SGR-based Reporter to Assay Plant Transcription Factor-promoter Interactions

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[Abstract] We developed an *in vivo* method to assay plant transcription factor (TF)–promoter interactions using the transient expression system in *Nicotiana benthamiana* (*N. benthamiana*) plants. The system uses the *Arabidopsis stay green* (*SGR*) gene as a reporter. Induction of *SGR* expression in *N. benthamiana* causes chlorophyll degradation and causes leaves to turn yellow.

Materials and Reagents

A. Plant material
   1. 4 to 5 week old healthy *N. benthamiana* plants

B. Vectors and bacteria strains
   2. pDONR221 or other gateway DONR vector (Life Technologies, Invitrogen™, catalog number: 12536-017)
   3. *Escherichia coli* (*E. coli*) DH10B or similar cells for molecular cloning
   4. SGR reporter destination vector, SPDK 2388
      Note: This vector contains the reporter gene SGR without promoter.
   5. Binary vector for TFs over-expression (*e.g.* LIC6 from *Arabidopsis* Biological Resource Center)
   6. Binary vector for expression of negative control protein [*e.g.* Actin7 (At5g09810) in LIC6]
   7. *Agrobacterium tumefaciens* (*A. tumefaciens*) strain GV2260

C. Other materials
   8. BP and LR clonase enzyme Kit (Life Technologies, Invitrogen™, catalog numbers: 11789-013 and 11791-043)
   9. LB media
   10. Acetosyringone (Sigma-Aldrich, catalog number: D134406)
   11. Infiltration medium (see Recipes)

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Equipment

1. Incubator (42 °C)
2. 1 ml Tuberculin syringes without needle (Tyco, catalog number: 8881501400)
3. Controlled environment plant growth chamber (12 h of light per day, 21 °C)

Procedure

A. Cloning of transcription factor of interest into *SGR* reporter vector
   1. Amplify promoter fragments (1-2 kb) from plant genomic DNA by high-fidelity PCR, and clone them into Gateway entry vector (pDONR221 or pDONR207) using BP reaction according to manufacturer's instruction. This will generate the entry vector containing the promoter fragments.
   2. Transfer the promoter fragments into the destination vector SPDK2388 via LR reaction. The LR reaction should be set up as below:
      100 ng entry vector containing promoters
      100 ng destination vector SPDK2388
      2 µl LR enzyme buffer
      2 µl LR enzyme
      ddH_2O to 10 µl
      Incubate at 25 °C overnight. Add 1 µl Proteinase K, incubate at 37 °C for 10 min. Transform the reaction mix into chemical competent cells of *E. coli* DH10B or similar cells, and select the transformants on 50 µg/ml spectinomycin (spectinomycin^{50}) containing LB plates.

B. Prepare plants and *Agrobacterium* strains
   1. Grow *N. benthamiana* plants with 12 h day light, 22 °C in the growth chamber. At 4 to 5 weeks, the plants should have three to four large healthy leaves suitable for infiltration.
   2. Transform separately the destination vector SPDK2388 with the TF of interest and the control into *A. tumefaciens* GV2260 by using chemical or electro competent cells. Typically, 200-500 ng plasmids and 50 µl competent cells are used for the transformation. Plate all the transformed cells and select the transformants on spectinomycin^{100}, streptomycin^{100}, Rifampicin^{25}, and Carbenicillin^{50} (SSRC) containing LB plates. More than 100 transformants should be expected.

C. Evaluate background expression of the promoter::SGR reporter constructs in *N. benthamiana* leaves

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1. Grow *Agrobacterium* GV2260 strains harboring the promoter::SGR construct overnight in ~20 ml LB media with SSRC.
2. Spin down the *Agrobacterium* culture at 3,000 *x g* for 10 min, discard the supernatant, and suspend in infiltration media. Adjust the concentration of the culture to a series of OD_{600}. For example, 0.05, 0.1, 0.3, and 0.6 OD. Incubate the culture at the room temperature for 3 h.
3. Infiltrate *N. benthamiana* leaves with different concentration of *Agrobacterium* culture prepared in step C2 above. Make a small cut on the abaxial side of the leaves, use 1 ml syringes without needle to infiltrate *Agrobacterium* culture into the leaves. The culture should cover a spot of ~1 cm in diameter (see Figure 1). Infiltrate 1-2 spots for each OD concentration and infiltrate all samples with different ODs onto a single leaf. Repeat the infiltration onto a second leaf within the same plant.
4. Keep infiltrated plants for 48 or 72 h in the same growth chamber and check the spots for signs of yellowing. Choose the highest OD concentration that shows no or slight yellowing for the following experiment.

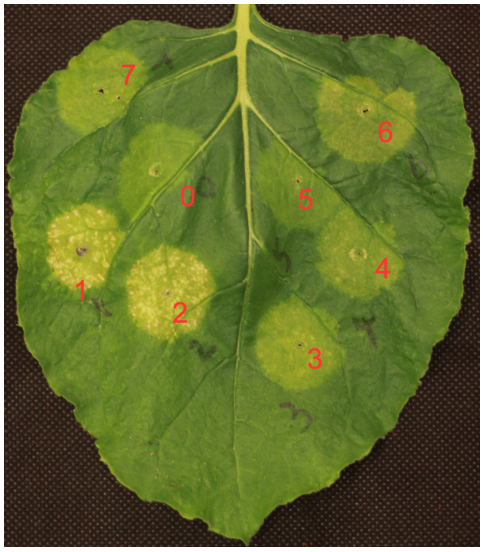

Figure 1. SGR reporter-based transcription factors and promoter interaction assay in *Nicotiana benthamiana*. The SUR1 promoter::SGR reporter construct was co-infiltrated with different MYB TFs (spots 1 to 7) or with a negative control Actin (spot 0). Only two MYBs (spot 1 and 2) showed severe yellowing signs indicating that these two MYBs activate SUR1 promoter.

D. Assay for TF-promoter interaction

1. Grow separately *Agrobacterium* GV2260 strains harboring the promoter::SGR construct, TF construct, and actin control overnight in LB liquid media with SSRC.

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Note: We have constructed an ATEC library in LIC6 binary vector for over-expression of 15,000 Arabidopsis genes, which include more than 700 transcription factors. All these clones are deposited to and can be ordered from ABRC. In ABRC, the ATEC clones are names as "DKL" plus AGI numbers. See Reference 3 for more details.

2. Spin down the cultures min and re-suspend them in infiltration media. Adjust the OD of the reporter culture to the concentration determined in step C4. Adjust the OD of TF and actin control to 1.0.
3. Mix *Agrobacterium* culture with the reporter and the TFs or control with equal volume (~ 1 ml each). Incubate at room temperature for 3 h.
4. Infiltrate *N. benthamiana* leaves with the mixed cultures from above onto 1-2 spots of ~1 cm onto the same leaf. Control and treatment samples should be present on the same leaf to overcome leaf-to-leaf variability.
5. Keep infiltrated plants for 48 to 96 h in the growth chamber. Record signs of yellowing of each spot at 24, 48, 72, and 96 h post infiltrations. The severity of yellowing indicates the strength of reporter gene expression by the corresponding TF. The spots containing TFs should be compared to the control spots to assess specificity of TF-promoter interactions. See example shown in Figure 1. Photograph the leaves to document the results.
6. Further validation of the observed results could be performed using luciferase-based reporter assay as described in Ma *et al*. (2013).

Recipes

1. Infiltration medium
   Note: Prepare fresh media every time; sterilization is not required.
   10 mM $MgCl_2$
   10 mM MES
   200 µM acetosyringone

Acknowledgments

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References

1. Hortensteiner, S. (2009). Stay-green regulates chlorophyll and chlorophyll-binding protein degradation during senescence. *Trends Plant Sci* 14(3): 155-162.
2. Ma, S., Shah, S., Bohnert, H. J., Snyder, M. and Dinesh-Kumar, S. P. (2013). incorporating motif analysis into gene co-expression networks reveals novel modular expression pattern and new signaling pathways. *PLoS Genet* 9(10): e1003840.
3. Popescu, S. C., Popescu, G. V., Bachan, S., Zhang, Z., Seay, M., Gerstein, M., Snyder, M. and Dinesh-Kumar, S. P. (2007). Differential binding of calmodulin-related proteins to their targets revealed through high-density *Arabidopsis* protein microarrays. *Proc Natl Acad Sci U S A* 104(11): 4730-4735.

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