Mammalian Two-Hybrid Assay Kit

INSTRUCTION MANUAL

Catalog #211344 (Mammalian Two-Hybrid Assay Kit), #211342 (pCMV-BD Vector), and #211343 (pCMV-AD Vector)
BN #211344-12
Revision #103002i

For In Vitro Use Only

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Mammalian Two-Hybrid Assay Kit

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Mammalian Two-Hybrid Assay Kit

MATERIALS PROVIDED

Materials provided[a]	Quantity		
	Catalog #211344	Catalog #211342	Catalog #211343
Vectors (1 µg/µl in TE buffer)			
pCMV-BD, supercoiled	20 µg	20 µg	—
pCMV-AD, supercoiled	20 µg	—	20 µg
Control plasmids (1 µg/µl in TE buffer)			
pBD-p53, supercoiled (positive interaction with SV40T)	20 µg	—	—
pBD-NF–κB, supercoiled (positive fusion control)	20 µg	—	—
pAD-SV40T, supercoiled (positive interaction with p53)	20 µg	—	—
pAD-TRAF, supercoiled (negative interaction with p53)	20 µg	—	—
pFR-Luc reporter plasmid (1 µg/µl in TE buffer)	50 µg	—	—

[a] On arrival, store the plasmids at –20°C. For short-term storage, store at 4°C for 1 month.

STORAGE CONDITIONS

All Components: –20°C

ADDITIONAL MATERIALS REQUIRED

Mammalian cells (e.g., HeLa, CHO, CV-1, COS, 293 and NIH3T3)
Cell culture medium [e.g., Dulbecco's minimum essential medium (DMEM)]
DMEM containing 10% fetal bovine serum (FBS), 1% L-glutamine, 1% penicillin and streptomycin, and 50 µM β-mercaptoethanol
5-ml BD Falcon polystyrene round bottom tubes (BD Biosciences catalog #352054)
Calcium- and magnesium-free PBS
Tissue culture dishes (24-wells)
Transfection reagents
Luciferase assay buffer[§] containing luciferin
Luminometer

[§] See *Preparation of Media and Reagents.*

Revision #103002i

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INTRODUCTION

Background

Stratagene's Mammalian Two-Hybrid Assay Kit is a powerful method for detecting protein-protein interactions in vivo in mammalian cells. The two-hybrid assay kit utilizes hybrid genes to detect protein-protein interactions via the activation of reporter-gene expression. Stratagene's mammalian two-hybrid reporter plasmid, pFR-Luc (see Figure 5), contains a synthetic promoter with five tandem repeats of the yeast GAL4 binding sites that control expression of the *Photinus pyralis* (American firefly) luciferase gene. Luciferase reporter-gene expression occurs as a result of reconstitution of a functional transcription factor caused by the association of two-hybrid proteins.

In this assay, a gene encoding a protein of interest (protein X) is fused to the DNA-binding domain of the yeast protein GAL4 while another gene (protein Y) is fused to the transcriptional activation domain of the mouse protein NF-κB. These two-hybrid constructs are cotransfected into a suitable mammalian host cell line with the reporter plasmid. If protein X and protein Y interact, they create a functional transcription activator by bringing the activation domain into close proximity with the DNA-binding domain; this can be detected by expression of the luciferase reporter gene.

A major advantage of Stratagene's mammalian two-hybrid assay kit over yeast two-hybrid systems[1] is that protein-protein interactions are studied in mammalian cell lines. As a result, this assay kit enables researchers to study interactions between mammalian proteins that may not fold correctly in yeast or that require post-translational modification or external stimulation that is not present in yeast.

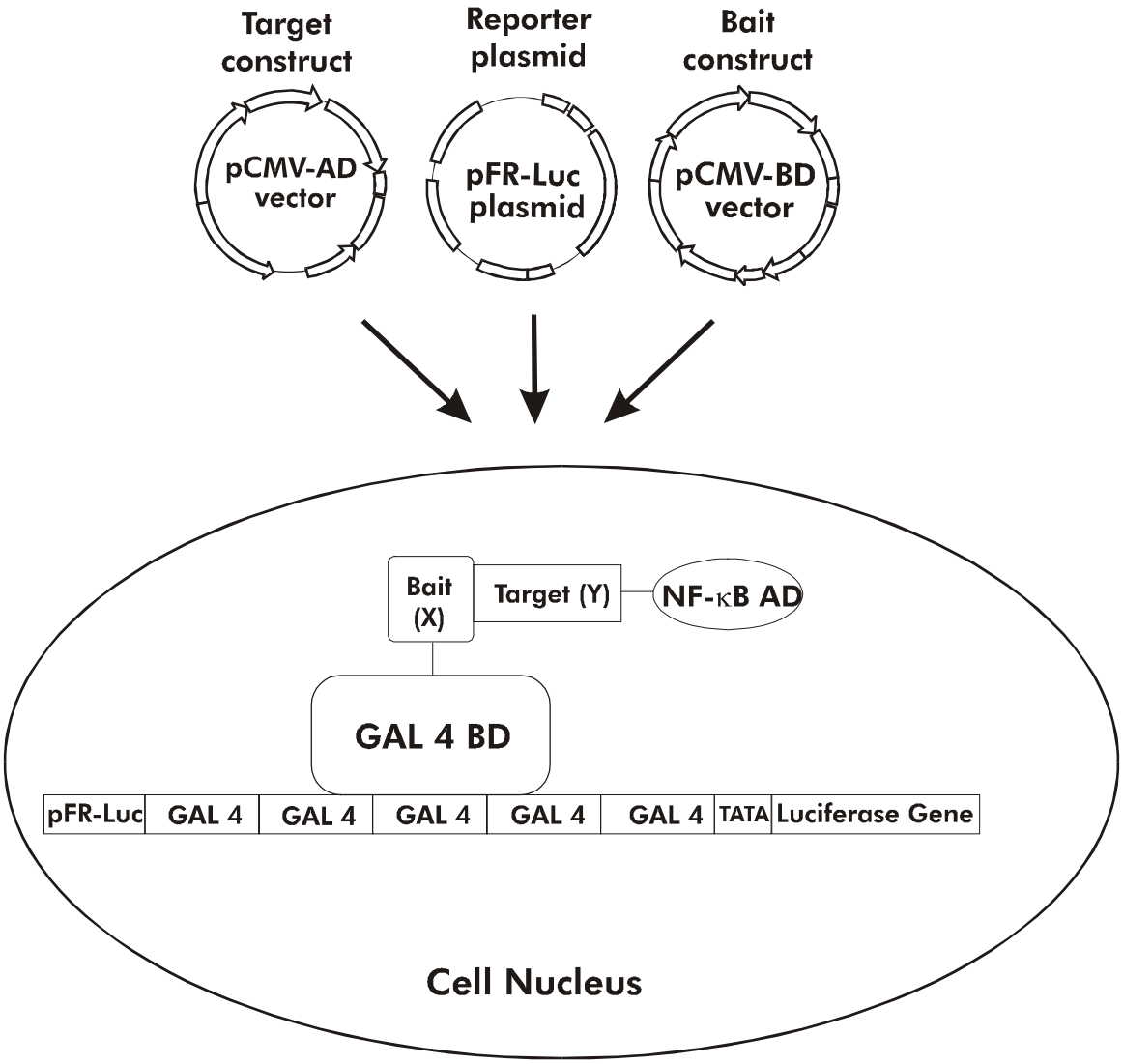


FIGURE 1 Schematic diagram of the Mammalian Two-Hybrid Assay Kit. Interaction between protein X (GAL4 BD-X fusion protein) and protein Y (NF-κB AD-Y fusion protein) brings the activation domain (AD) into close proximity with the DNA-binding domain (BD), resulting in activation of reporter gene expression through GAL4 binding sites.

PREPROTOCOL CONSIDERATIONS

Choosing a Cell Line

Stratagene's Mammalian Two-Hybrid Assay Kit may be used to screen for protein-protein interactions in various mammalian cell lines. The cells must not contain molecules or proteins that interfere with the interactions between the proteins of interest, and must contain proteins or enzymes that may be necessary for the interactions. Background expression levels of the luciferase reporter gene, and therefore the sensitivity of the assay, will vary in different cell lines. Although Stratagene's mammalian two-hybrid assay kit has been found to work in various cell lines including HeLa, CHO, COS, NIH 3T3, and 293; certain protein-protein interactions may be stronger in a particular cell line under defined conditions. Use the provided control plasmids to determine the optimal experimental conditions for the mammalian two-hybrid assay kit in your cell line.

Choosing a Transfection Method

As with all transfection assays, the sensitivity of an assay using the mammalian two-hybrid assay kit is greatly influenced by the transfection efficiency. High transfection efficiency generally provides a more sensitive assay that requires a smaller volume of sample. Transfection conditions should be optimized with a reporter plasmid before performing the assays. Sufficient quantity of plasmid is included for several optimization experiments.

Because the luciferase assay is very sensitive, various transfection methods, such as calcium phosphate precipitation and lipid-mediated transfection, may be used. Lipid-mediated transfection generally results in higher and more consistent transfection efficiency than other chemical methods in many cell lines.

Tissue Cultureware

The protocols given are based on 24-well tissue culture dishes with a well diameter of ~15 mm and a surface area of ~1.88 cm^2. When dishes with larger wells are used, increase the number of cells per well and the volume of reagents according to the surface area of the wells.

VECTORS

The pCMV-BD and pCMV-AD vectors are designed for the construction and expression of gene fusions with the GAL4 DNA binding domain and NF-κB transcriptional activation domain, respectively (Figures 2 and 3). The pCMV-BD vector contains DNA encoding amino acids 1–147 of the GAL4 gene (DNA binding domain)[2] and unique 3′ cloning sites. It is used for the construction of bait plasmids containing a DNA insert encoding a bait protein. The pCMV-AD vector contains DNA encoding the nuclear localization sequence (NLS) from SV40 large T-antigen (PKKKRKV), amino acids 364–550 of the mouse NF-κB gene (transcriptional activation domain)[3] and unique 3′ cloning sites. It is used for constructing a target plasmid containing a DNA insert encoding the interacting proteins. Both pCMV-BD and pCMV-AD contain the pUC origin for replication in *E. coli*. The pCMV-BD vector contains the kanamycin-resistance gene and the pCMV-AD vector contains the ampicillin-resistance gene. The constitutively-active cytomegalovirus (CMV) promoter governs the expression of the bait and target proteins in the pCMV-BD and pCMV-AD vectors. The SV40 poly (A) provide the signals necessary for transcriptional termination and polyadenylation of the bait and target genes in mammalian cells.

Cloning Considerations for the pCMV-BD Vector

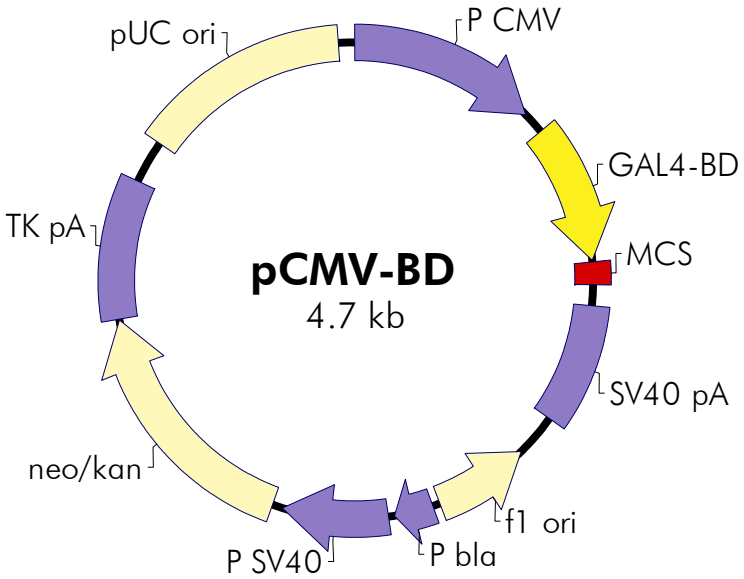
Figure 2 shows the multiple cloning site region of the pCMV-BD vector in frame with the GAL4-BD protein. In the absence of an inserted gene, there is no in-frame stop codon in this region. To minimize the addition of vector-derived amino acids at the C-terminus of the expressed protein, ensure that the inserted gene of interest contains in-frame stop codon(s).

Sequencing Primers

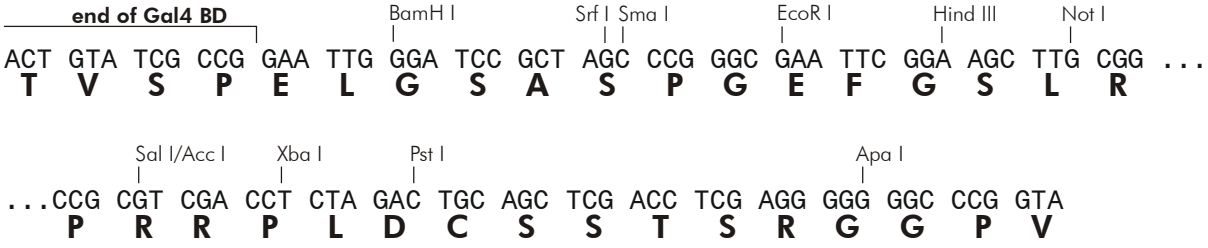
Sequencing Primers[a]	Bases	Sequence
BD 5′ primer	1045–1063	5′ AGC ATA GAA TAA GTG CGA C 3′
BD 3′ primer (T7 primer)	1231–1250	5′ TAA TAC GAC TCA CTA TAG GG 3′
AD 5′ primer	1192–1210	5′ GGA GAT GAA GAC TTC TCC T 3′
AD 3′ primer (T7 primer)	1364–1383	5′ TAA TAC GAC TCA CTA TAG GG 3′

[a] The 5′ primer is at the 5′ end of the MCS and 3′ primer is at the 3′ end of the MCS.

pCMV-BD Vector Map



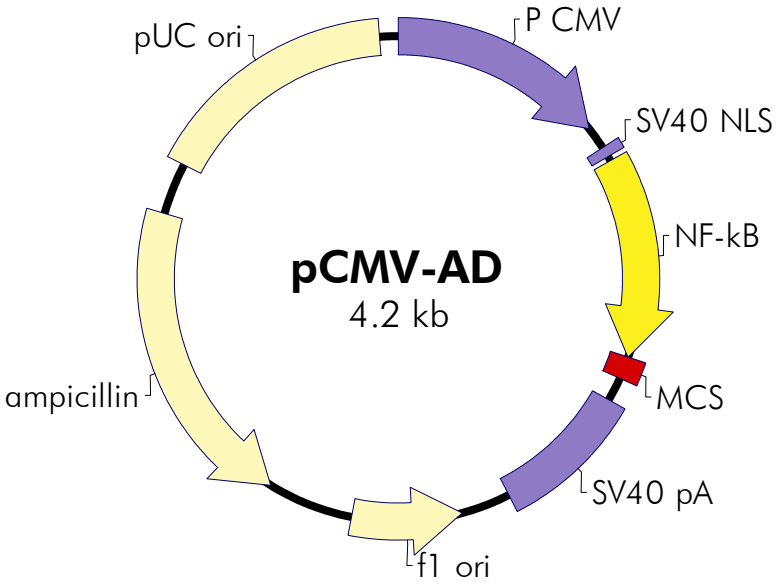
pCMV-BD Multiple Cloning Site Region
(sequence shown 1107–1208)



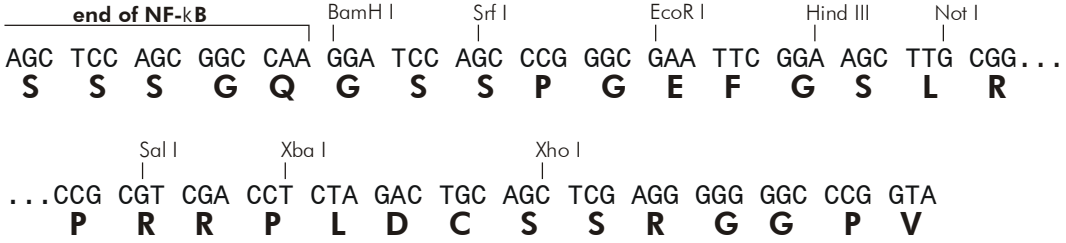
Feature	Nucleotide Position
CMV promoter	1–602
T3 promoter	620–639
GAL4 DNA-binding domain	675–1118
multiple cloning site	1125–1195
T7 promoter	1232–1250
SV40 polyA signal	1263–1646
f1 origin of ss-DNA replication	1784–2090
bla promoter	2115–2239
SV40 promoter	2259–2597
neomycin/kanamycin resistance ORF	2632–3423
HSV-thymidine kinase (TK) polyA signal	3427–3872
pUC origin of replication	4011–4678

FIGURE 2 Circular map and polylinker sequence of pCMV-BD vector.

pCMV-AD Vector Map



pCMV-AD Multiple Cloning Site Region
(sequence shown 1252–1341)



Feature	Nucleotide Position
CMV promoter	1–602
T3 promoter	620–639
SV40 nuclear localization signal (NLS)	667–688
NF-κB activation domain	709–1266
multiple cloning site	1267–1328
T7 promoter	1365–1383
SV40 polyA signal	1396–1779
f1 origin of ss-DNA replication	1917–2223
ampicillin resistance (*bla*) ORF	2464–3321
pUC origin of replication	3468–4135

FIGURE 3 Circular map and polylinker sequence of the pCMV-AD vector.

CONTROL PLASMIDS

Description

Stratagene's mammalian two-hybrid assay kit contains four control plasmids (Figure 4 and Table I). The pBD-NF-κB control plasmid expresses the GAL4 DNA binding domain and the transcription activation domain of NF-κB as a hybrid protein. The pBD-p53 control plasmid expresses the GAL4 binding domain and amino acids 72–390 of murine p53 as a hybrid protein.[4] The pAD-SV40T control plasmid expresses a hybrid protein which contains the NF-κB transcription activation domain fused to amino acids 84–708 of the SV40 large T-antigen.[5] The pAD-TRAF control plasmid expresses the NF-κB transcriptional activation domain and amino acids 297–503 of TRAF2.

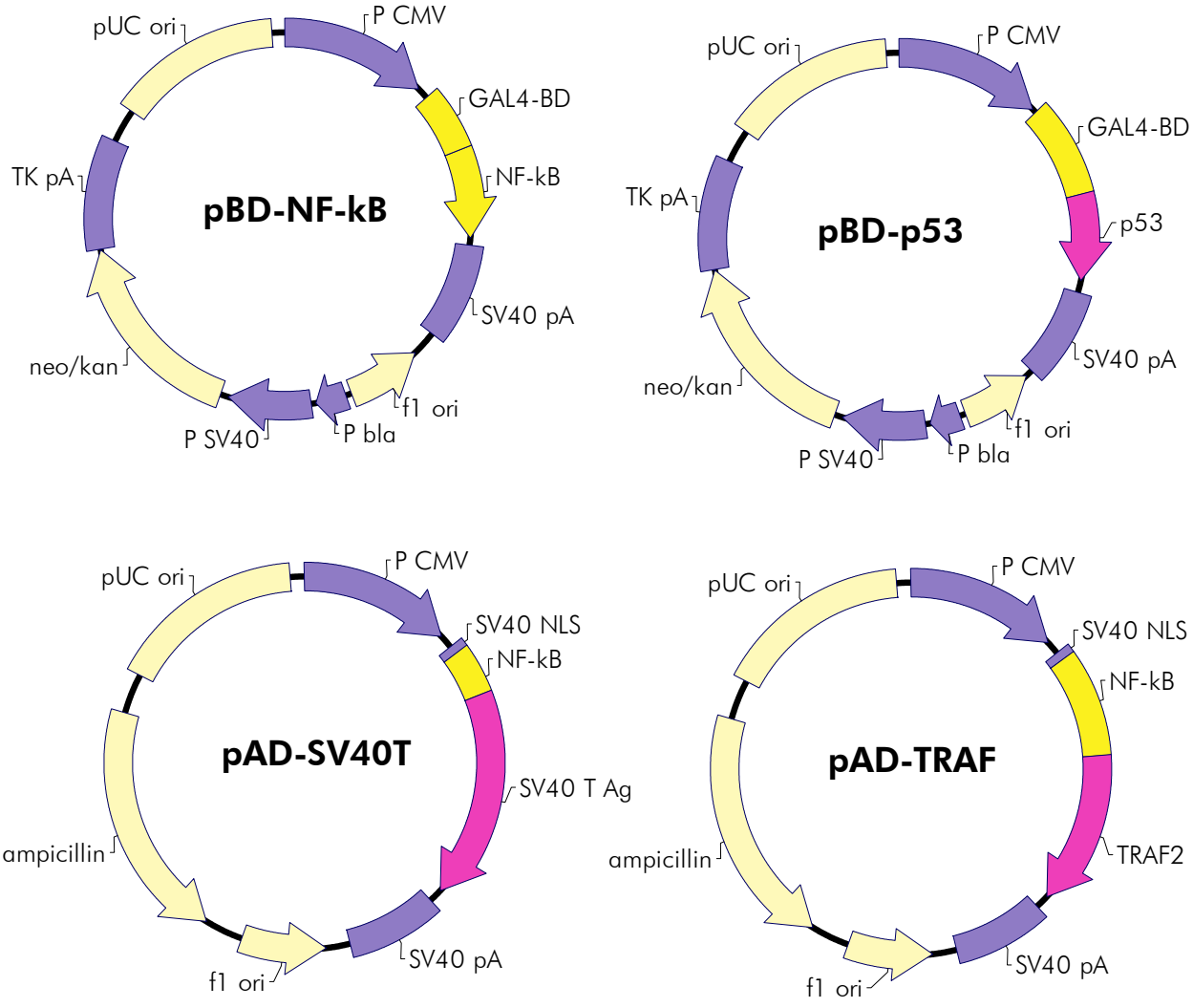


FIGURE 4 Circular maps of the pBD-NF-κB, pBD-p53, pAD-SV40T, and pAD-TRAF control plasmids.

TABLE I

Description of Control Plasmids

Control plasmid	Insert description	Genotype
pBD-NF-κB	NF-κB AD (aa 364–550)	Kan^r
pBD-p53	murine p53 (aa 72–390)	Kan^r
pAD-SV40T	SV40 large T-antigen (aa 84–708)	Amp^r
pAD-TRAF	TRAF2 (aa 297–503)	Amp^r

Applications

These plasmids are used alone or in pairwise combination as positive and negative controls for the induction and detection of the luciferase reporter gene. The pBD-NF-κB plasmid can be used to verify that induction of the luciferase reporter gene has occurred and that the gene products are detectable in the assay used. The pBD-p53 and pAD-SV40T control plasmids, whose expressed proteins interact in vivo, can be used to verify that induction of the luciferase reporter gene has occurred. The pBD-p53 and pAD-TRAF control plasmids, whose expressed proteins do not interact in vivo, can be used to verify that the luciferase gene is not induced in the absence of a two-hybrid interaction.

Expected Results

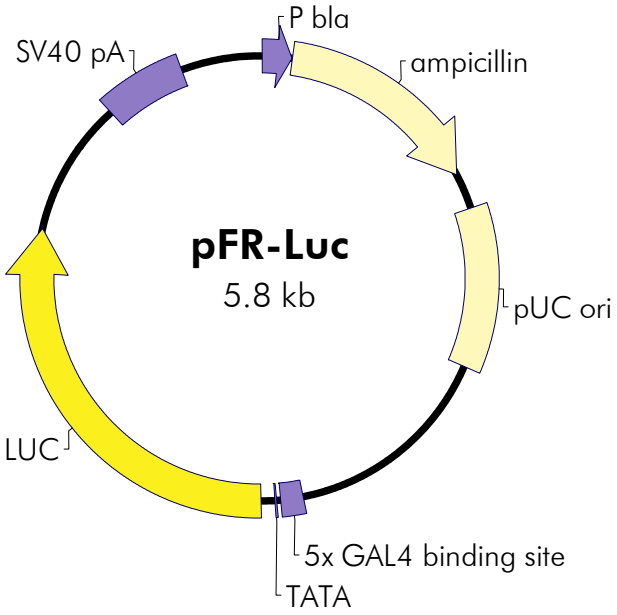
The expected results for the transfection of the control plasmids alone or in pairwise combinations with the pFR-Luc reporter plasmid into the cell line of choice (such as CHO or 293) and assayed for the luciferase gene expression, are outlined in Table II.

Table II

Expected Results for Interaction of Control Plasmids Cotransformed with pFR-Luc

Control Plasmids		Expected Results
BD fusion	AD fusion	
pBD-NF-κB	—	strong signal
pBD-p53	pAD-SV40T	positive interaction
pBD-p53	pAD-TRAF	negative interaction

REPORTER PLASMID



Sequence of GAL4 Binding Element in pFR-Luc

AT CTTATCATGTCTGGATC CA AGCTTGCATGCCTGCAG
GT **CGGAGTACTGTCCTCCG** AG **CGGAGTACTGTCCTCCG**
AG **CGGAGTACTGTCCTCCG** AG **CGGAGTACTGTCCTCCG**
AG **CGGAGTACTGTCCTCCG** AG CGGAGACTCTAGAGGG
TATATAATGGATCCCCGGGT AC CGAGCTCGAATTC...

5× GAL4 binding element

...CAGCTTGGCATTCCGGTACTGTTGGTAAAATG luciferase

Figure 5 Circular map and sequence of the 5× GAL4 binding element for the pFR-Luc reporter plasmid.

DNA BINDING- (pCMV-BD) AND ACTIVATION-DOMAIN (pCMV-AD) VECTOR CONSTRUCTION

DNA encoding the bait protein is prepared for insertion into the pCMV-BD vector and DNA encoding the target protein is prepared for insertion into the pCMV-AD vector either by restriction digest or PCR amplification. DNA encoding the bait or the target protein must be inserted so that the bait and the target protein are expressed in the same reading frame as the GAL4 DNA binding domain and the NF-κB transcriptional activation domain respectively (consult Figures 2 and 3 for information on reading frames). In the MCS of the pCMV-BD vector there are 11 unique sites, and in the MCS of the pCMV-AD vector there are 8 unique sites for convenient cloning options.

Preparing the pCMV-BD and pCMV-AD Plasmids

- Stratagene suggests dephosphorylating the digested plasmids with CIAP prior to ligating with the insert DNA. If more than one restriction enzyme is used, the background can be reduced further by electrophoresing the DNA on an agarose gel and removing the desired plasmid band through electroelution, leaving behind the small fragment originating between the two restriction enzyme sites.

- After purification and ethanol precipitation of the DNA, resuspend in a volume of TE buffer (see *Preparation of Media and Reagents*) that will allow the concentration of the plasmid DNA to be the same as the concentration of the insert DNA (~0.1 µg/µl).

Ligating the Insert

For ligation, the ideal insert-to-vector molar ratio of DNA is variable; however, a reasonable starting point is a 2:1 insert-to-vector ratio. The ratio is calculated using the following equation:

$$\text{Picomole ends / microgram of DNA} = \frac{2 \times 10^6}{\text{number of base pairs} \times 660}$$

where *X* is the quantity of insert (in micrograms) required for a 1:1 insert-to-vector molar ratio. Multiply *X* by 2 to get the quantity of insert required for a 2:1 ratio.

1. Prepare three control and two experimental 10-µl ligation reactions by adding the following components to separate sterile 1.5-ml microcentrifuge tubes:

	Control				Experimental	
Ligation reaction components	**1**[a]	**2**[b]	**3**[c]		**4**[d]	**5**[d]
Prepared plasmid (0.1 µg/µl)	1.0 µl	1.0 µl	0.0 µl		1.0 µl	1.0 µl
Prepared insert (0.1 µg/µl)	0.0 µl	0.0 µl	1.0 µl		*Y* µl	*Y* µl
rATP [10 mM (pH 7.0)]	1.0 µl	1.0 µl	1.0 µl		1.0 µl	1.0 µl
Ligase buffer (10×)[e]	1.0 µl	1.0 µl	1.0 µl		1.0 µl	1.0 µl
T4 DNA ligase (4 U/µl)	0.5 µl	0.0 µl	0.5 µl		0.5 µl	0.5 µl
Double-distilled (ddH_2O) to 10 µl	6.5 µl	7.0 µl	6.5 µl		*Z* µl	*Z* µl

[a] This control tests for the effectiveness of the digestion and the CIAP treatment. Expect a low number of transformant colonies if the digestion and CIAP treatment are effective.

[b] This control indicates whether the plasmid is cleaved completely or whether residual uncut plasmid remains. Expect an absence of transformant colonies if the digestion is complete.

[c] This control verifies that the insert is not contaminated with the original plasmid. Expect an absence of transformant colonies if the insert is pure.

[d] These experimental ligation reactions vary the insert-to-vector ratio. Expect a majority of the transformant colonies to represent recombinants.

[e] See *Preparation of Media and Reagents*.

2. Incubate the reactions for 2 hours at room temperature (22°C) or overnight at 4°C. For blunt-end ligation, reduce the [rATP] to 5 mM and incubate the reactions overnight at 12–14°C.

Transformation

Transform competent bacteria with 1–2 µl of the ligation reaction, and plate the transformed bacteria on LB-kanamycin (pCMV-BD) or LB-ampicillin (pCMV-AD) agar plates (see *Preparation of Media and Reagents*). Refer to reference 6 in *References* for a transformation protocol.

Verifying the Presence and Reading Frame of the Insert

Select isolated colonies for miniprep analysis to identify transformed colonies containing the pCMV-BD or the pCMV-AD vector with the DNA insert. The nucleotide sequence of the DNA insert should be determined to verify that the DNA insert will be expressed as a fusion protein with the GAL4 BD or the NF-κB AD and that the DNA inserts do not contain mutations. In addition, expression of the GAL4 BD-bait fusion protein can be verified by western blot analysis with an antibody that immunoreacts with either the protein expressed from the DNA insert or the GAL4 BD. Similarly, the expression of the NF-κB AD-target protein can also be verified by western analysis with an antibody that immunoreacts with either the protein expressed from the DNA insert or the NF-κB AD.

Recommended Sequencing Primers

Sequencing Primers[a]	Bases	Sequence
BD 5′ primer	1045–1063	5′ AGC ATA GAA TAA GTG CGA C 3′
BD 3′ primer (T7 primer)	1231–1250	5′ TAA TAC GAC TCA CTA TAG GG 3′
AD 5′ primer	1192–1210	5′ GGA GAT GAA GAC TTC TCC T 3′
AD 3′ primer (T7 primer)	1364–1383	5′ TAA TAC GAC TCA CTA TAG GG 3′

[a] The 5′ primer is at the 5′ end of the MCS and 3′ primer is at the 3′ end of the MCS.

CELL CULTURE AND TRANSFECTION

Note *The DNA used for transfections must be of high quality (i.e., double cesium chloride banded). Ensure that the plasmid DNA has an $OD_{260/280}$ of ~1.8–2.0 and is endotoxin free. The plasmids supplied with this kit are of high quality and are ready for transfection.*

Growing the Cells

Note *The following protocol is designed for adherent cell lines such as CHO, COS, NIH 3T3, HeLa, and 293. Optimization of media and culture conditions may be required for other cell lines.*

1. Thaw and seed frozen cell stocks in complete medium in 50-ml or 250-ml tissue culture flasks.

2. Split the cells when they just become confluent.

3. Subculture the cells at an initial density of ~1×10^5 –2×10^5 cells/ml every 3–4 days.

Preparing the Cells for Transfection

1. Seed 0.5–1.0×10^5 cells in 1 ml of complete medium in each well of a 24-well tissue culture dish.

2. Incubate the cells at 37°C in a CO_2 incubator for 24 hours.

Preparing the DNA Mixtures for Transfection

Combine the plasmids to be cotransfected in a sterile 5-ml BD Falcon polystyrene round bottom tube as indicated in Table III. As each assay is run in triplicate, the amount of plasmid DNA in each tube should be sufficient for three transfections.

For example, to prepare the first sample **in triplicate** as indicated in Table III, combine the following components in a 5-ml BD Falcon polystyrene tube and then proceed to *Transfecting the Cells*.

0.03 µg of pBD-p53
0.03 µg of pAD-SV40T
0.75 µg of pFR-Luc

TABLE III

Purpose	BD fusion	Amount of DNA (µg)[a]	AD fusion	Amount of DNA (µg)[a]	pFR-Luc Reporter Plasmid	Expected Results
Interaction positive control	pBD-p53	0.01	pAD-SV40T	0.01	0.25 µg	+
	pBD-p53	0.1	pAD-SV40T	0.1	0.25 µg	+
	pBD-p53	0.5	pAD-SV40T	0.5	0.25 µg	+
Interaction negative control	pBD-p53	0.01	pAD-TRAF	0.01	0.25 µg	–
	pBD-p53	0.1	pAD-TRAF	0.1	0.25 µg	–
	pBD-p53	0.5	pAD-TRAF	0.5	0.25 µg	–
Reporter gene activity positive control	pBD-NF-κB	0.01	-	-	0.25 µg	+
	pBD-NF-κB	0.1	-	-	0.25 µg	+
	pBD-NF-κB	0.5	-	-	0.25 µg	+
Experimental two-hybrid assay	pCMV-BD-Bait[b]	0.01	pCMV-AD-Target[c]	0.01	0.25 µg	+ for interaction – for no interaction
	pCMV-BD-Bait	0.1	pCMV-AD-Target	0.1	0.25 µg	+ for interaction – for no interaction
	pCMV-BD-Bait	0.5	pCMV-AD-Target	0.5	0.25 µg	+ for interaction – for no interaction
Verification of dependence of reporter expression on both bait and target fusions	pCMV-BD-Bait	0.01	pAD-TRAF	0.01	0.25 µg	–
	pCMV-BD-Bait	0.1	pAD-TRAF	0.1	0.25 µg	–
	pCMV-BD-Bait	0.5	pAD-TRAF	0.5	0.25 µg	–
	pBD-p53	0.01	pCMV-AD-Target	0.01	0.25 µg	–
	pBD-p53	0.1	pCMV-AD-Target	0.1	0.25 µg	–
	pBD-p53	0.5	pCMV-AD-Target	0.5	0.25 µg	–

[a] A range of DNA concentrations (usually from 10–500 ng) should be used in the pilot experiment to determine the optimal DNA concentration for a particular cell line.

[b] pCMV-BD-Bait corresponds to the pCMV-BD vector containing the bait gene of interest.

[c] pCMV-AD-Target corresponds to the pCMV-AD vector containing the target gene of interest.

Transfecting the Cells

A number of transfection methods, including calcium phosphate precipitation and lipid-mediated transfection, may be used. Transfection efficiencies vary between cell lines and according to experimental conditions. Transfection procedures should be optimized for the cell line chosen. Perform the transfections according to the manufacturer's instructions.

Extracting the Luciferase

1. Remove the medium from the cells and carefully wash the cells twice with 0.5 ml of 1× PBS buffer.[§]

2. Remove as much PBS as possible from the wells with a Pasteur pipet. Add 100 µl of 1× cell lysis buffer[§] to the wells and swirl the dishes gently to ensure uniform coverage of the cells.

3. Incubate the dishes for 15 minutes at room temperature. Swirl the dishes gently midway through the incubation.

4. Assay for luciferase activity directly from the wells within 2 hours.

5. To store for later analysis, transfer the solutions from each well into a separate microcentrifuge tube. Spin the samples in a microcentrifuge at full speed. Store the supernatant at –80°C. **Each freeze–thaw cycle results in a significant loss of luciferase activity (as much as 50%).**

   Note *If this passive lysis method does not yield satisfactory results, refer to the instructions for an active lysis method in* Troubleshooting.

Performing the Luciferase Activity Assay

1. Mix 5–20 µl of cell extract equilibrated to room temperature with 100 µl of room temperature 1× assay buffer[§] in a 5-ml BD Falcon polystyrene tube.

2. Measure the light emitted from the reaction with a luminometer using an integration time of 10–30 seconds.

3. Luciferase activity may be expressed in relative light units (RLU) as detected by the luminometer from the sample. The activity may also be expressed as RLU/well, RLU/number of cells, or RLU/mg of total cellular protein.

[§] See *Preparation of Media and Reagents*.

TROUBLESHOOTING

Observations	Suggestions
The background luciferase activity is too low to calculate	Increase the concentration of cell lysate used in the assay
	Use more pFR-Luc plasmid for transfection
	Plot and compare the absolute luciferase activity rather than the activation fold increase
Results vary among triplicate samples	Avoid variations in pipetting, growth conditions, extraction efficiency of luciferase, etc.
	Use the same DNA–transfection reagent mixture for the three wells
	Take care when washing the cells to avoid removing the cells from the wells
The activity increase of the luciferase over the background is low	Confirm expression of the fusion protein by running a western blot of the cell lysate (the GAL4-dbd protein expresssed from pCMV-BD is 187 aa and is approximately 21 kDa))
	Ensure excess pCMV-BD or pCMV-AD construct is not used; use only 10–500 ng of the pCMV-AD or pCMV-BD construct in the experiment
All samples exhibit very low or no luciferase activity	To ensure complete lysis, perform the following active lysis. Scrape all surfaces of the tissue culture dish, pipet the cell lysate to microcentrifuge tube and place on ice. Lyse the cells by brief sonication with the microtip set at the lowest setting **or** freeze the cells at –80°C for 20 minutes and then thaw in a 37°C water bath and vortex 10–15 seconds. Spin the tubes in a microcentrifuge at high speed for 2 minutes. Use the supernatant for the luciferase activity assay
	Optimize the transfection procedure with a reporter plasmid such as pCMV-βGAL
	Cell line used may contain incompatible molecules or proteins; use an alternate cell line

PREPARATION OF MEDIA AND REAGENTS

Assay Buffer (1×)

40.0 mM tricine (pH 7.8)
0.5 mM ATP
10 mM $MgSO_4$
0.5 mM EDTA
10.0 mM DTT
0.5 mM coenzyme A
0.5 mM luciferin

Cell Lysis Buffer (5×)

40 mM tricine (pH 7.8)
50 mM NaCl
2 mM EDTA
1 mM $MgSO_4$
5 mM DTT
1% Triton® X-100

LB Agar (per Liter)

10 g of NaCl
10 g of tryptone
5 g of yeast extract
20 g of agar
Add deionized H_2O to a final volume of 1 liter
Adjust pH to 7.0 with 5 N NaOH
Autoclave
Pour into petri dishes (~25 ml/100-mm plate)

LB–Ampicillin Agar (per Liter)

1 liter of LB agar, autoclaved
Cool to 55°C
Add 10 ml of 10-mg/ml filter-sterilized ampicillin
Pour into petri dishes (~25 ml/100-mm plate)

LB–Kanamycin Agar (per Liter)

Prepare 1 liter of LB agar
Autoclave
Cool to 55°C
Add 1 ml of 50-mg/ml-filter-sterilized kanamycin
Pour into petri dishes (~25 ml/100-mm plate)

Ligase Buffer (10×)

500 mM Tris-HCl (pH 7.5)
70 mM $MgCl_2$
10 mM dithiothreitol (DTT)

Note *rATP is added separately in the ligation reaction*

1× PBS Buffer

137 mM NaCl
2.6 mM KCl
10 mM Na_2HPO_4
1.8 mM KH_2PO_4
Adjust the pH to 7.4 with HCl

TE Buffer

10 mM Tris-HCl (pH 7.5)
1 mM EDTA

REFERENCES

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ENDNOTES

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MSDS INFORMATION

The Material Safety Data Sheet (MSDS) information for Stratagene products is provided on Stratagene's website at *http://www.stratagene.com/MSDS/*. Simply enter the catalog number to retrieve any associated MSDS's in a print-ready format. MSDS documents are not included with product shipments.