**MASS CLONAL PROPAGATION OF ARABIDOPSIS ROOTS**

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* This protocol (Czako, M. & Marton, L.; manuscript in preparation) has been developed for the 'RLD' ecotype, both wild-type and transgenic, but has also been successfully used for the Columbia ecotype and its NR deficient mutant G-5. We have maintained one of the cultures for 18 months and others for more than a year.

1. **MEDIUM**
   (i) **VITAMIX** (500X stock solution) 100 ml-1
   - Vitamin B1 500 mg
   - Vitamin B6 50 mg
   - Glycine 100 mg
   - Nicotinic acid 50 mg
   - Folic acid 25 mg
   - Biotin (Vitamin H) 50 mg

   (ii) **ARABIDOPSIS ROOT CULTURE MEDIUM (ARC)** 1000 ml-1
   - MS salts 4.3 g
   - Miller's solution [6% (w/v) KH2PO4 stock solution] 3 ml
   - myo-inositol 200 mg
   - Vitamix stock 2 ml
   - Sucrose 30 g
   - pH 5.8 (1 M NaOH)
   * Dispense liquid in 25 ml aliquots into 125 ml Erlenmeyer flasks.

2. **PROCEDURE**
   (i) Inoculate 25 ml liquid MS or ARC medium with one or more seedlings or rooted shoots. Incubate at rt on an orbital shaker at approximately 70-80 rpm. They will develop an extensive root system in about two weeks.
   (ii) The roots are then excised and transferred into fresh ARC liquid medium and given a 2 d treatment with 0.05 mg-1 IAA.
   (iii) The roots are transferred to fresh ARC medium.
   (iv) The IAA treatment is repeated every 2-4 weeks.

3. **COMMENTS**
   (i) The sustained root culture has become hormone independent, because it has been growing without IAA treatment for months now.
   (ii) If the roots are chopped into smaller segments at the time of IAA treatment, they tend to form a tangle of old and fresh roots, from which the fresh roots are difficult to separate. If a 10-15 mm diameter size disc of the root mat is excised from the mass and transferred into fresh medium roots will grow out from the 'disc', and the fresh roots are easy to separate.
   (iii) These roots are suitable for transformation by our root transformation protocol, though the frequency of shoot regeneration is not as high as in the cold-stored seedling-derived roots (see Marton & Browse 1991, Plant Cell Reports, 10, 235-239), but still very good. So far that has been the only way of clonal (mass) propagation of some important (e.g., heterozygotes in our lab) material for transformation and biochemical analysis (J. Browse, WSU) purposes. A further improvement is on the way to restore or even to surpass the original morphogenic potential and therefore the transformation efficiency.