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Direct clone characterization from plaques and colonies by the polymerase chain reaction

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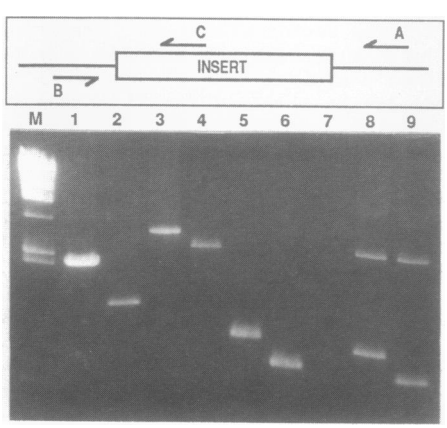
We show that PCR[1] can be performed directly[2] on bacterial plaques or colonies, circumventing all DNA preparation. This allows the presence, size and orientation of inserts to be determined rapidly by amplification with flanking primers, and (for orientation) by including a single internal primer. For M13mp and pUC-based vectors, inserts of up to 3.7 kb are readily amplified using primers (A and B in upper figure) flanking the insert. The orientation of the insert may be screened using a primer (C) within the insert. The amplified material can be used directly for restriction digestion, cloning[3], and sequencing[4].

Amplification reactions. 20 µl aliquots of 10mM Tris/HCl pH 8.3 at 25°C, 50mM KCl, 1.5 mM $MgCl_2$, 0.1 mg/ml gelatine, 0.25 mM dNTP, 10 pmol each oligonucleotide, 2.5 U Taq polymerase (Cetus), and template DNA were overlaid with paraffin oil, and subjected to 30 rounds of temperature cycling: 94°C 1 min, 55°C 1 min, 72°C 2 min (3 min for inserts > 2.5 kb) and a final 5 min 55°C step, on a PHC-1 programmable heating block (Techne). 5 µl of the reaction mixture was analysed on a 1-2% agarose gel containing 1 µg/ml EtBr.

PCR on colonies. Colonies were resuspended in 0.5 ml water (live bacteria could be rescued at this stage) and boiled in a water bath for 5 minutes. After centrifugation for 2 min at 13000 rpm, 5 µl of the supernatant was used in the PCR.

PCR on plaques. Phage were transferred from a single plaque to a 20µl PCR reaction mix using a fresh toothpick, briefly swirled in the mixture. The toothpick could then be used to innoculate bacterial cultures for preparation of sequencing template.

Primers. A and B primers for M13mp and pUC were 5'-GTAAAACGACGGCCAGT and 5'-CAGGAA-ACAGCTATGAC, and for pSV2gpt were 5'TCCAAACTCATCAATGTATC and 5'-CTCACAGT-CTCCTCAGGTGAG (flanking the *Bam*HI cloning site in a vector containing the mouse V_HNP gene[5]). Primer C was a sequencing primer for the mouse γ1 genomic insert, 5'-GTGAACCCCTCCTCCCT.



Upper fig. Positions of screening primers. (see text).

Lower fig. 1% agarose gel of PCR products from plaque and colony screens. Lanes 1 and 2: inserts of 2.1 and 1.2 kb identified from pUC colonies; lanes 3-5: inserts of 3.7, 2.5 and 0.9 kb identified from M13 plaques; lanes 6 and 7: orientation screen (B,C-type reaction) of M13 plaque clones positive and negative respectively for the desired orientation; lanes 8 and 9: combined screen (A,B,C-type reaction) of pSV2gpt colony clones harbouring a 2.5 kb insert in opposite orientations. M=lambda-*Hin*dIII size markers.

References.

1. Saiki *et al* (88), Science 239 487-491.
2. Saiki *et al* (86), Nature 324 163-166.
3. Orlandi *et al* (89), Proc. Nat. Acad. Sci. USA, in press.
4. Innis *et al* (88), Proc. Nat. Acad. Sci. USA 85 9436-9440.
5. Neuberger (83), EMBO J. 2 1373-1378.

Note added in proof: colonies transferred directly (see 'PCR from plaques') are also efficient PCR substrates.