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Screening for recombinant plasmids using MacConkey agar

Pendola and Palis[1] recently reported that when screening recombinant plasmids for inserts in the *lac*Zα gene, MacConkey–lactose agar can be used instead of agar that contains X-Gal–IPTG. However, this method is not equally suitable for use with all plasmid vectors that carry this marker. Moreover, the results can vary depending on the source of the MacConkey medium used. In addition to the pUC9, pBluescript and pGEM4 plasmids mentioned by Pendola and Palis[1], MacConkey–lactose plates can be used to screen for inserts in pUC8, pUC9-1, pUC13, pUC19, pUCHinEco, pGEM3Z, pK18, pK19, pWM528 and pWM529 (Ref. 2). In our experience, the method failed with pUC8-1, pUC8-2, pUC9-2, pUC12 and pUC18 (Ref. 2). The different results obtained with very similar vectors, such as pUC18 and pUC19, are probably the result of sequence differences in the amino termini of the LacZα proteins they produce; these regions are encoded by the polylinkers of the vectors.

In addition to the pUC family, multipurpose pBluescript-series vectors are frequently used in experiments involving DNA cloning, construction of nested deletions, site-directed mutagenesis and RNA transcription *in vitro*. We tested *E. coli* DH5α carrying either plasmid pBluescript II SK+ (pSK) or pBluescript II KS+ (pKS), which differ only in the orientation of their multiple cloning sites[3], on MacConkey–lactose medium. Appropriate dilutions of liquid cultures in broth with ampicillin were plated and grown at 37°C. MacConkey agar from two vendors was used: Difco (Detroit, USA) and Merck (Darmstadt, Germany). Insertions were simulated by cloning Ω interposon (a selectable DNA fragment flanked by transcription and translation stop signals[4]) into the *Bam*HI site of pSK and pKS, to make pSKΩ and pKSΩ. Remarkable differences were observed in the results obtained, both between the two orientations of the polylinker and between media from different vendors.

In general, the Difco agar performed better: pSK and pSKΩ plasmids could be clearly differentiated on Difco plates after only an overnight incubation, with colonies being intensely red or white. Colonies carrying either pKS or pKSΩ were white after 1 d of growth, but could be differentiated from one another after 2–3 d of growth. For both plasmid pairs, colonies with inserts were slightly smaller than those with none, as might be expected for strains metabolizing an extra substrate.

These differences in colony colour were not as profound on the Merck agar as on the Difco agar. Colonies without inserts did not appear red until 2–3 d of growth: pSK-bearing colonies were pink, and pKS-bearing colonies were red in the middle with a white margin. Even colonies with inserts were faintly pink after prolonged incubation on Merck agar. Nevertheless, this agar offered an additional opportunity to identify clones with inserts. The inhibition of colony growth by expression of LacZα, described with pUC plasmids[5], was very conspicuous in colonies grown on Merck agar, especially those that carried the pSK or pSKΩ plasmid. Colonies bearing pSK were small (0.2–0.3 mm) and white after 24 h, while those carrying pSKΩ were larger (0.9–1.0 mm) and white. Although less pronounced in colonies that carried pKS or pKSΩ, this size difference was still sufficiently large to allow the colonies to be distinguished from one another. The specific enhancement of growth inhibition that was seen on Merck agar was puzzling since, according to the manufacturers' declarations, the composition of the

Merck and Difco media was identical, with one minor exception: lactose was added after the Difco agar was autoclaved, but was already included in the Merck agar.

The efficiency with which a defective β-galactosidase LacZ M15 protein is complemented by different LacZα proteins may fall above or below the sensitivity of MacConkey plates, depending on the source of the medium, the sequence of the LacZα protein (determined in part by the sequence of the polylinker) and on experimental conditions. On the other hand, the superior sensitivity of X-Gal–IPTG agar can detect complementation with all suitable vectors under most conditions. When cloning in LacZ-complementing plasmids without using X-Gal–IPTG, the following is generally recommended: plate a *lac*Z M15 strain transformed with your ligation mixture on MacConkey–lactose agar, examine both the colour and morphology of colonies and use any phenotypic difference found to choose clones for plasmid isolation.

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