Corrigendum


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1. Introduction

In a previous publication [1], it was reported that small molecule probes could be used in combination with cDNA phage display libraries to identify cellular protein receptors. This proof-of-concept was performed with FK506 as the small molecule probe in order to clone FKBP12, the FK506 binding protein. It was recently discovered that the FKBP clone reported in figure 6 of the original paper actually originated from a contamination by a positive-control FKBP clone, rather than the cDNA library.

Although this control-FKBP clone appears to have been amplified in the original selection, and has been successfully amplified in subsequent optimization experiments, it was important to ascertain whether the Display Cloning technique is efficient enough to isolate a cDNA clone directly from a cDNA phage display library. Therefore, the cloning experiment was repeated using the same cDNA library, FK506-biotin probe and selection conditions as described in the original paper, with careful precaution to avoid contamination. From this reselection, a native FKBP gene was isolated as the predominant clone (5 out of 16), after six rounds of amplification. The details and analysis of the reselection experiment are described below.

2. Reselection of human brain cDNA library

The FK506 reselection was performed essentially as described [1], using the same human brain cDNA library, monomeric avidin resin with biotin-FK506 as the affinity probe and biotin as the specific eluent. The addition of a blocking step (with 1% BSA) prior to phage incubation was added in order to reduce non-specific background binding events. To avoid contamination, disposable plasticware was used throughout the experiment. The selection was performed for a total of seven consecutive rounds.

In order to follow the progress of the selection, specific primers were designed that recognize the FKBP12 gene. Fig. 1a shows the PCR analysis of the first five rounds of selection, along with an analysis of the entire library prior to selection. No FKBP12 is noted in the library prior to selection, presumably due to a limitation in the sensitivity of the PCR method. However, a faint band corresponding to approximately 300 bp is noted in selection round two, with bands of increasing intensity through round five, suggesting amplification of the FKBP12 gene.

Analysis of individual clones from round six showed that 6 out of 16 randomly selected clones responded to the FKBP primers (Fig. 1b). Five of these were found to be identical (lanes 1, 4, 7, 10 and 15) and contain the entire FKBP12 coding sequence (Fig. 2). One clone was different (lane 11) and did not contain nucleotide sequence corresponding to any coding region. In round seven, the number of FKBP-positive clones increased to 11 out of 16 (lanes 4–14), all of which were identical to the clone from the previous round, indicating a selection for this clone as the predominant selected phage.

The entire nucleotide sequence of the selected clone is shown in Fig. 2 [2]. The sequence shows that the predominant phage contains the EcoRI restriction site, a large amount of 5' UTR, the start codon and entire FKBP12 coding sequence, followed by a STOP codon, 3' UTR and the HindIII restriction site. It should also be noted that the 5' UTR does not contain any random STOP sequences

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1 Selected clone matches codons 19–432 of GenBank accession number M34539 (coding sequence: codons 79–405).
and that the FKBP12 coding region is in frame with the T7 coat protein.

3. Conclusion

The Display Cloning protocol can be used to select proteins directly from cDNA phage display libraries using small molecule probes, as originally claimed. The number of selection rounds is greater than initially reported, but remains within useful parameters. It should be noted that since the original publication, two other reports of cDNA phage display cloning have been described [3,4].

References