LUCIFERASE ASSAY PROTOCOL FROM TRANSFORMED TISSUE

Special Note:
The Luciferase enzyme is unstable in a protein extract: it loses enzyme activity quickly. Therefore, extracts should always be:
- ground up in small numbers (<24 at a time)
- ground up in a COLD mortar
- ALWAYS kept chilled on ice or 4C room until ready to assay; prechill all tubes if possible
-assayed in luminometer as soon as possible and PRIOR to freezing
-Bradford assays can be done before or after freezing at -80C

Protocol
1. Place small mortars, pestles and Luciferase Extraction Buffer (all kept in 4C walk-in) on ice. Use a second ice bucket for post-ground samples.

2. Collect liquid nitrogen from downstairs and place 24 frozen tissue samples inside.

3. Place a small amount of autoclaved sand in each mortar.

4. Add 500ul - 1ml of Extraction Buffer per mortar. Grind for 30seconds. Pour back into original eppendorf tube and place on ice until all ground.

5. Centrifuge in 4C walk-in room, microfuge 10krpm, 10minutes.

6. During spin, label a new batch of eppendorf tubes and place in coldroom or on ice.

7. In cold room, pour supernatant into newly labelled tubes slowly. If there are several particles poured into the new tube, it should be centrifuged again for 3minutes.

Place tubes on ice.

8. Label luminometer cuvettes with at the top rim only (lower down will block light emission).

9. Add 50ul of extract into each cuvette. Leave at room temperature 10minutes to briefly warm up the samples. **You must be very efficient after this point.**

10. Immediately, remove frozen luciferase assay reagent (LAR) from -80C freezer. (this contains the luciferase substrate, luciferin, plus a buffer and cofactors such as ATP, all of which are unstable). *There is batch to batch variation of LAR, so make sure to use the same batch only*. Each tube contains 1ml, enough for measuring 5 samples.

Place tubes in 37C water bath for 9-10minutes to thaw, then at room temperature.

11. While waiting, turn on luminometer and set to: 25C, 10s, Integ.. The luminometer will count photons for a period of 10seconds and integrate the total output. Without any samples, but just an empty cuvette, press start and record this blank. It should be less than 20counts ---it it’s more, then tell Manish --the rubber 0-ring probably needs to be replace as it is letting in too much light.

12. At the 10minute mark, add 200ul of LAR into each cuvette containing 50ul of extract, pipet up and down 3X rapidly and quickly measure. Record the stabilized measurement and quickly move on to the next sample.

13. At the end, remember to turn off the power.

14.The **luminometer cuvettes** can be reused, BUT ONLY if they have been thoroughly soaked and rinsed to remove residual protein. So immediately pour out and rinse a few times in water, then let soak overnight in water, rinse again a few times, spray with ethanol and let dry upside down on paper towels. If the cuvettes are scratched or look old, throw them away.

15. Proceed with with the Bradfords (see other protocol) or freeze. With the embryo or aleurone samples, 1.5ul of sample may not be enough: if the samples are not turning blue, use your own judgement and use more extract, and of course, record the amount(s) you use.