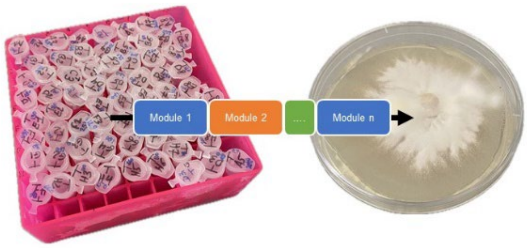
MoClo 4 Phanero



Production of MoClo (Molecular Cloning) vectors for the genetic transformation of *Phanerochaete chrysosporium*

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Thematic action concerned: *WP1*

Context —
The lack of genetics tools limits experimental strategies for studying *Basidiomycota*, and in particular for studying wood-decaying white-rot fungi. This technological bottleneck is a particular obstacle to molecular approaches in wood and forest science, as the main fungi that support the functioning of forest ecosystems or threaten the wood industry belong to the *Basidiomycota* phylum. Recent experiments at UMR IAM have enabled us to genetically transform the white-rot fungus *Phanerochaete chrysosporium*, using MoClo (Molecular Cloning) vectors and Agrobacterium-mediated transformation. These results are a proof of concept: the combination of custom-designed binary vectors and agro-transformation can generate stable transgenic lines in *Basidiomycota*.

Objectives —
The "MoClo 4 Phanero" project proposes to capitalize on this recent success, by developing a complete collection of vectors in the form of a set of modular elements to be assembled specially dedicated to the study of basidiomycetes, following the example of recent MoClo kits made available to other research communities studying plants, bacteria or *Ascomycota*.

Approaches —
The first stage of the project involved the identification of sequences of interest (promoters, 5' untranslated sequences, addressing peptides, tags, 3' untranslated sequences, terminator sequences) absent from collections already available. This step is followed by the production of these sequences and their cloning into a modular vector system. Thanks to the assembly of modular system elements, the number of possible transcriptional units grows exponentially. These transcriptional units are then combined into a binary vector for Agrobacterial transformation of fungi. Binary vectors are produced on a custom basis, depending on the biological question, and at least contain a gene of interest and a selection gene to identify the transformed fungi.

Key results —
- Three new resistance cassettes for the selection of transformation events are available:

Carboxin resistance,
Hygromycin resistance,
Itraconazole resistance.

- In order to study the localization of proteins of interest, 6 modules have been created and should enable the production of as many markers for cellular compartments.
- A series of modules is currently being produced to enable protein purification from transformed mushrooms.
- Intended for the transformation of *Phanerochaete chrysosporium*, the vectors already produced have enabled the transformation of *Trametes versicolor* and *Fomitiporia mediterranea*. Trials will soon be carried out for the fungus *Laccaria bicolor*.

Perspectives —
Ultimately, we will have a set of modules to enable the construction of binary vectors containing constructs optimized for expression in *Basidiomycota*. These vectors will enable us to:
- Study the phenotypic effects of ectopic expression of a gene of interest,
- Study the subcellular localization of a protein of interest.
- Produce recombinant proteins in fungal systems.