Supplementary material for Barnett *et al*. (July 31, 2001) *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.161294798.

Purification and cloning of pSymA DNA. *Sinorhizobium meliloti* strain 1021 (1) was grown in TY broth to an $OD_{600} = 1$ (2). Agarose-embedded DNA was prepared by using a CHEF bacterial genomic DNA plug kit (Bio-Rad) with the modification that more cells were used, approximately 2×109 cells for each milliliter of agarose plugs. Because the circular replicons of *S. meliloti* are not easily separated, even by pulsed-field gel electrophoresis (PFGE; refs. 3 and 4), agarose-embedded DNA was digested with the restriction enzyme *Swa*I (Boehringer Mannheim, 40 units enzyme per plug). *Swa*I linearizes pSymA into a 1.4-Mb DNA that is easily separated from the chromosomal and pSymb DNAs by using a CHEF-DR III apparatus (Bio-Rad) and the following conditions: 1% pulsed-field certified agarose (Bio-Rad) in $1/2 \times$ TBE buffer (90 mM Tris/64.6 mM boric acid/2.5 mM EDTA, pH 8.3), a switch time ramped from 60 to 120 s over 24 h, 120 degrees field angle, and a field strength of 6 V/cm. After PFGE, the pSymA band was excised and electroeluted in a dialysis bag by using PFGE and the same conditions described above. The yield of purified pSymA DNA per 14 cm gel was approximately 0.3 μg. The pSymA DNA was purified either once or twice by this procedure. DNA from multiple gels was pooled, concentrated, and sheared randomly (5). A portion of this DNA, 1–2 kb in size, was purified by HPLC, cloned into a linker/adaptor version of M13mp18 to minimize chimeras (R.W.H., unpublished work), and sequenced by using the BigDye terminator technology on ABI377-XL sequencers.

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