

Construction of a set of vectors allowing inducible production of siRNA in *Schizosaccharomyces pombe*.

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RNA interference (RNAi) is a sequence-specific gene silencing mechanism. It is induced by the formation of dsRNA that are recognised by the Dicer complex and processed into 21-23 long oligonucleotides called siRNA (short interfering RNA). Subsequently, RISC (RNA-Inducing Silencing Complex) binds siRNA that targets the complex towards its homologous mRNA (DYKXHOORN *et al.*, 2003) which is eventually degraded.

In contrast to budding yeast, the entire pathway is conserved in the fission yeast *Schizosaccharomyces pombe*, making it a valuable organism to both study physiological RNAi and to use it as a inducible gene knock-down tool.

In an attempt to apply this method in the fission yeast, we are using three different approaches to produce siRNA. In each case, a vector containing a regulatable promoter activated in presence of tetracycline (tTA') (GOSSEN *et al.*, 1995) is generated and the *ura4* marker required for growth on medium lacking uracil serves as reporter. First, a vector expressing the full length antisense RNA of *ura4* (800 nucleotides) (RAPONI and ARNDT, 2003) is used. Second, we are trying to generate much shorter dsRNA where both strands are linked by either a short hairpin of 25 nucleotides (BRUMMELKAMP *et al.*, 2002) or a longer one of 350 nucleotides (SCHRAMKE and ALLSHIRE, 2003). The ability of these different dsRNA to induce silencing of *ura4* will be presented.

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