BioISI - Biosystems & Integrative Sciences Institute

Construção de vectores de transformação genética para sobreexpressão e análise de perfis de expressão de genes envolvidos na produção de cortiça.

Place of work: *ForGen Lab – Forest Genomics and Molecular Genetics Laboratory* - Faculdade de Ciências da Universidade de Lisboa (FCUL)/ Instituto de Tecnologia Química e Biológica António Xavier (ITQB-NOVA); **Supervisors:** Célia Miguel (cmmiguel@fc.ul.pt FCUL), Ana Milhinhos (afmilhinhos@fc.ul.pt ITQB-NOVA)

The student will be part of a project that aims at understanding the role of previously identified genes during cork cambium formation and/or activity. The project has several tasks intertwined that will allow the student to have contact with different technologies used in any molecular genetics lab, including learning the basis of primer design, of molecular cloning and more specifically learn plant genetic transformation technologies. The student will be responsible for generating a set of expression vectors that will be used to transform *Arabidopsis thaliana*. The techniques used in the work plan include:

Task 1. Primers design

Oligonucleotides for amplifying the genomic sequences of the genes of interest and promoter regions will be designed using Primer3 software tool. Optimization of PCR conditions to amplify the candidate genes will be ensued.

Task 2. Plasmid construction

Constructs for reporter gene constructs and overexpression will be generated by cloning the genomic sequence of the promoter and corresponding candidate genes, which will be fused in-frame to a reporter tag (for overexpression under CaMV 35S promoter), in a binary based destination vector, through Gateway recombination. Colony PCR and Sanger sequencing will be used for selection of positive clones.

Task 3. Genetic transformation of *Agrobacterium*

Generated plasmids in *E.coli* will be transfected into *Agrobacterium* to use in plant transformation.

Task 4. Genetic transformation and/or selection of transformant seedlings of *Arabidopsis*

Arabidopsis plants will be transformed with Agrobacterium carrying the constructs of interest through the floral dip method. *Agrobacterium* will be applied to *Arabidopsis* plants at a flowering stage and the generated transgenic plants will be selected among the progeny seedlings.

Task5. Analysis of the expression patterns of the *Arabidopsis* GFP/GUS reporter lines generated.

The *Arabidopsis* plants generated will be analysed under confocal microscopy and real-time qPCR as to the isolated and cloned genes expression profiles *in vivo* and *in planta*; to understand the role of the candidate genes in suberization and cork formation in plants.