

Supplementary material

Fosmid library construction and clone isolation for physical mapping

Genomic *C. porosus* DNA was physically sheared to generate random fragments. The extent of DNA shearing was ascertained by resolving the sheared DNA via pulse-field gel electrophoresis (PFGE) using the CHEF DRIII Pulsed Field Electrophoresis System (Bio-Rad), and comparison against a Pulse Marker™ 0.1-200 KB (Sigma). The gel-run parameters were as follows: 1.1% low melting-point (LMP) agarose (Promega)/1 X TBE buffer, current; 6 volts/cm, included angle, 120°; switch time linearly ramped from 1-9 seconds; run time, 15 hours; temperature, 14°C. After completion of the run time, the LMP agarose gel was stained with SYBR-Gold, and visualized under UV light.

Sheared DNA having a large proportion of 30-50 kb fragments was end repaired to generate blunt-ended 5'-phosphorylated DNA. End repaired DNA was resolved by PFGE against a Pulse Marker™ 0.1-200 KB (Sigma) to ensure correct DNA size selection, the LMP gel was stained with SYBR-Gold, and visualized under blue light to avoid DNA degradation. End-repaired DNA fragments migrating to 30-50 kbp in length were excised and recovered by gel purification.

Size fractionated, end-repaired DNA was ligated into vector PCC1FOS™, packaged using MaxPlax Lambda Packaging Extracts and transformed into Phage T1-resistant EP1300™-T1^R *E. coli* plating strain according to the manufacturer's instructions (CopyControl™ Fosmid Library Production Kit, Epicentre® Biotechnologies, Madison, Wisconsin USA). The titer of the phage particles was then determined. A random selection of the fosmid clones were induced to high copy number for high yields of DNA, and minipreps were performed according to Sambrook et al. (1989) after growing and

inducing these fosmid clones in 5 ml of media. This procedure was also performed in 800 μ l of media in 1.5 ml deep 96-well plates. The proportion of clones with inserts and the average size of inserts was then determined by restriction digestion.

Following validation of the fosmid library's quality and induction, approximately 1000 fosmid clones were plated at optimal density for picking. Clones were picked into 96-well plates with 300 μ l of LB broth supplemented with chloramphenicol (12.5 μ g/ml) and incubated at 37°C overnight. These cultures were then used to inoculate duplicate cultures induced for high copy number. The eight deep-well plates used contained 768 selected fosmid clones and were arranged in a 4 \times 2 (row \times column) array. A portion of the medium from each well was pooled into its respective row and column (32 rows, 24 columns). For quality control, an additional pool was made for each plate. DNA was purified from each of the pools (32 + 24 + 8 = 64), as well as for a combined pool of the overall plate array.

Small insert libraries enriched for microsatellite repeats were constructed using DNA from the combined pools of the fosmid plate array following the protocol of Glenn and Schable (2005), and primers were then designed and optimized according to Miles et al. (2009b).

To identify individual fosmid clones harbouring microsatellite DNA loci, the fosmid plate array was screened by PCR amplification of row and column DNA pools using the respective microsatellite primer sets. The PCR conditions are as described in Miles et al. (2009b). Fosmid clones positive for microsatellite repeats were purified to provide cloned DNA fragments of sufficient size to be used as probes in fluorescent in situ hybridization (FISH) assays.