

Supplementary Methods

Library Construction. The *A. oryzae* nuclear DNA was sheared randomly into pieces of approximately 2 kb in size using HydroShear® (GeneMachines). The fragments were blunted using BAL 31 nuclease (Takara) and Klenow Fragment (Takara), ligated with adaptors prepared by annealing 5'CGAGAGCGGCCGCTAC and 5'CTCGCCGGCGATG. The adaptor modified inserts were ligated at 16°C overnight with pUC19 prepared by digestion with *SalI*, filled in with dT and treated with calf intestine alkaline phosphatase (Takara Bio, Otsu, Japan). The ligation mixture was transformed into DH10B cells (Invitrogen) by electroporation yielding whole genome shotgun library. A cosmid library was prepared using SuperCos 1 (STRATGENE, La Jolla, CA) according to the manufacturer's instructions. A bacterial artificial chromosome (BAC) library with 75.7 kb of an average insert length was prepared by MacroGen (Seoul, Korea).

Sequencing. High quality sequences were acquired by sequencing both ends of the inserts of approximately 250,000 WGS clones using ABI PRISM 3700 capillary DNA sequencers. Phred/Phrap software was used to assemble the raw sequence reads. Long-read sequencing of bridging WGS clones by a LI-COR Model 400L DNA sequencer was applied to fill short gaps. Sequences of both ends of inserts of approximately 5,500 cosmid and 6,700 BAC clones were incorporated into WGS. A part of the clones including PCR amplicons using *A. oryzae* genomic DNA as a template were used to connect WGS contigs and were sequenced to fill corresponding gaps. The assembly of raw sequences (501,985 reads) including the cosmid- and BAC-end sequences reached over 9X depth of coverage. The assembly consisted of 6 scaffolds and 10 contigs (or 24 contigs in total), and has a total contig length of 37,047,050 bases (see Supplementary Table S1). The assembly was validated by the presence of the end-sequenced BAC or cosmid clones that supports the assembly (approximately 15X

and 5X coverage of the genome size by the inserts, respectively, in total). Consequently, 99.37% of the assembly was covered by at least one BAC or cosmid clone. All the uncovered regions were located only at the termini (i.e. not inside) of the contigs.

Mapping Contigs. Contigs were mapped to each chromosome by hybridization using probes prepared at the regions close to each terminus and the middle of the contig. The relative positions of contigs were determined by the finger printing method. Probes were used for the Southern hybridization analysis with *NotI*, *PmeI*, *PacI*, *BamHI*, *EcoRI*, *KpnI*, *SacI*, *Sall*, *SphI* or *XbaI* digests of *A. oryzae* genomic DNA.

Southern Hybridization. Agarose-embedded *A. oryzae* chromosomal DNA was prepared from protoplasts. Electrophoretic separation of chromosomes was accomplished in 0.8% agarose (Certified Megabase Agarose, Bio-Rad, Hercules, CA) with 1x TAE buffer using CHEF-Mapper (Bio-Rad) (constant power 1.5 V/cm, 14°C, an angle of 106° between the field vectors, 73 h with 3,000 s pulse time, 18 h with 2,700 s pulse time and 73 h with 2,300-2,200 s of linear ramping pulse time). After soaking the gel in 0.25 N HCl for 30 min, the DNA was transferred to Hybond-N+ nylon membrane (Roche Diagnostics, Basel, Switzerland) by a capillary transfer method. The probe DNA was prepared by PCR using *A. oryzae* genomic DNA as a template and were labeled by PCR DIG Synthesis Kit (Roche, Diagnostics). Hybridization and detection were performed according to the manufacturer's instructions.

Pulse Field Gel Electrophoresis. *A. oryzae* genomic DNA prepared in the agarose plug described above was digested by *NotI*, *PacI* or *PmeI* and electrophoresed on 1.0% agarose (Pulsed Field Certified Agarose, Bio-Rad) with 0.5x TBE buffer using CHEF-Mapper (constant power 6.0 V/cm, 14°C, an angle of 120° between the field vectors, 26 h with 12-35 sec of linear ramping pulse time). The transfer onto nylon membrane, hybridization and detection were carried out as described above.