showing how each GFP band peak was closed. (C) Quantification results.

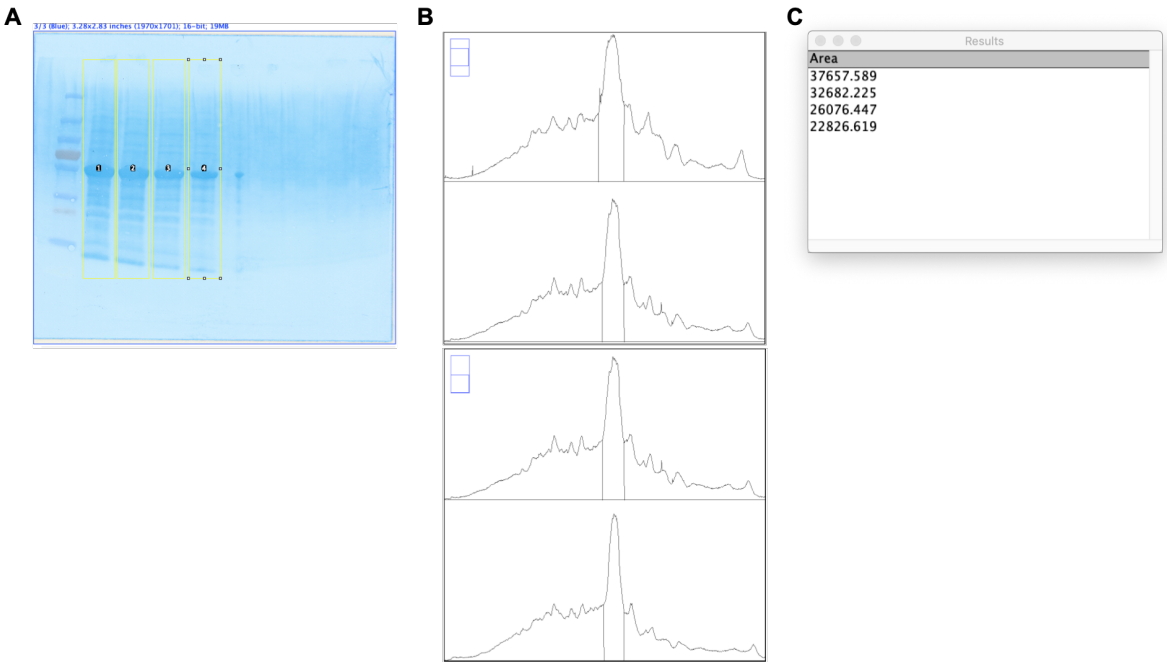

Figure 3. **Quantification of CBB membrane Rubisco bands using ImageJ.** (A) Lane selection. (B) Plot of lanes, showing how each Rubisco band peak was closed. (C) Quantification results.

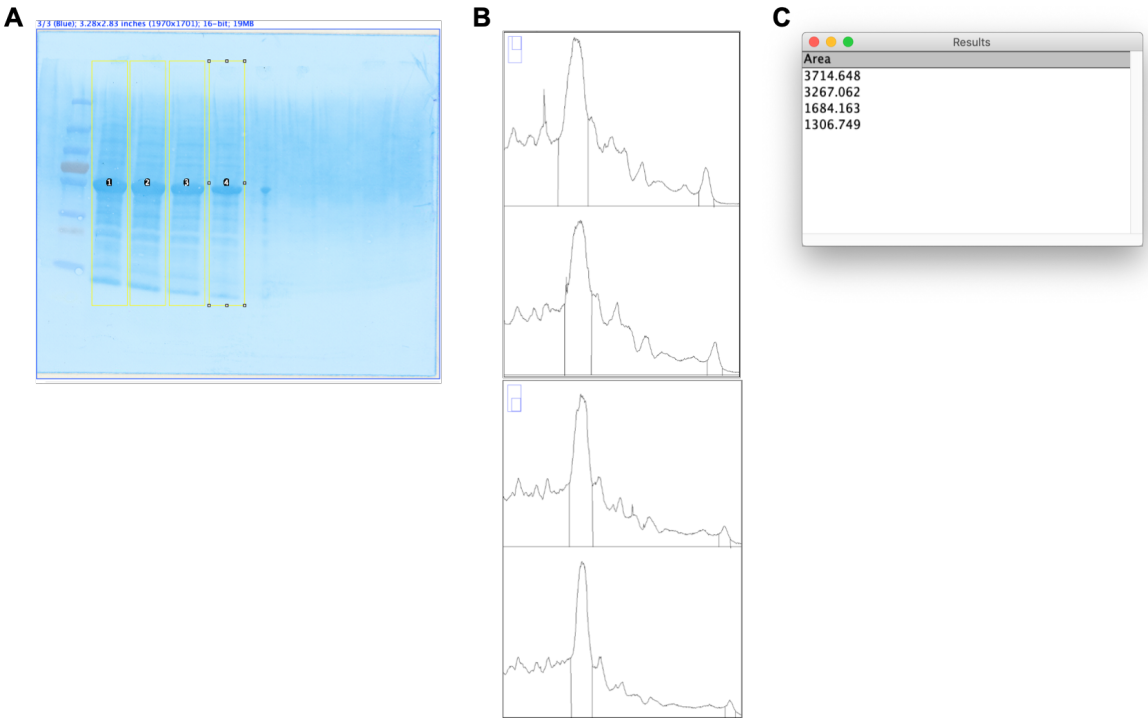

Figure 4. **Quantification of CBB membrane bottom bands using ImageJ.** (A) Lane selection. (B) Plot of lanes, showing how each bottom band peak was closed (rightmost peak). (C) Quantification results.