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A LIVING COLOR TAGGED PLASMID FOR ANALYSING HORIZONTAL GENE TRANSFER

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Genetic transfer in ecosystems usually occurs as a rare event. Therefore it can be difficult and time consuming to select transconjugants by using markers like antibiotic or heavy metal resistance. Additionally a lot of environmental micro-organisms are known to possess resistances against these markers or live under conditions where antibiotics do not work.

Therefore we are searching for a permanent living marker like Green Fluorescent Protein to tag the degradative plasmid pJP4. The 80 kb plasmid pJP4 was isolated in 1981 by Don and Pemberton as a 2,4-D converting broad host range plasmid with a catabolic operon of 22 kb fully expressed only in *Ralstonia eutropha*. The Hg-resistance gene outside the catabolic region is active also in other species. While the catabolic operon is well characterized, little is known about other regions outside the operon. Using the suicide plasmid pAG408 in transposon mutagenesis experiments we got different hints on promoter regions at the plasmid pJP4 by insertion of the promoter-less *gfp*. One of these regions was located out of the catabolic operon and was therefore further analyzed. The insertion was shown to be without any influence on the expression of the 2,4-D pathway in *Ralstonia eutropha* by RTPCR analysis. The tagged pJP4-promoter was active not only in *Ralstonia eutropha* but also in *E. coli* and different haloalkaliphilic transconjugants like *Halomonas* and *Pseudomonas*. Our studies yielded permanent glowing transconjugants with this *gfp*-tagged pJP4 for use in further genetic transfer experiments and for single cell analysis. Now we are searching for the sequences of the tagged promoter and the gene(s) connected with it.

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