

## Fusion Reporter Approaches to Monitoring Transmembrane Helix Interactions in Bacterial Membranes

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### Abstract

In transenvelope multiprotein machines such as bacterial secretion systems, protein–protein interactions not only occur between soluble domains but might also be mediated by helix–helix contacts in the inner membrane. Here we describe genetic assays commonly used to test interactions between transmembrane  $\alpha$ -helices in their native membrane environment. These assays are based on the reconstitution of dimeric regulators allowing the control of expression of reporter genes. We provide detailed protocols for the TOXCAT and GALLEX assays used to monitor homotypic and heterotypic transmembrane helix–helix interactions.

**Key words** Membrane protein, Protein–protein interaction, Transmembrane segment, Helix–helix interaction, One-hybrid, Two-hybrid, cI repressor, TOXCAT, GALLEX

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### 1 Introduction

The proper assembly of multiprotein complexes such as bacterial secretion systems requires specific interactions between the different subunits. While most of the interactions involve contacts between soluble domains of these subunits, the transmembrane helices (TMHs) of inner membrane proteins are also key players in membrane protein complex formation. For examples, the Type II secretion (T2SS)-associated GspC, GspL, and GspM proteins interact with each other via their TMHs [1]. A similar situation has been evidenced for the Type VI secretion system (T6SS) TssLM complex [2–4]. The TMH could be involved in homotypic interaction, i.e., participate in the formation of dimers such as the Type IV secretion (T4SS) and T6SS-associated VirB10 and TssL inner membrane proteins [4, 5] or in heterotypic interactions with other subunits [1–3]. Monitoring interactions between TMHs is not an easy task because mutations within or swapping of the TMH could interfere with the conformation of the soluble domains and therefore may indirectly affect protein–protein interactions. Thus, genetic one- or two-hybrid approaches based on fusion to transcriptional