A simple method for screening of NotI linking clones

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NotI linking clones are valuable for ordering of DNA fragments fractionated by Pulsed field gel electrophoresis(1) and especially interested in that they are often associated with transcribable genes(2,3). To facilitate the isolation of NotI linking clones, we have devised a method which based on the insertion of Kanamycin cassette bearing NotI sites at both ends into cleavable NotI sites of genomic DNA fragments already cloned into plasmid vectors. The Kanamycin cassette used was constructed by ligation of NotI linker(BamHI-SacII-NotI-SfiI-BamHI: Boeriger mannheim) to Kanamycin Block(Kan.Gen.Block: Pharmacia) commercially obtained. The resultant fragment was digested with NotI and cloned into NotI site of Bluescript-KS. One of transformants, designated pBS-Kan2, was found to contain the Kanamycin block having heterologous NotI-BamHI fragments at both ends(Fig. 1A). DNA was extracted from approximately 10000 clones of human DNA library(Insert length:2-6Kb) in pUC13 vector and digested with NotI. Kanamycin cassette excised with NotI from pBS-Kan2 was ligated to these linear DNA and subjected to transformation of JM103. Analysis of 10 Kanamycin resistant clones, which were randomly picked up, have showed that they included the human genomic sequences linked to NotI site, inserting Kanamycin cassette into their sites(Fig.1B) and seven of them represented single copy per haploid genome. Furthermore, all of NotI sites lying them appeared to be methylation-free in placenta DNA. Unique site of SfiI and SacII at each end of Kanamycin cassette will be useful in physical mapping of recombinant plasmids. If Kanamycin block is cut out with SacII, it might be applicable to screen the clones containing sequences around SacII sites which also distributed in vertebrate DNA less frequently(1,3).

Figure 1. **A. Structure of pBS-Kan2** capital letters: introduced NotI linker, lowercase letters: polylinkers in Kanamycin Block and Bluescript-KS **B. Gel analysis of NotI linking clones** (1)-(10): transformants digested with NotI, (M): Lambda-HindIII as size marker. Bands of 1.5Kb observed in all clones indicate the Kanamycin cassette.

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