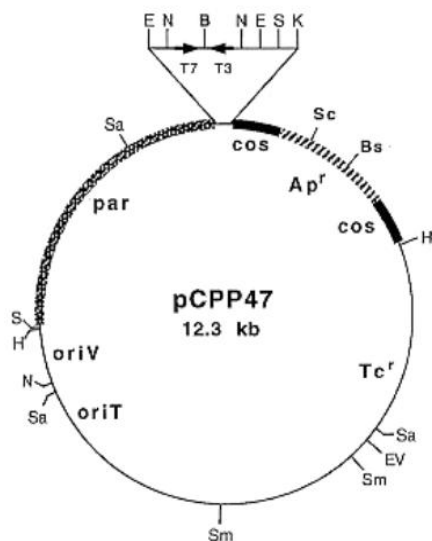


**USDA-ARS Root Disease and Biological Control Research Unit
Thomashow group**

P.fluorescens Q8r1-96 genomic library.

We are using an ordered library of Q8r1-96 genomic DNA in the cosmid vector pCPP47(1), which contains a *par* locus and is stable in strain Q2-87 in the absence of selection.



This library consists of 1536 clones with inserts averaging 30 kb in size, and for the estimated 6-Mb genome of Q8r1-96 has a 99.9% probability of containing a given DNA sequence. The library is suitable for both PCR- and hybridization-based screening. For PCR screening, the library was divided into 16 "primary" pools (i.e. all clones from a defined microtiter plate) that were further divided into a series of "secondary" pools (containing all clones in the same row or column from a defined microtiter plate). Positive primary pools are subsequently analyzed by row and column PCR, enabling us to pinpoint specific clones.

For screening by hybridization, the library was arrayed on sets four, 7.4 x 11.4-cm nylon membranes in a 386-sample format that allows one to screen 1536 clones (4 membranes) in a single experiment. The clones were replicated manually from glycerol stocks onto membranes with a 96-pin Multi-Blot Replicator (1.58 mm diameter solid pins, 0.2 ml delivery per sample) (V&P Scientific, Inc., San Diego, DA) and a library copier that permits arraying in a 386-sample format. These membranes can be repeatedly hybridized with ³²P-labeled probes.

References

- Bauer, D. W., and A. Collmer. 1997. Molecular cloning, characterization, and mutagenesis of a *pel* gene from *Pseudomonas syringae* pv. *lachrymans* encoding a member of the *Erwinia chrysanthemi pelADE* family of pectate lyases. *Mol. Plant-Microbe Interact.* 10:369-379.

