Large-Scale Colony Screening and Insert Orientation Determination Using PCR

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Though many diagnostic PCR methods have been described, few techniques have been developed to rapidly and efficiently screen transformed bacterial colonies for plasmid presence (1). Unfortunately, PCR screening of a large number of colonies is time consuming and requires substantial amounts of polymerase and primers. In this report, we describe a two-step large-scale bacterial colony screening method that we have labeled “cracking PCR”. Cracking PCR includes (i) size screening of cracked bacterial colonies (i.e., selecting lysed clones displaying a band similar to the expected recombinant size) in an agarose gel and (ii) PCR screening of gel-determined potential positives directly from the cracking buffer/colony mixture. This method offers several advantages. First, the initial use of conventional cracking methods permits many colonies to be screened quickly in agarose, rather than the several hours required for PCR amplification. Second, since only the apparent positive clones will be used as template in PCR, fewer reactions will be necessary, thus reducing time and cost requirements. Finally, cracking PCR serves to reconfirm the presence of positive recombinants. The initial cracking step assists to verify that a band present in the PCR amplification reaction is a true band, rather than an artifact resulting from contamination from another colony or an anomaly of the reaction.

Figure 1 illustrates a flowchart of the cracking PCR procedure. Bacterial colonies are lysed using a protocol based on a previously described method (2). This method has been significantly modified. Briefly, 1 mm² of each colony is picked from a masterplate with a sterile toothpick and twisted in 50 µL cracking buffer (in 200 µL: 50 mM Tris-HCl, pH 7.5, 2 mM EDTA, 1% SDS, 27.4 g sucrose, 20 mg bromophenol blue, and 20 mg xylene cyanol) in 0.5-mL microcentrifuge
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Figure 2. PCR products from a bacterial transformation (TOP10F strain) after performing the cracking PCR method. Lane 1 is a 1-kb ladder (Life Technologies). Products in lanes 2–5 were amplified using one forward primer internal to the insert (GB5-3) and one reverse primer internal to the vector (3’AOX1; Invitrogen, Carlsbad, CA, USA). Lane 2 is transformed pGAPZaA (Invitrogen). Lanes 3 and 4 contain a 1928-bp fragment amplified from two different clones of transformed pGAPZaA•GB5-3 [pGAPZaA containing a glucocerebrosidase (EC 3.2.1.45.) insert] with the insert in the correct orientation. Lane 5 contains a 1932-bp fragment amplified from transformed pGAPZaA•GB5-3 with the insert in the incorrect orientation. Products in lanes 6–8 were all amplified using two reverse primers: one internal to the vector (3’AOX1), the other internal to the insert (GB2). Lane 6 is transformed pGAPZaA•GB5-3 with the insert in correctly. Lane 7 is pGAPZaA•GB5-3 with the insert in incorrectly. Lane 8 is the negative control. Lane 9 is the positive control of transformed pGAPZaA•GB5-3 confirmed by sequence analysis, containing a 1928-bp segment amplified with primers GB5-3 and 3’AOX1.

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