

## Detection for quorum sensing inhibitor: *in vitro* Dimerization of TraR proteins and its use as screening system of AHL antagonist

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### Abstract

Acylhomoserine lactones (AHL) are known to be the triggering molecules in the quorum sensing mechanism of many gram-negative bacteria. In order to detect AHL inhibitors that are potential biofilm inhibitors, a sensitive *in vitro* bioassay was developed based on a transcriptional activator protein, TraR from *Agrobacterium tumefaciens*. Two TraR and cognate AHL complexes form a dimer to function as the QS specific transcriptional activator. The protein was produced in a His<sub>6</sub> tagged form in recombinant *E. coli* strain harboring the corresponding gene and purified using a Ni<sup>2+</sup> affinity column. Dimerization of the protein-signal complexes was measured with size exclusion chromatography based on the size difference between the monomer and dimer. When tested against several AHLs, the complexes exhibited an excellent dimerization activity. To test the *in vitro* dimerization system, fimbrolide, a competitive inhibitor of AHL, was applied to the system and a strong inhibition on the TraR dimerization was confirmed.

### Reference

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