A polymerase chain reaction approach for constructing jumping and linking libraries

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The applications of jumping libraries have been limited due to technical difficulties in their preparation; in particular the requirement for substantial amounts of size selected high molecular weight DNA and selectable marker DNA (1–4). In this report we demonstrate the usefulness of the polymerase chain reaction (PCR) for performing predetermined jumps or constructing generalized hopping and linking libraries. The principle of the technique is schematically depicted in the figure and was applied for jumping from one end to the other of a 150 kb long *MluI* genomic DNA fragment of the human MHC region (5). Genomic DNA, prepared from JY cells in agarose blocks, was digested with *MluI* and circularized at a concentration of 0.2 $\mu\text{g}/\text{ml}$ at 4°C using 1600 units/ml of T4 DNA ligase (NEB). The ligated DNA was precipitated by ethanol, dissolved in TE and subsequently digested with *Sau3AI*. The *Sau3AI* digested DNA was again circularized at a concentration of 0.2 $\mu\text{g}/\text{ml}$ as before. The ligated DNA was precipitated by ethanol, dissolved in TE and passed through a G-50 sephadex spin column. The eluate was amplified with specific primers, from within the HLA-B7 gene, so constructed that they amplify a stretch of DNA from the *MluI* site to the *Sau3AI* site that borders the fragment at the end point of the jump. The figure shows amplification of 40 ng of total genomic DNA (very limited or extensive *MluI* digests) using 100 ng of primers for 45 cycles at 94°C, 1 min.; 55°C 1 min. 30 sec.; 72°C, 2 min. The amplified product, ≈ 400 bp long, was digested with *MluI* and *Sau3AI* and cloned in the Bluescript vector pKS(–). A total of 38 clones were sequenced and compared with the HLA-B7 gene, from where the jump originated and with the HLA-cW gene, the concluding point of the jump. A summary of this data is presented in the table.

The limited digest yielded clones containing sequences adjacent to the initial jumping point; presumably arising from the undigested DNA. In the extensive digest, almost half the clones contain sequences adjacent to the initial jumping point but most of the other half come from the concluding point of the jump. This technique is simple and practical for constructing general jumping and linking libraries and for directionally hopping from a defined sequence. Several applications of this approach for human genome mapping are also apparent.

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TABLE

<i>MluI</i> digest	clones	B7-like	cW-like	New
Very limited	8	5	none	3
Extensive	30	13	12	5

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