nies and a corresponding reduction in the number of blue colonies by 50%. The optimum amount of genomic DNA will vary for each mini-library created because it is dependent on the number of restriction fragments of that size present in the genome of interest and must be determined empirically.

The average insert size was checked in six colonies picked at random (Figure 1). The DNA was digested with PvuII, which removes the insert and 500 bp of flanking vector DNA. Of the six colonies, four have inserts of around 3.0–3.5 kb, as expected. Two clones contained much smaller fragments of DNA. This could reflect contamination of the size-selected insert in combination with preferential insertion of smaller fragments. Alternatively, it could represent instability of the AT-rich Dictyostelium DNA when present in bacteria. We favor the second possibility because this is a known feature of Dictyostelium genomic DNA.

**Isolation of the mo15 gene.** Colonies derived from the the most efficient insertion ligation were screened using the mo15 cDNA clone as probe (3), and out of approximately 8000 white colonies screened, 20 came through two rounds of screening containing the mo15 gene. This was confirmed by PCR analysis using primers based on the known coding sequence (data not shown). When DNA was isolated from two of these, the insert size was found to be approximately 3 kb, as expected, and DNA sequence analysis confirmed the presence of the mo15 gene. These are independent isolates because restriction analysis revealed that each was in a different orientation relative to the vector sequences (data not shown).

This quick and efficient method for mini-library creation increases the range of restriction enzymes that can be used to isolate a fragment of suitable size and circumvents the necessity to generate a batch of digested, phosphatased vector with a low background of self-ligation for each restriction enzyme used. It is particularly useful when isolating clones from organisms whose DNA is unstable in bacteria, because the desire not to amplify libraries means that it is often necessary to generate a fresh library several times to isolate more than one gene.

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Hao-Jen Huang and Catherine Pears
University of Oxford
Oxford, England, UK
chimeric, and the isolation of pure, cloned DNA can prove difficult (6).

The bacterial artificial chromosome (BAC) system has recently been used to overcome many of the problems presented by YACs. The BAC system allows the stable maintenance of relatively large (>300 kb) genomic DNA fragment libraries as single-copy supercoiled plasmids in an E. coli recombination-deficient host. BAC libraries have been successfully constructed using human, bovine and plant DNAs and have proven to be mostly free of chimeric clones (2,3,5,6,8).

Although the construction of BAC libraries is relatively easy, several steps can be tedious. The single-copy nature of the vector pBel0BAC11 makes purification of this plasmid laborious (3,8). The method of Woo et al. (8) required growing 4 L of E. coli culture, using several large-scale QIAGEN® Plasmid Maxi Kits (Qiagen, Chatsworth, CA, USA), followed by a three-day cesium chloride gradient ultracentrifugation. The final yield was 50–70 µg of vector DNA. Because only 25–40 ng of vector DNA are required per ligation reaction (6), we reasoned that a medium-scale plasmid preparation (midiprep) should result in sufficient vector for several ligation reactions. For example, 100 mL of culture would yield 1–1.5 µg of vector, enough for approximately 40 ligations. However, initial attempts to obtain the pBel0BAC11 vector by pooling multiple alkaline lysis miniprep extractions were not successful because the plasmid preparations were contaminated with significant amounts of genomic DNA and low-molecular-weight RNA. Pure plasmid vector was obtained by performing a novel and rapid boiling lysis that removed most of the genomic DNA and high-molecular-weight RNA. The low-molecular-weight RNA was removed by a low-salt alcohol precipitation and the remaining high-molecular-weight genomic DNA by agarose gel purification. This rapid technique can be performed in one day and yields approximately 1 µg of pure pBel0BAC11 vector. Specifically, the protocol entails the following steps.

First, the pBel0BAC11 vector in the E. coli strain DH10B™ (Life Technologies, Gaithersburg, MD, USA) was streaked out on LB plates containing 12.5 µg/mL of chloramphenicol, 50 µg/mL isopropyl β-d-thiogalactopyranoside (IPTG), 25 µg/mL of 5-bromo-4-chloro-3-indolyl-β-d-galactopyranoside (X-gal). The plate was incubated overnight at 37°C.

Second, a single blue colony was picked and inoculated into 100 mL of Super Broth (4) containing 20 µg/mL of chloramphenicol and incubated for 20 h with shaking at 37°C.

Third, the cells were harvested by transferring 2-mL aliquots of the culture into 24 × 2-mL Eppendorf tubes (Hamburg, Germany). The tubes were centrifuged at 12 000× g for 10 s to pellet the cells, and the supernatant was removed by aspiration. An additional 2 mL of culture were added to each tube, the cells pelleted and the supernatant aspirated once more.

Fourth, the cell pellets were resuspended in 100 µL of TER buffer (200 mM Tris-HCl, pH 7.4, 20 mM EDTA, pH 8.0, 200 µg/mL RNase A) and 950 µL of XS buffer (1% potassium ethyl xanthogenate [Fluka, Buchs, Switzerland], 100 mM Tris-HCl, pH 7.4, 20 mM EDTA, pH 8.0, 1% sodium dodecyl sulphate [SDS], 800 µm ammonium acetate). The tubes were inverted several times and heated in a boiling water bath for 45 s. The tubes were removed and incubated on ice for 30 min to allow the cell debris to precipitate.

Fifth, the tubes were centrifuged at 12 000× g for 15 min. The supernatants were transferred to fresh 24 × 2-mL Eppendorf tubes containing 1 mL of isopropanol. The tubes were inverted several times and left to incubate on ice for 10 min.

Sixth, the tubes were centrifuged at 12 000× g for 10 min. The DNA pellets were washed once with 1 mL of 70% ethanol and resuspended in 80 µL of TE buffer (10 mM Tris-HCl, pH 7.4, 1 mM EDTA, pH 8.0). The DNA solutions were pooled into 2 × 2-mL Eppendorf tubes, and 10 µL of 5 M NaCl were added to each tube. The tubes were centrifuged at 12 000× g for 5 min and the supernatant transferred to two fresh tubes containing 950 µL of isopropanol. After being inverted several times, the tubes were incubated at room temperature for 10 min, followed by centrifugation at 12 000× g for 8 min. The DNA pellets were washed once with 70% ethanol, lyophilized in a SpeedVac® (Savant Instruments, Farmingdale, NY, USA) and resuspended in 90 µL TE buffer.

Seventh, 10 µL of 10× loading buffer (50% glycerol, 0.1% bromophenol blue) were added to each tube and
Eighth, the gel slices were placed in a 2-mL Eppendorf tube containing 1 mL of 500 mM NaCl and allowed to incubate for 30 min at room temperature. The equilibrated gel pieces were transferred without salt solution into 4 empty Wizard® DNA Purification Columns (Promega, Madison, WI, USA). The Wizard columns containing the gel pieces were frozen at -70°C for 10 min. The columns were placed in 1.5-mL Eppendorf tubes and spun at 12,000×g for 12 min. The supernatant was pooled into two tubes and extracted twice with 700 µL of chloroform/isoamyl alcohol (24:1). A 1/10 vol of 3 M sodium acetate and 1 vol of isopropanol were then added to each tube and inverted several times. The tubes were incubated at -20°C for 30 min, then spun at 12,000×g for 15 min. The DNA pellets were washed twice with 70% ethanol, lyophilized and resuspended in 100 µL of TE buffer. The concentration, purity and yield of the pBeloBAC11 vector were determined by electrophoresing 2 µg of DNA into yeast by means of artificial chromosome vectors. Science 236:806-812.

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Daniel Tillett and Brett A. Neilan
The University of New South Wales
Sydney, NSW, Australia