

Plant Transformation Project

At the beginning of the semester you sprouted tobacco seeds on MSO medium (no hormones). Since then small plants have grown in those dishes.

Today you will surgically remove leaves from several different plants, cut them down to about 1 cm pieces with exposed cut edges, and place them in a tube of growing *Agrobacterium tumefaciens*. These bacteria have been electroporated with plasmids that include the virulence genes, the Ti DNA borders, and two structural genes driven by the 35S promoter from the cauliflower mosaic virus. One structural gene is Phantastica in an antisense orientation (to silence native Phantastica expression), and the other is for gentamicin resistance. Maintaining these cultures as axenic is critical, so all surgical manipulations will be carried out in a sterile Petri dish and with flamed instruments.

After shaking the leaf explants in the bacterial shake for about 10 minutes, you will pour the shake into an empty sterile Petri dish bottom or cover. You will remove the explants with forceps into a plate of medium that you found gave good regeneration of plantlets in your tissue culture hormone project.

After 2 days, the explants will be moved to a medium containing Timentin to execute any remaining *Agrobacterium*.

Next week we will move the explants onto gentamicin medium to select for plantlets that are truly transformed. Any that produce roots in gentamicin medium would be considered likely to be transgenic. Verifying their transgenic status would require some other steps probably beyond the scope of this class.

However, with some luck we should be able to recover transgenic plants showing the phenotype expected for silencing of native myb genes. These include ectopic blade ridges along the midribs, etc.