

DUALhybrid System

Vector selection guide

Available DUALhybrid vectors

In the classical yeast two-hybrid system, baits are expressed as fusions to the C-terminus of a DNA binding domain (DBD), for example those derived from the yeast GAL4 protein or from the *E. coli* LexA repressor protein.

By cloning the cDNA encoding your protein of interest into a classical bait vector, you create a DBD-bait fusion protein (Figure 1A). The DBD locates your bait to the promoter region upstream of the reporter genes present in the yeast genome by binding to specific sites (a GAL4 upstream activating sequence or a series of LexA operator sites), leaving your bait free to interact with any binding partners present in the yeast nucleus.

The DBD-bait orientation is the classical orientation and is used in the overwhelming majority of all yeast two-hybrid screens. If you have no data indicating that the N-terminus of your protein of interest is involved in crucial interactions, we recommend to use this orientation. The corresponding vector to clone your cDNA into is pLexA-dir.

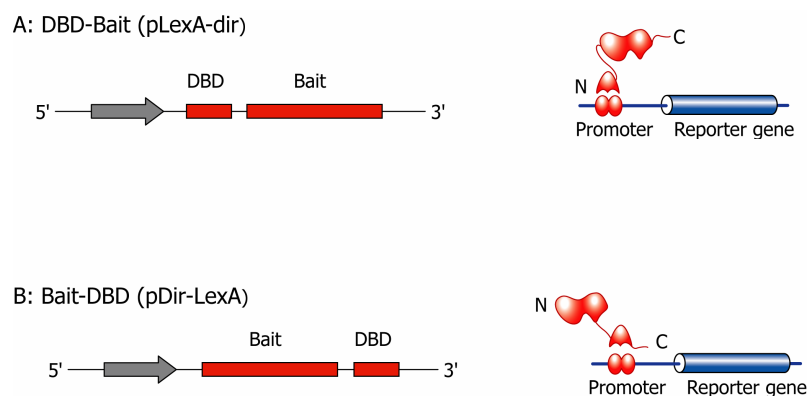


Figure 1

However, there may be some baits where you would like to avoid a fusion to the N-terminus. For instance, you may suspect that the N-terminus is involved in interactions and you are afraid that fusing the DBD to it may block interactions. Alternatively, a free N-terminus may be crucial for stability or proper folding of the bait. Maybe you have already tried a yeast two-hybrid screen with your protein of interest in a DBD-bait orientation and found no interactors.

In these cases, we recommend that you try a bait-DBD fusion by cloning your cDNA into pDir-Lex (Figure 1B).

Most importantly, switching the orientation of **DBD** and bait may help to avoid self-activation issues. A bait is self-activating when it activates the reporter genes on its own (in the absence of a protein-protein interaction). Transcriptional activators or proteins involved in DNA replication or repair are often self-activating in the yeast two-hybrid system, but even proteins that are not normally found in the nucleus may turn out to be self-activating. The regions responsible for this activation are not well defined, but it is generally thought that stretches of acidic residues or amphipathic helices may add to the propensity of a protein to non-specifically activate transcription. Commonly, around 25% of all baits turn out to be self-activating when first tested in a yeast two-hybrid assay.

We have found that switching the position of the **DBD** in a self-activating bait may reduce or sometimes even abolish its self-activating ability. Often, self-activation can be reduced to a point where it becomes possible to carry out a library screen. This procedure is often better than modifying the bait by N- or C-terminal truncation since you do not lose any potential binding regions and avoid the risk of constructing a bait that does not fold properly anymore.

If you have further questions or would like advice on how to clone your proteins, please visit our support section at <http://www.dualsystems.com> or contact us at support@dualsystems.com. We are happy to answer your questions.

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