mately 10 U. This weak fluorescence was not detectable by microscopic analysis and indicated that no significant GFP induction occurred by expression of single fusion proteins.

Expression of pACT-TRAF2 and pBIND-LT β R together with the pGal4p-GFP yielded a strong mean fluorescence of 210 U, which is similar to the expression of the positive controls VP16/MyoD and Gal4p/Id fusion proteins and yielded a relative mean fluorescence value of 180 U. Bicistronic expression of the VP16/TRAF2 and the Gal4p/LT β R fusion proteins resulted in a mean fluorescence value of 200 U similar to that obtained by coexpression of fusion proteins cloned in different plasmids. To compare relative transcriptional activation, activation factors were estimated by normalizing values against background fluorescence (Table 1). Expression of VP16/TRAF2 and Gal4p/LT β R fusion proteins led to a 21-fold enhancement of GFP fluorescence in co-transfection as well as in the bicistronic expression system. This indicates a strong intracellular association between TRAF2 and the intracellular domain of LT β R receptor, leading to high transcriptional GFP induction. The transcriptional activation of MyoD with Id is characterized by a GFP activation factor of 18 (Table 1).

With the GFP-based reporter gene system described here, two-hybrid protein interactions can be directly characterized in living mammalian cells by the use of a standard device to measure cellular fluorescence (e.g., EPICS or FACS). In contrast to the commonly used stable expression system in yeast, this system offers a faster analysis of novel protein interactions by its transient expression mode and allows the comparison of the relative transcriptional activation of a given interaction. Compared to other enzyme-based assay systems used to characterize mammalian two-hybrid interactions (4), the direct measurement of protein interactions using GFP avoids the need of enzyme substrates and therefore provides a more economical test system. Used with a cell sorter, this method can be further extended to select single GFP-expressing cells.

The use of the bicistronic expression system facilitates the coordinated expression of two-hybrid fusion proteins

expressed on one plasmid together with different reporter genes such as GFP variants. With this pIRES-based bicistronic expression system, stable cell lines expressing the two-hybrid interaction-dependent GFP can be established and used for the identification of inhibitors of a given protein pair.

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Titration of Infective and Noninfective Ff Filamentous Bacteriophages Using a Monoclonal Antibody against g3p

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Phage display technology is now widely used as a rapid and powerful approach for identifying and optimizing binding antibodies, peptides and other biomolecules (14). Phage display selection and screening can be performed in microplates and is therefore amenable to automation. However, one time-consuming step is the determination of phage titers, which is necessary before and during panning. Titration of Ff filamentous bacteriophages is usually done by the determination of plaque-forming units (pfu) or colony-forming units (cfu), which requires serial dilution infection of an *E. coli* test strain, plating of the cells, overnight growth and subsequent counting of colonies or plaques (7).

For titration of noninfective phages as used in selectively infective phage (SIP) technology (11), this method can obviously not be used. Alternative approaches such as ELISA using anti M13 antiserum (7) and spectrophotometry (10) are not very accurate; it has been shown that a phage population

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usually contains a high proportion of polyphages (phages of more than one unit length having more than one genome packaged) (6), especially if the widely used truncated versions of minor phage coat protein III (g3p) are expressed (5,8,9). Therefore, we aimed to develop a method that accurately determines the phage titer without relying on infectivity and that can be used in high-throughput experiments. Since in all types of phage display systems the concentration of the C-terminal domain of the phage g3p protein is directly correlated with the number of phages, we have established an ELISA method us-

Figure 1. 10C3 ELISA using fCKK phage. Polystyrene microplate wells (F96 Maxisorb™ Immuno plate; Nalge Nunc International, Roskilde, Denmark) were coated in triplicate for 1 h at 37°C with serial dilutions of an fCKK phage preparation of known titer (determined by conventional pfu titration). Phages were either diluted in PBS buffer (open circles) or in PBS buffer containing 8 M guanidine HCl (filled circles). Blocking was for 1 h at room temperature using PBS containing 5% skimmed milk powder. After washing three times with PBS containing 0.05% Tween® 20, the mouse monoclonal IgG₁ anti-g3p antibody, 10C3 (12) (purchased from MoBiTec GmbH, Göttingen, Germany), diluted 1:250 in 2% milk powder in PBS was added to the wells for 1 h at room temperature. After washing, bound 10C3 was detected with a sheep anti-mouse antibody conjugated to horseradish peroxidase (Sigma, St. Louis, MO, USA) using BM blue soluble (Roche Molecular Biochemicals, Mannheim, Germany) as substrate. Absorbance at 370 nm was measured photometrically, and data were analyzed using Origin™ (Microcal Software, Northampton, MA, USA). Error bars represent the standard deviation of the mean ($n = 3$). Data points obtained from phages diluted in PBS containing 8 M guanidine were fitted to a sigmoidal curve.

ing the commercially available mouse monoclonal anti-g3p antibody, 10C3 (12), which recognizes a linear epitope of the C-terminal domain of g3p (1). The g3p is present most likely in five copies on every phage, reflecting the fivefold symmetry of the phage coat and the pilus (4).

Phage fCKK (3), which is derived from phage fd by an insertion of a kanamycin resistance gene, was used as the standard phage. While the ELISA signal obtained after the direct coating of the phage solution onto microplate wells and subsequent detection with the 10C3 antibody did not correlate well with pfu values (data not shown), we subsequently found that an accurate and reproducible correlation could be obtained when the phages were denatured in 8 M guanidine HCl before coating (Figure 1). Interestingly, M13 phages such as VCSM13 and M13K07 can be titrated even without prior denaturation with guanidine (12) (data not shown). The few differences in amino acid sequence between the M13 and fd phage proteins (13) seem to cause a difference in accessibility of the epitope to 10C3 during coating. Using this method, the titers of phage preparations can be rapidly determined from a fit of the linear part of the standard titration curve. Moreover, aliquots of the standard phage preparation can be used many times once the titer has been determined because it can be stored in 8 M guanidine at -80°C for at least several months without losing reactivity in ELISA experiments.

For two different phage preparations of the HuCAL single chain Fv phagemid library (2), phage concentrations determined by counting pfu were 5×10^{12} and 1.8×10^{12} phages/mL, and 7.1×10^{12} and 7.5×10^{12} phages/mL when determined by g3p ELISA, using VCSM13 as a helper phage and using fCKK as the standard phage. Hence, the ELISA method described here is also useful for the widely established phagemid-based systems. We cannot, however, completely rule out that the nature of the partner fused to g3p may have an influence on the number of infective phages, which means that standard titration curves might not be transferable between different phage libraries. Therefore, we recommend that

for each individual phage library under study, a specific standard curve should be generated.

In conclusion, the described method for the titration of filamentous bacteriophages used in phage display or SIP technology is rapid; it can be performed in less than 5 h, is accurate and reproducible and can be applied in a high-throughput fashion.

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Contact Printing of Lift Filters for Alignment of Plaque and Colony Lifts

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A time-consuming step in isolating clones by plaque or colony lifts is the need to align the exposed X-ray film to the petri plates from which the lifts were made. This step is carried out to pick the plaque, plaque region or colony that is positive for the radioactive probe. As is typically done, this alignment is a two-step procedure: (i) the film is first aligned to the lift filters and marks are transferred to the film; and (ii) the film is then aligned to the plates. These steps add to the time required and increase the possibility of errors in the final alignments.

The modified procedure is a single-step alignment: it is rapid, convenient and very accurate, and was effective in aligning several hundred films with the

petri plates from which lifts were made. It is equally efficient for aligning films of duplicated lifts to each other to determine if signals are in precisely the same place.

Alignment between petri plate and lift filter is established using the standard ink spot method. Ink is introduced into a 23-gauge needle by immersing its tip to a 5-mm depth in India ink (Rapidograph 3084-F) in a microcentrifuge tube. The agar of the petri plate is stabbed shallowly in numerous spots, moving quickly so the ink fills the holes but does not usually make large drops on top of the agar. The filter is then placed on the agar where ink transfers to the filter.

Ink marks on the filter are transferred directly to the film by contact printing (or shadowing). This is carried out by a brief exposure to white light after the filters are placed on top of the film. With appropriate exposure, the film becomes slightly darkened under most of the filter while the ink spots are clear; the parts of the film not covered by filters are quite dark (Figure 1). If filters are labeled with dark ink (e.g., VWR lab marker 52877-150; Chicago, IL, USA), then the labels also transfer as clear marks and can usually be read di-

rectly from the film. If properly done, the exposure from visible light is nearly uniform across the filter except at ink spots or at the label. The petri plate is aligned by placing the ink spots in the agar directly above the clear spots in the film. Alignment is sufficiently precise that parallax distortion becomes a concern but can be minimized using numerous ink spots and precisely vertical observation. Occasionally, if the exposure is insufficient or the agar is too opaque, it may be necessary to highlight the clear spots with a colored marker to make them visible through the agar.

Positive colonies or plaques result in dark spots that are easily visible in the weakly exposed circle under each filter (Figure 1). The ability to detect a dark mark depends on two factors: (i) the radiation must be sufficient to cause detectable darkening of the film; and (ii) there must not be extraneous light or dark marks that camouflage the radiation-induced signals. Films have a "toe" region in the curve that relates exposure to the resulting optical density of the film (1). Faint exposures that fall in this toe region result in little or no optical density, and so are not detectable. The detection of radioactive signals is improved if the film is preflashed to bring

Figure 1. Contact-printed lift filters for lambda rescreens. The filter on the left shows no positives while the filter on the right shows many positives. Alignment marks (a) are clearly visible. Filter labels are legible except where they are obscured by dark positive signals. Water placed under the corner of the plastic wrap causes a large pale mark on the film (b), but both the alignment marks and the positive plaques remain easily visible. The edge of the plastic wrap sandwich (c, arrow) is also visible. Filters are 85 mm in diameter.