S021 DUALmembrane Plus Service Description

DUALmembrane Plus screening for protein interactions involving integral membrane proteins and membrane-associated proteins

What is the DUALmembrane system?

The DUALmembrane system is a genetic assay to identify interactions between an integral membrane protein or a membrane-associated protein and

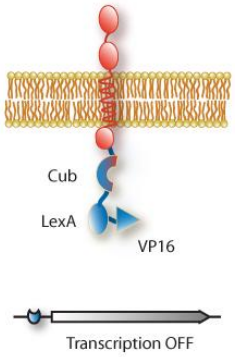
- other integral membrane proteins
- other membrane-associated proteins
- other soluble proteins

The DUALmembrane system is based on the split-ubiquitin system originally developed by Nils Johnsson and Alexander Varshavsky in 1994. It has the main advantage of detecting interactions *in situ* directly at the membrane.

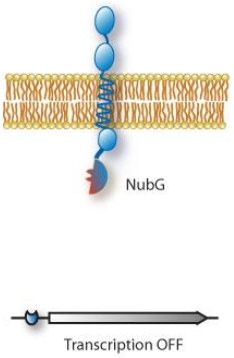
Why use a DUALmembrane Plus screen?

- Profit from a complete offer: The DUALmembrane Plus service includes bait design and cloning, a functional assay of the bait, the library screen in the most appropriate screening conditions, and the analysis of all prey clones.
- Choose a protein of interest and a cDNA library to screen and supply us with the necessary information using the DUALmembrane Plus Screen Request Form.
- We carry out the entire screen, including functional analysis of the bait, toxicity test, library transformation and analysis of up to 384 positive clones from the screen.
- You receive a detailed report with 5' and 3' sequences, BLAST analysis of all preys, and delineation of the interaction domains.

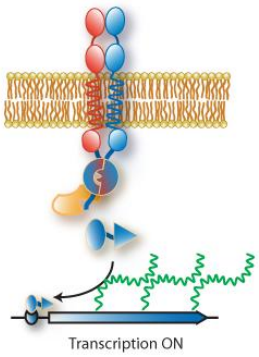
The DUALmembrane principle



A bait is constructed by fusing an integral membrane protein to the C-terminal half of ubiquitin (Cub) and a transcription factor (LexA-VP16). The transcription factor is immobilized at the membrane and cannot reach the nucleus to activate the reporter genes.



A prey is constructed by fusing a second protein to the N-terminal half of ubiquitin (NubG).



Interaction of bait and prey at the membrane leads to reconstitution of split-ubiquitin through the interaction of Cub and NubG. Split-ubiquitin is cleaved by proteases to release the transcription factor, which enters the nucleus and activates the reporter genes. Thus, an interaction between two proteins at the membrane leads to growth of the yeast harboring the interaction.

How does a DUALmembrane Plus screen work?

Part I: Bait construction and analysis

- We obtain the cDNA encoding your protein of interest from you and subclone your protein of interest into the bait vector of your choice
- A functional assay is performed to ensure that the bait is properly located within the membrane and is functional in the DUALmembrane assay
- The bait is transformed into yeast and tested for toxicity. The autoactivation properties of the bait are also tested and the most appropriate selective pressure is determined.

Part II: Library screen and prey analysis

- The bait is screened by sequential transformation with a cDNA library of your choice
- Up to 384 prey clones are picked up and arrayed
- You receive a complete report including raw 5' and 3' prey sequences, prey identification by BLAST analysis and prey interaction domains.
- All results and any derived intellectual property fully belong to you

How much time does a DUALmembrane Plus screen take?

- Part I 4-6 weeks
- Part II 8-10 weeks

Literature: examples of successful DUALmembrane screens

- Matsuda *et al.* (2005) **JBC** 280, 28912-28916
- Vitale and Buxbaum (2004) **Biol. Bull.** 207, *167*
- Thaminy *et al.* (2003) **Genome Res.** 13, 1744-1753

Ordering information

Order number	Custom service
P021	DUALmembrane Plus custom screening service

Version 2009