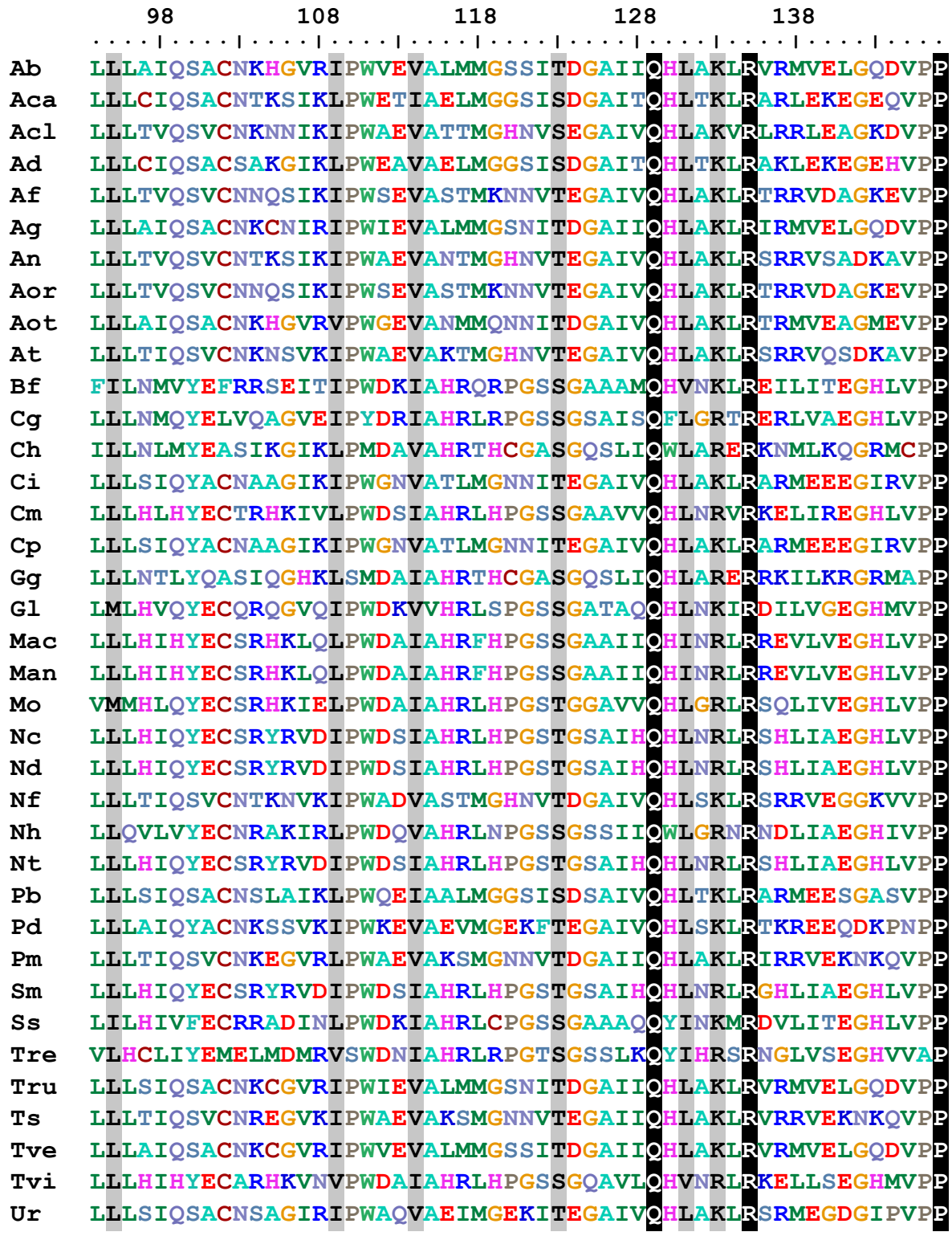

Figure S1 Conserved region in SAD-4 homologs. Positions are relative to *N. crassa* SAD-4. Strain names and sequence accession numbers are listed in Table S2.

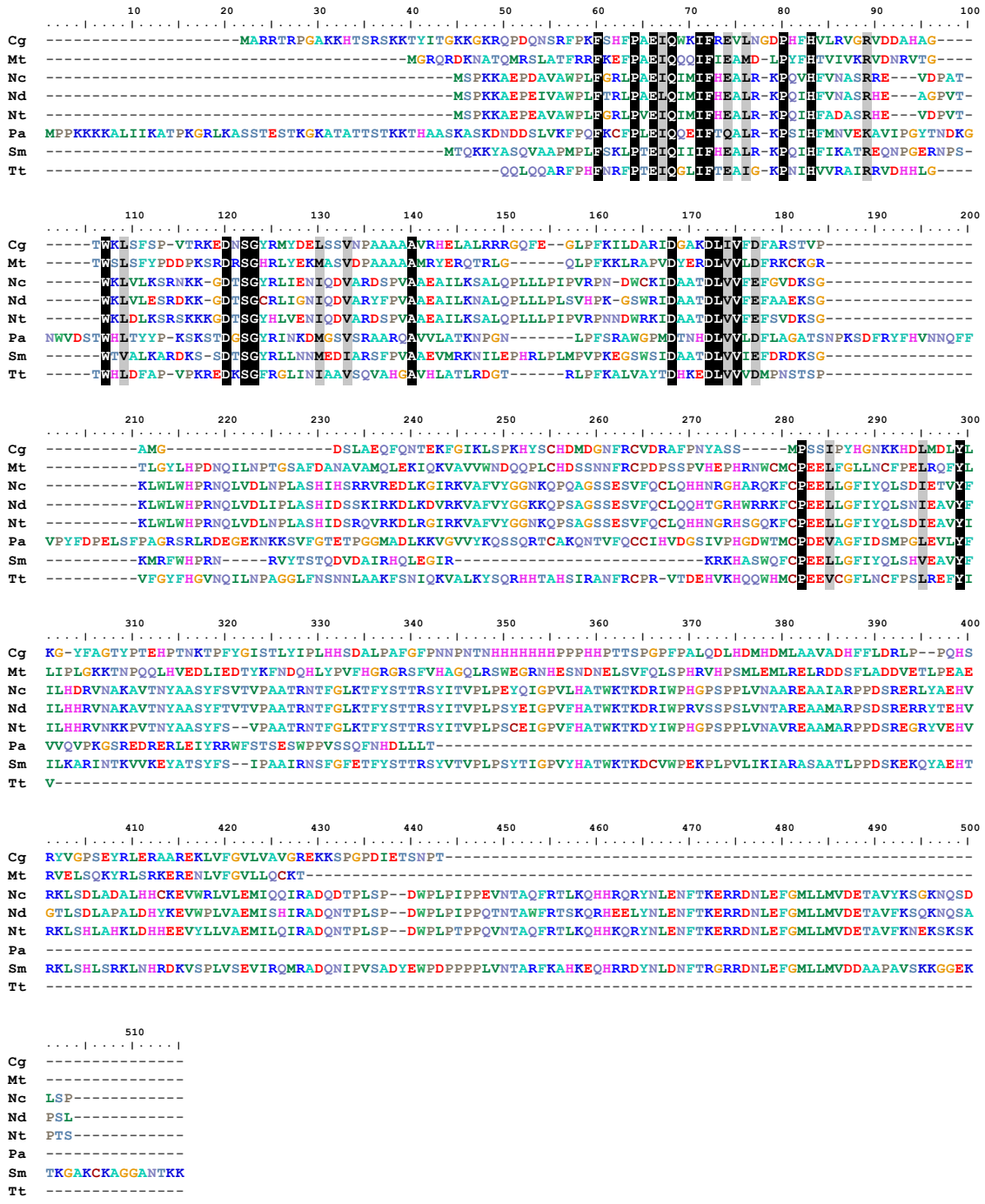

Figure S2 Alignment of SAD-5 homologs. Strain names and sequence accession numbers are listed in Table S2.

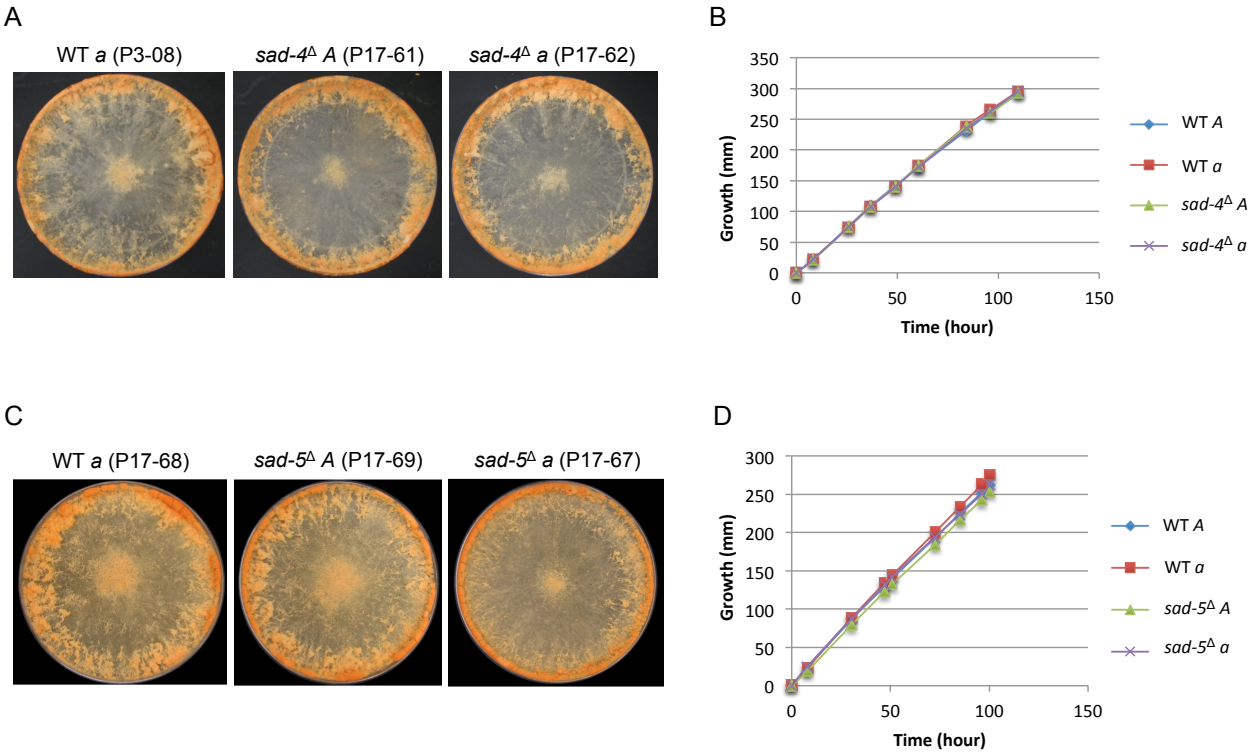

Figure S3 *sad-4* and *sad-5* mutants appear normal during the vegetative phase. *sad-4*$^{\Delta}$ and *sad-5*$^{\Delta}$ strains have normal mycelial morphology (A and C) and growth (B and D).

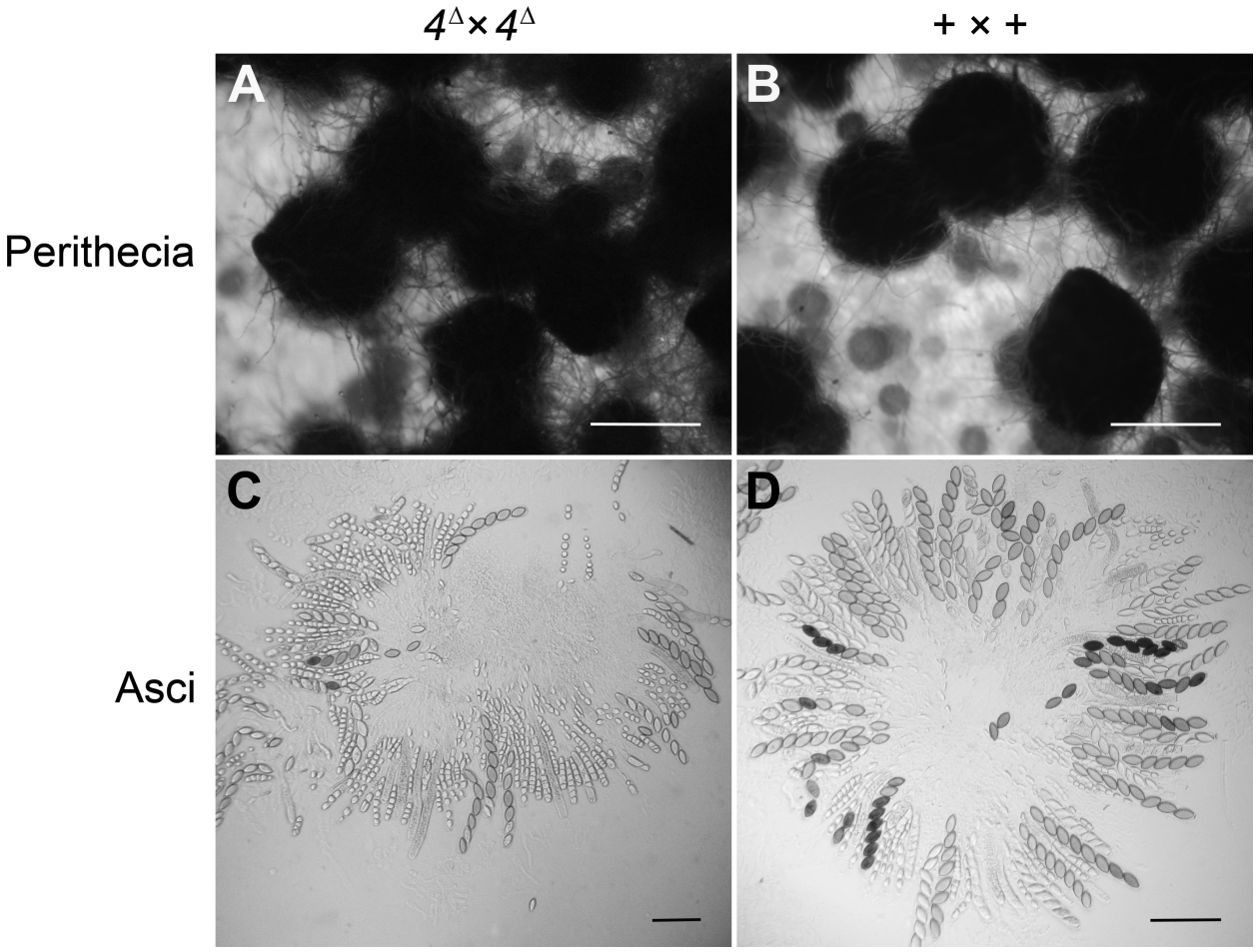

Figure S4 Crosses homozygous for *sad-4*$^{\Delta}$ produce fewer ascospores. (A and B) No differences in perithecial development and abundance were detected between *sad-4*$^{\Delta}$ (F5-32 × P17-62) and control (F5-23 × P3-08) crosses. Bars, 500 µm. (C and D) *sad-4*–null crosses produce more aborted (bubble) asci. Bars, 100 µm.

Table S1 Primers used in this study

Purpose	Primer	Sequence (5' to 3')
sad-4$^{\Delta}$::*hph* confirmation	NCU01591-294825F	TGGCATGAGACTGGCATCTGAG
	NCU01591-299764R	GCAGAAGCGGAAGACATGTCAAG
sad-5$^{\Delta}$::*hph* confirmation	NCU06147-438124F	GACCATGGCCACGACTACAGCTAC
	NCU06147-442142R	ATATGAGACCTGCGCGCTAGCCAAC
rfp-sad-4 construction	NCU01591-296360F*NotI	GTCTAGCGGCCGCGATCATATCAAG
	NCU01591-298299R*SpeI	CCAGACTAGTCTTCCGAATACTAAAATG
gfp-sad-5 construction	NCU06147-E	TCTGCGGAACAATATGAACAACCTG
	NCU06147-F	AGGACAACCCTCCACTCGCAAAG
	NCU06147-G	AGGCTCGAACTCCCGACCTCA
	NCU06147-H	CCGCTTCACGGGCTGCGTTGAC
	NCU06147-NGFP1	GCAGCCTGAATGGCGAATGGACGCGCAGCTGAGACGAGGTGCCGAAAG
	NCU06147-NGFP2	CAGGAGCGGGTGCGGGTGCTGGAGCGATGAGTCCCAAAAAGGCCGAGCCTGAC
sad-1 cDNA amplification	SAD1-4489F	ATGCTCATCTGGCGACAGCAGATG
	SAD1-4796R	TTCTCTTTCATGTCGAATGTTTCCC
sad-4 cDNA amplification	NCU01591-296801F	CTGGTGTAGCCGTTGTGGTTCG
	NCU01591-298048R	GCGTCGAAGGCAGGAACTTGAT
sad-5 cDNA amplification	NCU06147-F2-TH	CGCTTTCGTTTACGGTGGGAAC
	NCU06147-R2-TH	GGCCAGATGCGATCCTTTGTCT
actin cDNA amplification	ACT-507F	CGTTGGTCGTCCCCGTTATCATG
	ACT-811R	TGGGAGCCTCGGTAAGAAGGACG

Table S2 Sequences used in this study

Protein	Abbreviation[1]	Accession #[2]	Strain
SAD-4	Ab	XM_003014454.1	*Arthroderma benhamiae* CBS 112371
	Aca	XM_001539307.1	*Ajellomyces capsulatus* NAm1
	Acl	XM_001267850.1	*Aspergillus clavatus* NRRL 1
	Ad	XM_002623645.1	*Ajellomyces dermatitidis* SLH14081
	Af	XM_002377995.1	*Aspergillus flavus* NRRL3357
	Ag	XM_003173571.1	*Arthroderma gypseum* CBS 118893
	An	XM_001395616.2	*Aspergillus niger* CBS 513.88
	Aor	XM_001826292.2	*Aspergillus oryzae* RIB40
	Aot	XM_002844811.1	*Arthroderma otae* CBS 113480
	At	XM_001218470.1	*Aspergillus terreus* NIH2624
	Bf	CCD55484.1	*Botryotinia fuckeliana*
	Cg	XP_001228162.1	*Chaetomium globosum* CBS 148.51
	Ch	CCF43159.1	*Colletotrichum higginsianum*
	Ci	XM_001242382.1	*Coccidioides immitis* RS
	Cm	EGX93189.1	*Cordyceps militaris* CM01
	Cp	XM003069573.1	*Coccidioides posadasii* C735 delta SOWgp
	Gg	EFQ30009.1	*Glomerella graminicola* M1.001
	Gl	EHK98895.1	*Glarea lozoyensis* 74030
	Mac	EFY84375.1	*Metarhizium acridum* CQMa 102
	Man	EFY96871.1	*Metarhizium anisopliae* ARSEF 23
	Mo	XM_360623.1	*Magnaporthe oryzae* 70-15
	Nc	BK006474	*Neurospora crassa* OR74A
	Nd	102414[3]	*Neurospora discreta* 8579 *mat A*
	Nf	XM_001260425.1	*Neosartorya fischeri* NRRL 181
	Nh	XP_003051566.1	*Nectria haematococca* mpVI 77-13-4
	Nt	EGO56400.1	*Neurospora tetrasperma* FGSC 2508
	Pb	XM_002789947.1	*Paracoccidioides brasiliensis* Pb01
	Pd	EKV11485.1	*Penicillium digitatum* PHI26
	Pm	XM_002149363.1	*Penicillium marneffei* ATCC 18224
	Sm	XP_003350666.1[4]	*Sordaria macrospora* k-hell
	Ss	XP_001597890.1	*Sclerotinia sclerotiorum* 1980
	Tre	EGR52832.1	*Trichoderma reesei* QM6a
	Tru	XM_003235041.1	*Trichophyton rubrum* CBS 118892
	Ts	XM_002484729.1	*Talaromyces stipitatus* ATCC 10500
	Tve	XM_003019899.1	*Trichophyton verrucosum* HKI 0517
	Tvi	EHK24463.1	*Trichoderma virens* Gv29-8
	Ur	XM_002544541.1	*Uncinocarpus reesii* 1704
SAD-5	Cg	XP_001227787.1	*Chaetomium globosum* CBS 148.51
	Mt	XP_003665043.1	*Myceliophthora thermophila* ATCC 42464
	Nc	BK006779	*Neurospora crassa* OR74A
	Nd	62731[3]	*Neurospora discreta* 8579 *mat A*
	Nt	EGO55361.1	*Neurospora tetrasperma* FGSC 2508
	Sm	XP_003344714.1	*Sordaria macrospora* k-hell
	Pa	XP_001905702.1	*Podospora anserina* S *mat+*
	Tt	XP_003654540.1[5]	*Thielavia terrestris* NRRL 8126

[1]Abbreviations are used in Figures S1 and S2. [2]GenBank accession numbers are listed unless otherwise stated. [3]*N. discreta* database at DOE-JGI (http://genome.jgi-psf.org/). [4]A putative intron was removed from its ORF. [5]This sequence is incomplete.

Table S3 Small RNA sequencing and alignment data

	$r^{\Delta} \times r^{+}$	$r^{\Delta}4^{\Delta} \times r^{+}4^{\Delta}$	$r^{\Delta}5^{\Delta} \times r^{+}5^{\Delta}$	$r^{+} \times r^{+}$
Raw reads	58,558,484	31,162,221	31,162,221	36,467,267
≥14 nt after adapter trimming	55,022,756	26,001,036	22,318,233	32,655,370
Homology with the genome	51,641,029	24,578,034	20,920,863	30,650,403
18-30 nt long	33,392,504	15,763,605	14,098,686	20,701,772
No mismatches	30,016,594	14,093,205	12,738,940	18,758,512

Shown are numbers of small RNA reads after each processing step. See Figure 6 for cross information.