Vector-Alu PCR: a rapid step in mapping cosmids and YACs

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Economical procedures for mapping large numbers of cosmids and YAC's, using panels of somatic cell hybrids are of practical importance in genome mapping. We developed a method based on direct PCR of individual cosmid colonies with primers complementary to the cosmid vector and 2 primers complementary to the extreme 5 prime and 3 prime parts of the Alu consensus sequence (1). We also applied this method on samples of YAC DNA, either liquid or in agarose blocks. The cosmid vector is p2CpG, a derivative of pCpG and pKNUN1 (2; Dauwerse, unpublished), its primers: ML and RL. The YAC vector is YAC4, its primers: YL5 and YL3. The primers for the ALU repeat: ALU5 and ALU3. The primers PDJ33 and PDJ34 suggested by Dr P. de Jong (personal communication) are also used for YAC's. The sequences of the primers are depicted in Table 1. The method was used to map the human positive cosmid and YAC clones isolated from libraries generated from two hamster−human radiation hybrids, both harbouring the adenomatous polyposis coli (APC) gene (located on chr. 5). The PCR products of these clones (see Fig. 1A) were (competition) hybridised to filters with a chr. 5 regional mapping panel of radiation hybrids (3; see Fig. 1B). The results are presented in Table 2. There is a four-fold rise in the number of specific PCR products after adding the vector primers compared to the procedure with ALU5 and ALU3 alone. Vector-Alu PCR was found to be a rapid and easy step in the mapping of cosmids and YAC's, it omits the isolation of a great number of cosmids and YAC's. The co-amplification of the multiple cloning site of p2CpG facilitates specific (end) cloning. The PCR product can also be used directly for RFLP screening.

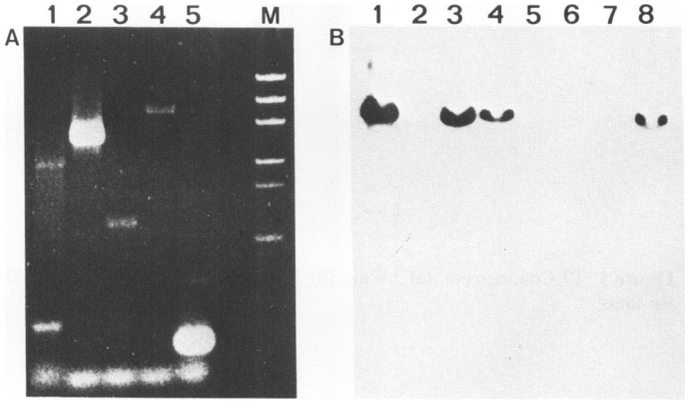

Figure 1. A) Gel pattern of PCR products after vector-Alu PCR. Lane 1: YAC JW12; primers PDJ33/PDJ34, lane 2; cosmid cB65; ML/ALU5/ALU3, lane 3: cB65; RL/ALU5/ALU3, lane 4: cB66; ML/ALU5/ALU3, lane 5: cB66; RL/ALU5/ALU3, lane 6: marker; Phi-X digested with HaeIII. B) Autoradiogram after hybridisation of a Southern blot with radiation hybrids with the PCR product of cB 65 (1 A, lane 2). Lane 1: Human control, lane 2: Chinese hamster, lanes 3−8: 6 different radiation hybrids, the hybrid in lane 3 was used as cloning source. The procedure for cosmids: each colony is rinsed twice *in situ* with 10 μl water, the washings (i.e. bacterial suspension) are enlarged with water to 50−100 μl per amplification to be performed, boiled for 10 min. and centrifuged for 3 min at 1200g. The supernatant is used directly for vector-Alu PCR using two primer combinations, ML/ALU5/ALU3 or RL/ALU5/ALU3; a combination of four primers resulted in the formation of homoduplexes. Temperature/time profile: 1′ 95°C, 1′30″ 65°C, 2′ 72°C. The PCR product is run on a low-melting agarose gel. The sliced agarose with DNA is melted at 65°C and a 10 ng aliquot is labelled through multipriming and used for hybridisation studies. The procedure for YAC's is the same as above with the exception that the YAC DNA is isolated first.

ACKNOWLEDGEMENT

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REFERENCES

1. Dauwerse *et al.* (1989) *Nucl. Acids Res.* **17**, 3603.
2. Willard *et al.* (1987) *J. Mol. Evol.* **26**, 180.
3. Tops *et al.* in preparation.

Table 1. Primer sequences

ML:	5′ GACGTTGTAAAACGACGGCCAGT 3′
RL:	5′ AACAGCTATGACCATGATTACG 3′
ALU5:	5′ GTGAGCCACCGCGCCCGGCC 3′
ALU3:	5′ ACAGAGCGAGACTCCGTCTC 3′
PDJ33:	5′ GCCTCCCAAAGTGCTGGGATTACAGG(C/T)(G/A)T GAGCCA 3′
PDJ34:	5′ TGAGC(C/T)(G/A)(A/T)GAT(C/T)(G/A)(C/T)(G/A)CC (A/T)CTGCACTCCAGCCTGGG 3′
YL3:	5′ AGTCGAACGCCCGATCTCAA 3′
YL5:	5′ AAGTACTCTCGGTAGCCAAG 3′

Table 2. Results

Probe library	No. clones	No. PCR products	No.[a] mapped
Cosmid	69	58	41
YAC	21	14	7

[a]Not all PCR products gave clear signals on Southern blots.