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Bacterial two-hybrid assay reagents

Strain and plasmids

Strain	**Relevant Details**	**Reference/Source**
FW102 O_L2–62	Bacterial-two hybrid reporter strain. FW102 containing an F' Kan bearing the p*lac*O_L2–62-*lacZ* fusion where the λCI operator is centered at position –62 upstream of the transcription start site	Deaconescu et al. 2002
Plasmids	**Relevant Details**	**Reference/Source**
pACλCI	P_{lacUV5}-directed synthesis of the λCI protein	Dove et al. 1997
pACλCI-β-flap (831-1057)	P_{lacUV5}-directed synthesis of the λCI protein fused via three alanines to residues 831-1057 of the β subunit of *E. coli* RNAP	Deighan et al. 2008
pBRα	P_{lacUV5}/P_{lpp}-directed synthesis of the full length α subunit of *E. coli* RNAP	Dove et al. 1997
pBRα-σ^{70} D581G	P_{lacUV5}/P_{lpp}-directed synthesis of the αNTD (residues 1-248 of the α subunit of *E. coli* RNAP) fused directly to *E. coli* σ^{70} region 4 (residues 528-613 of σ^{70}). The σ^{70} moiety also carries the D581G substitution.	Kuznedelov et al. 2002
pBRα-β flap (831-1057)	p*lacUV5*- and p*lpp*-directed synthesis of the αNTD (residues 1-248 of the α subunit of *E. coli* RNAP) fused via three alanines to residues 831-1057 of the β subunit of *E. coli* RNAP.	Deighan et al. 2008

User notes

- pACλCI-β-flap (831-1057) + pBRα-σ^{70} D581G = a positive control (about 800 MU β-gal activity in our hands with 20 μM IPTG)
- pACλCI-β-flap (831-1057) + pBRα = a negative control (about 80 MU β-gal activity in our hands with 20 μM IPTG)
- your pBRα fusion protein plasmid + pACλCI = negative control 1
- pBRα + your pACλCI fusion protein plasmid = negative control 2
- pBRα-β flap (831-1057) and pACλCI-β-flap (831-1057) are the 'source plasmids'- digest these with NotI-BamHI to generate a NotI-BamHI backbone for cloning your gene (gene fragment) of interest: The β-flap fragment (831-1057) is 682bp.
- For the positive control we routinely grow the overnight cultures with 20 μM IPTG and use 20 μM IPTG in the sub-cultures. However, some fusion proteins may be toxic; for these we suggest growing the overnight cultures without IPTG and testing various IPTG concentrations (up to 200 μM IPTG) in the sub-cultures.
- NotI is a 8bp cutter 5'-GCGGCCGC-3'. Since your protein of interest is going to be on the C-ter of the fusion proteins add an extra base onto the NotI site to maintain the reading frame (e.g., 'A'). This new NotI site, GCGGCCGCA, is translated into a 3 x Alanine linker.

- The forward primer for cloning by PCR would look like: **5'**-gaagGCGGCCGCA_your seq in frame-**3'**
- If cloning by PCR the reverse primer should incorporate a stop codon, and a BamHI recognition site (BstYI or BglII are also compatible and can be cloned into BamHI-digested pACλCI-β-flap or pBRα-β flap backbones). The reverse primer would look like: **3'**-your seq_stop codon_GGATCC_gaag-**5'**

Sequencing primers

- pBRa_F (approx 150bp upstream of NotI site in the pBRα-Y vector)
  5'-gaacagcgtaccgacctggac-**3'**
- pAC-cI_F (approx 100bp upstream of NotI site in the pACλCI-X vector)
  5'-gatcagggatagcggtcagg-**3'**

References

Dove, S. L., Joung, J. K., & Hochschild, A. (1997) Activation of prokaryotic transcription through arbitrary protein-protein contacts. *Nature* 386**,** 627-630

Deaconescu, A. M., Chambers, A. L., Smith, A. J., Nickels, B. E., Hochschild, A., Savery, N. J., & Darst, S. A. (2006) Structural basis for bacterial transcription-coupled DNA repair. *Cell* 124**,** 507-520

Deighan, P., Diez, C.M., Leibman, M., Hochschild, A., and Nickels, B.E. (2008) The bacteriophage λ Q antiterminator protein contacts the β-flap domain of RNA polymerase, *PNAS* 105: 15305-15310

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