YAC subclone contig assembly by serial interspersed repetitive sequence (IRS)-PCR product hybridizations

N.German Pasteris¹, Michael D.Bialecki¹ and Jerome L.Gorski^{1,2,*}

Departments of ¹Human Genetics and ²Pediatrics and Communicable Diseases, School of Medicine, University of Michigan, Ann Arbor, MI 48109, USA

Received September 1, 1993; Accepted October 1, 1993

Yeast artificial chromosomes (YACs) are presently the most effective means of cloning large contiguous regions of genomic DNA (1). However, performing a detailed molecular analysis of YAC clone insert DNA is constrained by inherent physical and technical limitations. Typically, an extended analysis of YAC DNA is facilitated by subcloning YAC insert DNA into lambda bacteriophage or cosmid vectors and constructing a contig map (2–4). In the past, a variety of strategies and techniques have been used to construct a contig map of YAC subclones; these have included the use of species-specific repetitive DNA to perform hybridization-based sequence fingerprint analyses (2) and the use of subclone-specific vector-Alu PCR products (3) and RNA end-clone fragments (4) to identify partially overlapping subclones. Each of these techniques, however, requires that individual subclones be isolated prior to analysis. Since, to ensure adequate representation, it is typical to construct a cosmid or bacteriophage library representing 5 to 10 genome equivalents of YAC insert DNA (3, 4), these techniques require the isolation and analysis of numerous individually purified clones.

The construction of a contig map would be expedited by delaying the isolation of individual clones until a minimal tile contig is deduced. Here we report on the use of interspersed repetitive sequence (IRS)-PCR products derived from YAC insert DNA to construct a contig map of bacteriophage subclones prior to the isolation of individual purified clones. To simultaneously identify phage clones containing an IRS-PCR product and localize the product to a specific restriction fragment within the YAC insert, each IRS-PCR product was concurrently hybridized both to filters containing phage plaques plated on numbered grids and blots containing digested YAC DNA.

High molecular weight yeast DNA was isolated by using the sucrose gradient method (1). DNA was partially digested with *Sau3A* by serial dilution (5) and fragments ranging from 15 to 20 kb were isolated from a 0.5% agarose gel by electrolution. Ligation products were packaged by using Gigapack II Plus Gold (Stratagene). Phage clones were plated at low density (1000 plaques/150 mm plate) and filters were prepared and hybridized as described (5). To ensure a five-fold representation of YAC DNA, 6,000 plaques were plated. Duplicate filters were screened with total human genomic DNA as probe. The resulting 55 human positive phage were arrayed in a numbered grid and replica-plated for further analysis.

To obtain numerous randomly-distributed YAC-specific markers, IRS-PCR amplification of YAC DNA was performed. PCRs were 100 µl in volume and contained 100 ng of YAC DNA, 50 mM KCl, 10 mM Tris-HCl, 1.6 mM MgCl₂, 0.01% gelatin, 250 µM each dNTP, and 0.5 µM primer; the primer, TC65, was directed against a consensus 3' *Alu* repetitive sequence and modified to contain a 5' *NotI* restriction endonuclease recognition sequence (6). PCR reactions were incubated at 94°C for 10 min and, after the addition of 3 U AmpliTaq DNA polymerase (Perkin-Elmer/Cetus), 35 cycles of 94°C denaturation (1 min), 55°C annealing (1 min), and 72°C extension (4 min) were performed as described (7). A portion of the amplification product was digested with *EagI* and cloned into a plasmid vector (pBluescript KS). Of 22 recombinants examined, 10 unique amplification products ranging from 0.5 to 3.5 kb in size were identified.

Radiolabeled PCR products were used as hybridization probes against replica filters containing arrayed grids of primary phage clones. IRS-PCR products were denatured for 10 min in 5×SSC, 3 mg/ml sonicated human placental DNA and allowed to preanneal at 65°C for 15 min prior to use as probes. Hybridizations were performed at 65°C in 5×SSPE, 5×Denhardt's solution (5), 0.5% SDS overnight and washed at room temperature in 2×SSC, 0.5% SDS for 15 min, at 65°C in 1×SSC, 0.5% SDS for 15 min, and finally in 0.1×SSC, 0.1% SDS for 15 min. Although the signal varied between probes due to the degree of blocking, generally, the results were robust and unambiguous (Figure 1).

To facilitate the construction of the contig map and address DNA markers (and the phage containing the markers) to a particular region of the YAC, each probe was simultaneously hybridized to PFGE fractionated YAC DNA. YAC DNA preparation, restriction enzyme digestions, PFGE fractionations, and DNA transfers were performed as previously described (8). By hybridizing these probes to YAC DNA digested separately with two different enzymes (*Bss*HII and *Sal*I), IRS-PCR products were unambiguously assigned to one of five distinct intervals (Figure 1).

The distribution of IRS-PCR products and the initial regional DNA marker (DXS323) was such that 47 phage clones were rapidly mapped and overlapped by performing eleven hybridizations. Only one resulting gap (phage 1 and 48) needed

*To whom correspondence should be addressed at: Division of Pediatric Genetics, Department of Pediatrics, 3570 Medical Science Research Building II, PO Box 0688, University of Michigan Medical Center, Ann Arbor, MI 48109-0688, USA

Figure 1. Contig of lambda bacteriophage clones from a 140 kb YAC in the Xp11.21 region (A) and hybridizations of IRS-PCR products to replica filters containing a numbered grid of 32 bacteriophage grown in an ordered array (B). A restriction map of the YAC and the relative localization of contained DNA markers is shown (A). For simplicity, only a subset of the phage clones comprising the contig are shown. Locus DXS323 was used to isolate the YAC clone; LCe5.5 is a 5.5 kb *EcoRI* fragment derived from phage 1; LE is a left YAC end-clone; the remaining DNA markers are isolated IRS-PCR products. The results of hybridizations using products p10, p23, and p28 are shown (B). Probes p10 and p23 both hybridize to phage 24, 27, and 49; probe p28 detects a different set of phage. These results show that the technique is reproducible, robust, and specific.

to be filled by using plaque purified flanking phage (phage 26 and 2) DNA as probe to detect partially overlapping phage clones and complete the contig. The assembled phage contig map across the YAC insert is illustrated in Figure 1A; an example of the actual data is provided in Figure 1B. In principle, this methodology can be applied to YACs of variable size and species, phage or cosmid subclones, and may be amendable to automation. A critical step in this procedure is the generation of the numerous IRS-PCR products. In our laboratory, the TC65 primer was sufficient to generate a relatively dense array (10 products per 140 kb YAC insert) of distributed DNA markers. Primers directed against other repeats may be required in regions with a diminished representation of Alu repeats. We are currently implementing this approach to generate a cosmid contig of an 740 kb YAC with success.

ACKNOWLEDGMENTS

We wish to thank D.Voss for assisting in preparing the manuscript. This work was supported in part by March of Dimes — Birth Defects Foundation Basic Science Grants 1-FY91-0067

and 1-FY93-0326 and National Institutes of Health (NIH) grants HD23768 and NS30771 to J.L.G.

REFERENCES

1. Burke, D.T., Carle, G.F. and Olsen, M.V. (1987) *Science* **236**, 906–912.
2. Bellanne-Chantelot, C., Barillot, E., Lacroix, B., Le Paslier, D., and Cohen, D. (1991) *Nucleic Acids Res.* **19**, 505–510.
3. Pieretti, M., Tonlorenzi, R. and Ballabio, A. (1991) *Nucleic Acids Res.* **19**, 2795–2796.
4. Whittaker, P.A., Wood, L., Mathrubutham, M. and Anand, R. (1993) *Genomics* **15**, 453–456.
5. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
6. Nelson, D.L., Ledbetter, S.A., Corbo, L., Victoria, M.F., Ramirez-Solis, R., Webster, T.D., Ledbetter, D.H. and Caskey, T. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 6686–6690.
7. Gorski, J.L., Burright, E.N., Reyner, E.L., Goodfellow, P.N., and Burgess, D.L. (1992) *Genomics* **14**, 649–656.
8. Gorski, J.L., Boehnke, M., Reyner, E.L. and Burright, E.N. (1992) *Genomics* **14**, 657–665.