



# Oligonucleotide Array Design for Identification and Detection of *Penicillium* Subgenus *Penicillium* using *CO1* DNA Barcodes

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This project is to demonstrate that cytochrome oxidase 1 (*CO1*) barcodes can be used to design a DNA array hybridization system to detect a mixture of species from the environment.

## Why *Penicillium* subgenus *Penicillium*?

- Availability of the *Penicillium CO1* database, representing 58 species of *Penicillium* subgenus *Penicillium* and 12 outgroup species (Figure 1, Seifert *et al.*, 2007. PNAS 104: 3901-3906).
- This is a very challenging taxonomic group. Need to develop a new oligonucleotide array with information from a second gene to allow detection of some species that are missed by the  $\beta$ -tubulin array previously developed (Seifert *et al.*, in prep).
- Feasibility of amplification of labelled PCR products and alignment of *CO1* in *Penicillium*.

## What have we done so far? (started in March 2007)

- Two software applications, the custom designed Sigoli and the commercially available Array Designer, were used to design oligonucleotides of various specificity levels from the *Penicillium CO1* sequence database.
- Oligonucleotides of *Penicillium CO1* were designed to achieve specificity at each cluster and sub-cluster of the *CO1* tree, *i.e.*, matching all the strains within a cluster while minimizing potential cross-hybridization patterns outside the cluster.
- A total of 181 oligos (20-41 bp in length) were selected and the BLAST analyses against 358 available *Penicillium CO1* sequences confirmed that these potential oligos were unique within each target clade (Figures 2 and 3).

## What's next?

- Synthesize selected oligonucleotides and 'micro-spot' onto membranes to create the first generation *CO1* array.
- Amplify and label the *CO1* region corresponding to the oligos on the array from DNA samples originating from pure cultures or previously characterized environmental samples.
- Validation will be done by hybridizing these labeled PCR products to the *CO1* DNA array and comparing hybridization data of each oligo with the *in silico* results presented here.

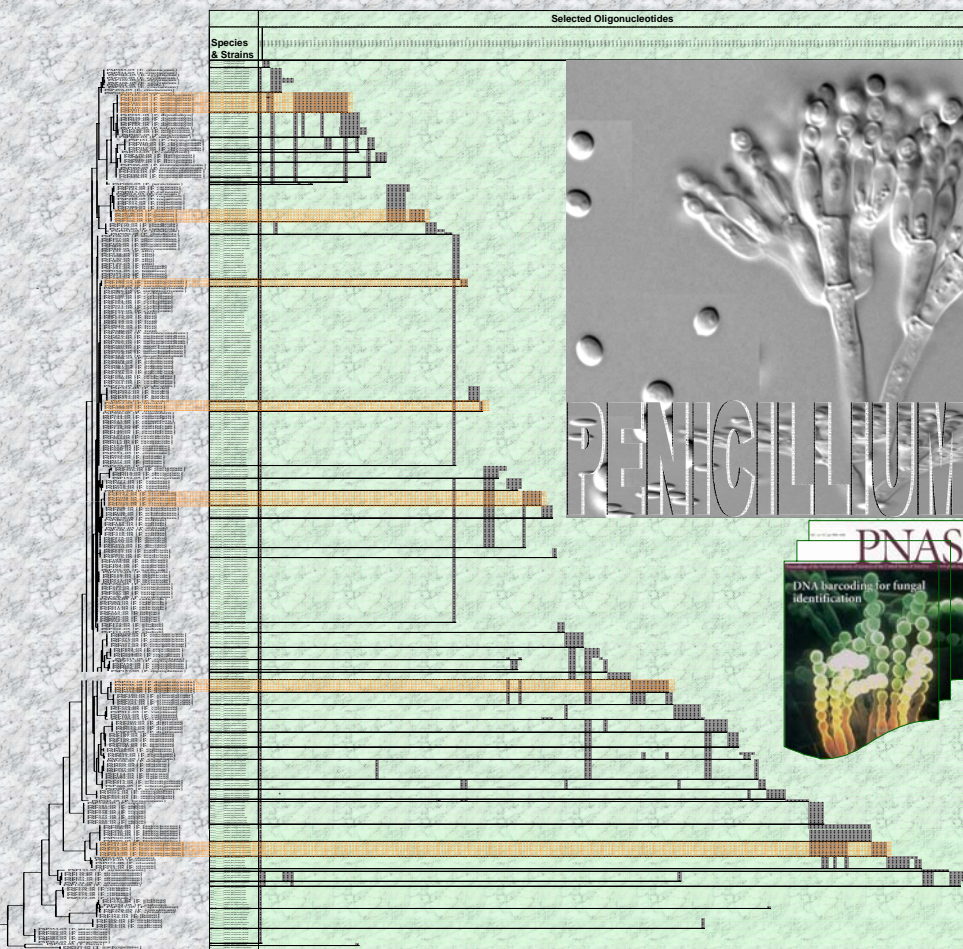


Figure 1. Neighbour-joining (NJ) tree of subgenus *Penicillium* and related species (Seifert *et al.*, 2007).

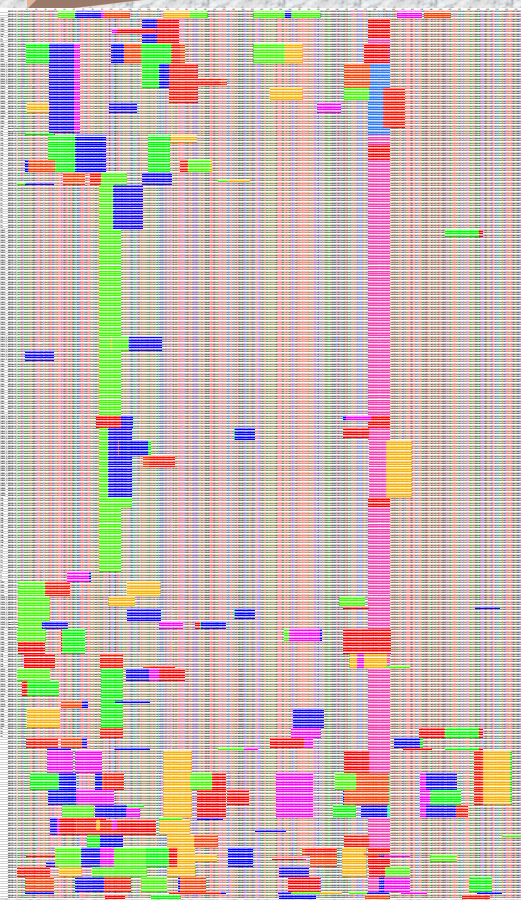


Figure 2. The *in silico* hybridization patterns for the *CO1 Penicillium* array for each selected oligonucleotide (horizontal) and different isolates of subgenus *Penicillium* and outgroup species. Both oligonucleotides and species are in the same order as the NJ tree.

Figure 3. The sequence position of each selected oligonucleotide in the *CO1* DNA sequence alignment of subgenus *Penicillium*.