Biotechnology

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During cloning an extra GET, which encodes for a valise residue in the proteins' primary structures, was inserted immediately prior to each stop code. As a universal stop code ATA was used, replacing other stop codes if present. This was mainly done to gain maximum flexibility during cloning as the Flexi vector system enables the direct transfer to other vectors with different tags. A GET is mandatory to later transfer the gene of interest to a vector encoding a C-terminal tag. He correct expression of the encoded fusion proteins was assessed by SD-Page.

Analyzing the gel under fluorescent conditions reveals protein bands which have thealigned attached. The Passenger Plus preplanned Protein ladder possesses two fluorescent bands, at 25 and 70 kid respectively.Standard Protein with a size of 60 kid was also analyzed and helps as an additional size reference.
Thefeatures a size of 34 kid alone. Agrees gel of PC products after amplification of Comparable jejune genes from genomic DNA. The band sizes match the respective length of each gene.

Refer to Table 2 for expected gene lengths. As markers Hyper Ladder I (M) and II (MM) were used both most of the fusion proteins investigated fall into a range between 61 ND 73 kid, namely Haloing fused to arc (73 kid), pyre (72 kid), esp. (71 kid), gap (70 kid), (65 kid), pebble (62 kid), hiss (62 kid) and flag (61 kid). Outside of this size range, only Haloing-flag (93 kid) and the small(52 kid) are found. For each protein, bands with the correct size could be detected, see Figure Figurer. 4.

Additionally, bands of smaller size are visible (34 kid) which might be due to untimely termination of translation, potentially comprising only the Haloing(D, which features the corresponding size. He municipality of the immobilizers proteins was assessed using polygonal antibodies raised against whole and partially lased attenuated cells of Comparable jejune. Secondary antibodies conjugated with a fluoroscope were used to detect signals. Rather verification of the results was performed by using a standard western blot experiment to test for municipality.

Figure Figure shows the results of the investigated proteins after purification with Haloing"; magnetic beads was performed prior to SD-PAGE and blotting. Only two of the Investigated Monogenic proteins (ca, and halls) show strong visible bands In western blot matching the expected sizes. The three remaining Monogenic proteins, pee la, flag and pal, cannot be distinguished in western blot analysis as well as all the other proteins. Which showed clear positive signals for ca and his], a rather weak signal for pebble and extremely low signals for flag and pal. N contrast, Figure Figurative shows a western blot performed directly with whole lactates after recombinant expression without further purification. At least four bands are visible in all the samples with the most prominent band at 70 kid. Bands of lower intensity appear at approximately 55 kid, 28 kid and 18 kid. The first five lanes, corresponding to the known monogenic proteins, ca, his], pebble, flag and pal, show bands of higher intensity than the remaining five lanes.

However, as the investigated fusion proteins fall either into the 70 kid or in case of HTH-pal into the 55 kid range, a clear differentiation between positive bands and background caused by KERR cross-reactive proteins is

hardly possible. Erectly analyzing the whole lactates by western blot failed to discriminate between positive bands and background as the whole lactates of KERR cells clearly show cross- reactive signals with the used polygonal antibody.

However, as these bands bear similar sizes as the investigated fusion proteins, a secure identification of true positives is difficult at best using standard western blot without previous purification methods. Even then identification of municipality is sometimes difficult at best as sensitivity might be low due to differences in expression rate and losses of protein content during purification. Still, the signal intensities of the five monogenic proteins were well represented by all three methods hea derivative of a Theologians - is fused during expression to the N-terminus of our protein of interest.

The expression of the fusion proteins is under control oft RNA Polymerase. Additionally, an noncompliance cassette is present to allow for antibiotic selection. Second, with commonly used BLOB(DEE) expression cells, induction is realized by spoilsport ;-D-1-disproportionately (PIPIT). However, the PIPIT-induced expression oft RNA Polymerase in BLOB(DEE) is not tightly regulated, I. E. The promoter is leaky, causing a basal expression even if cells are not induced.

This is a major problem especially if toxic substances are to be expressed [27]. In our method, we used KERR cells to counter this problem. These cells are under regulation of L-Ramose and induction can completely be turned off by addition of glucose to the medium during growth. In fact, for the proteins

investigated, expression has failed in BLOB(Dee) for all but two proteins (data not shown), whereas KERR cells were able to express all the proteins with satisfying yields as we showed by SD-PAGE.