**File S1**

**Materials and Methods**

***Manipulation of Neurospora strains***

Strains used in this study include P9-42 (Oak Ridge wild type *a*), P12-02 ($r^{\Delta}$::*hph a*), and F2-01 (*fl A*). Mutant alleles used, including $r^{\Delta}$ (which contains an *hph*-mediated gene replacement; Colot *et al*. 2006), were obtained from the Fungal Genetics Stock Center (McCluskey 2003) and are described in the *Neurospora* Compendium (Perkins *et al*. 2001) and e-Compendium (http://www.bioinformatics.leeds.ac.uk/~gen6ar/newgenelist/genes/gene_list.htm). Culturing and crossing media were prepared as previously described (Vogel 1964; Westergaard and Mitchell 1947). Standard procedures for growth, crosses, and other *Neurospora* manipulations were used during the course of this study (Davis and de Serres 1970).

***Small RNA library preparation***

Half a gram of four-and-a-half-day-old perithecia (fruiting bodies) were harvested as previously described (Shiu and Glass 1999). Small RNAs were extracted on a glass fiber filter using the *mir*Vana miRNA Isolation Kit (Ambion, Austin, TX). cDNA libraries were constructed with the Small RNA Sample Prep Kit v.1.5 (Illumina, San Diego, CA). Each purified library was evaluated using the Experion automated electrophoresis system (with the DNA 1K chip; Bio-Rad, Hercules, CA), quantified with the Qubit fluorometer using the Quant-iT dsDNA HS Kit (Invitrogen, Carlsbad, CA), and diluted according to Illumina's standard protocol for sequencing on the Genome Analyzer (GA) II. One sample was used per sequencing lane.

***Illumina sequencing***

cDNA molecules were ligated to adapters at both ends and subsequently attached to the solid surface of a reaction chamber (flow cell), which had been coated with oligonucleotides complementary to adapter sequences. Each DNA fragment bent over and the unattached adapter annealed with an oligonucleotide primer, forming a "bridge". After repeated cycles of polymerase-based denaturation and extension (bridge amplification), a "cluster" of homogeneous molecules (~1000 copies) was produced for each DNA fragment. A single flow cell contains 8 lanes (channels), and each can accommodate several millions of clusters. The molecules in each cluster were sequenced using a four-color DNA sequencing-by-synthesis approach that utilizes reversible terminators with removable fluorescent dyes. A high-resolution image of the flow cell was captured after each sequencing cycle, allowing the reading of nucleotides added to each individual cluster.

***Computational analyses***

Flow cell image files from the Illumina Genome Analyzer (GA) II were processed using the GA Pipeline v.1.4 software, yielding 10.2 ± 1.2 million reads per sequencing run (lane). These data are available through the NCBI Sequence Read Archive (SRA) database (SRX247455–247456). The 3'-adapters of the Illumina sequences were trimmed and all sites of alignment of the remaining sequence to the reference genome were identified using the program SOAP (Li *et al*. 2008), allowing for no mismatches or base call ambiguity in either adapter or insert. The reference genome (*N. crassa* OR74A, NC10) was obtained from the Broad Institute website (http://www.broadinstitute.org/annotation/genome/neurospora/MultiDownloads.html). Computation was performed using the High Performance Computing resources at the University of Missouri Bioinformatics Consortium. Downstream processing of the results of the analysis by SOAP was performed using custom Linux shell scripts. DNAPlotter and Tablet were used for whole genome sequence and alignment visualization, respectively (Carver *et al*. 2009; Milne *et al*. 2010).

**References**

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