Determination of Luciferase Activity in *Arabidopsis* Seedling

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[Abstract] There are different direct and indirect methods available to study gene expression in plant systems. In this protocol we describe a modified expression assay using transgenic plants expressing the luciferase gene under the control of phosphate transporter PHT1;1 promoter. This assay was originally optimized for analyzing the repression of PHT1;1 promoter in response to arsenate As (V) which can be adapted to study the kinetics of transcriptional regulation of any gene in response to biotic or environmental stimuli measuring Luciferase activity in *Arabidopsis thaliana*.

Materials and Reagents

1. Transgenic *Arabidopsis* seedlings expressing Luciferase fused to promoter of interest (*i.e.* PHT1;1:Luc in pGWB435 binary vector)
   Note: Vector information can be found at: http://shimane-u.org/nakagawa/pgwb-tables/2.htm.
2. KH_2PO_4 (Sigma-Aldrich, catalog number: P5655)
3. Sodium arsenate dibasic heptahydrate (Sigma-Aldrich, catalog number: A6756)
4. 30 mM arsenate (see Recipes)
5. 0.36 mM D-Luciferin (Sigma-Aldrich, catalog number: L9504) (see Recipes)
6. Johnson media (see Recipes)

Equipment

1. Growth chamber [16-h-light/8-h-dark regime (24 °C/21 °C)]
2. White 96-well plates (Greiner Bio-One GmbH, catalog number: 655074)
3. MicroAmp optical adhesive Film (Life Technologies, Applied Biosystem®, catalog number: 4311971)
4. Luminometer (centro LB 960) (Berthold Technologies)

Software

1. Mikro Win 2000 software

Procedure

1. Sow the transgenic seeds minimum (24 plants for each treatment) in Petri dish containing Johnson media (0.6% agar) with 1 mM phosphate and incubate in growth chamber with 16-h-light/8-h-dark regime (24 °C/21 °C) for 7 days.
2. Transfer the seedlings to Johnson media (0.6% agar) lacking phosphate and leave for 3 days.
3. Add 200 µl of Johnson liquid media (without agar) to White 96-well plate and then transfer single seedlings to each well by placing upside down.
   Note: Since PHT1;1 is prominently expressing in root we submerged the seedlings upside down.
4. Add D-Luciferin substrate (50 µM final concentration) to each well and incubate for 1 h.
5. Measure the initial luminescence *i.e.* Luminescence (0).
6. Add arsenate to each well (30 µM final concentration) and cover the plate with MicroAmp optical adhesive Film (refer to Reference 1 to understand the behavior of PHT1;1 in response to Arsenic).
7. Measure the luminescence of the plate in every 10 min interval with the luminometer using Mikron Win 2000 software (counting time for each well is two seconds).
8. Calculate the percentage of luciferase activity (%LUC activity) as follows:
   %LUC activity = [(Luminescence(n) – Luminescence(0))/ Luminescence(0)]*100]
   Luminescence (0) = Luminescence at time zero
   Luminescence (n) = Luminescence at 2^{nd} and consecutive readings
   Once we calculate the %LUC activity for each time point, we have to calculate the average Luminescence of all the independent plants at each time point.
9. Luciferase activity can be also determined in *Nicotiana benthamiana* (*N. benthamiana*) using 1 cm discs of agro infiltrated leaves transferred to a microplate and proceed as described above.

Recipes

1. 30 mM arsenate
   Dissolve 468 mg of sodium arsenate dibasic heptahydrate in 50 ml water
   Filter sterilize

2. 0.36 mM D-Luciferin

   Mix 1 mg of D-Luciferin with 8 ml dH_2O

   Warm at 80-90 °C for 30 to 40 sec to dissolve it

   Add dH_2O to 10 ml

   Filter sterilize (0.25 µm)

   Stored at -20 °C

3. Johnson media

Macronutrientes	**Stock**	**ml stock/L**
KNO_3 (4 °C)	100 g/L	6.06 ml
$Ca(NO_3)\cdot 4H_2O$ (4 °C)	100 g/L	9.446 ml
$MgSO_4\cdot 7H_2O$ (4 °C)	100 g/L	2.46 ml

Micronutrientes	**g for 100 ml stock 1000x**	**ml stock/L**
KCl (-20 °C)	0.378 g	1 ml
H_3BO_3 (-20 °C)	0.15457 g	
$MnSO_4.H_2O$ (-20 °C)	0.0845 g	
$ZnSO_4.7H_2O$ (-20 °C)	0.0575 g	
$CuSO_4.\ 5H_2O$ (-20 °C)	0.01248 g	
H_2MoO_4 (-20 °C)	0.00161 g	

	g for 200 ml stock 500x	**ml stock/L**
$FeSO_4.7H_2O$ (4 °C) (ajustar pH a 3.5 con H_2SO_4 diluido) Proteger de la luz	0.1112 g	2 ml

	Stock	**stock/L**
Mio-inositol (4 °C)	50 g/L	2 ml

MES	0,5 g/l
1% sacarosa	10 g/L
0.5% sacarosa	5 g/L

Make volume to 1 L using Milli Q water

Adjust the pH to 5.7 with KOH

0.6% Agar

After Autoclave add phosphate KH_2PO_4

1 M KH_2PO_4: 6.8 g/50 ml (filter sterilize)

Add 1 ml/L to get final concentration of 1 mM

Acknowledgments

This protocol is adapted from the original paper by Castrillo *et al.,* 2013. This work was supported by fellowships from the Spanish Ministry of Science and Innovation (G.C) and from La Caixa/CNB International PhD program (to M.T.C.) as well as research grants (BIO2004-03759, BIO2007-66104, BIO2010-16687, AGL2007-61705, AGL2010-15151, CSD2007-00057, EUI2009-03993, and BIO2011-29085) from the Spanish Ministry of Science and Innovation.

References

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