

# A rapid PCR-based method to determine the *Neurospora crassa* mating type

Anke Schäfer | Frank Kempken 

Abteilung Botanische Genetik und Molekularbiologie, Botanisches Institut und Botanischer Garten, Kiel, Germany

## Correspondence

Frank Kempken, Abteilung Botanische Genetik und Molekularbiologie, Botanisches Institut und Botanischer Garten, Christian-Albrechts-Universität zu Kiel, Olshausenstraße 40, Kiel 24098, Germany.  
Email: [fkempken@bot.uni-kiel.de](mailto:fkempken@bot.uni-kiel.de)

## Abstract

So far mating type determination in *Neurospora crassa* requires test crosses with strains of known mating type. We present a simple, quick, and reliable polymerase chain reaction-based method for mating type determination in *N. crassa*.

## KEYWORDS

mating type, *Neurospora crassa*, PCR-based mating type identification

Rapid mating type determination in *Neurospora crassa* currently requires the maintenance of cultures with viable protoperithecia and an overnight incubation [1]. We present a reliable polymerase chain reaction (PCR)-based alternative that can be completed in a few hours avoiding the need to maintain fresh cultures of tester strains of known mating type.

*N. crassa* was established as a model organism about a hundred years ago and a great wealth of data has been established which has been reviewed extensively [2, 3].

It has long been known that the heterothallic species *N. crassa* has two mating types designated “A” and “a.” The DNA sequences that determine mating behavior are completely different in *a* and *A* mycelia, and have been termed idiomorphs instead of alleles because the sequences at the same locus in different strains are dissimilar [4, 5]. As shown in Figure 1a, the 5.3 kb mating type locus *A* contains three genes *matA-1*, *matA-2*, and *matA-3* [6, 7]. The 3.2 kb mating type locus *a* was first thought to contain one gene only but a second one, *mata-2* (see Figure 1b), was discovered from a comparative study employing the fungus *Sordaria macrospora* [8, 9].

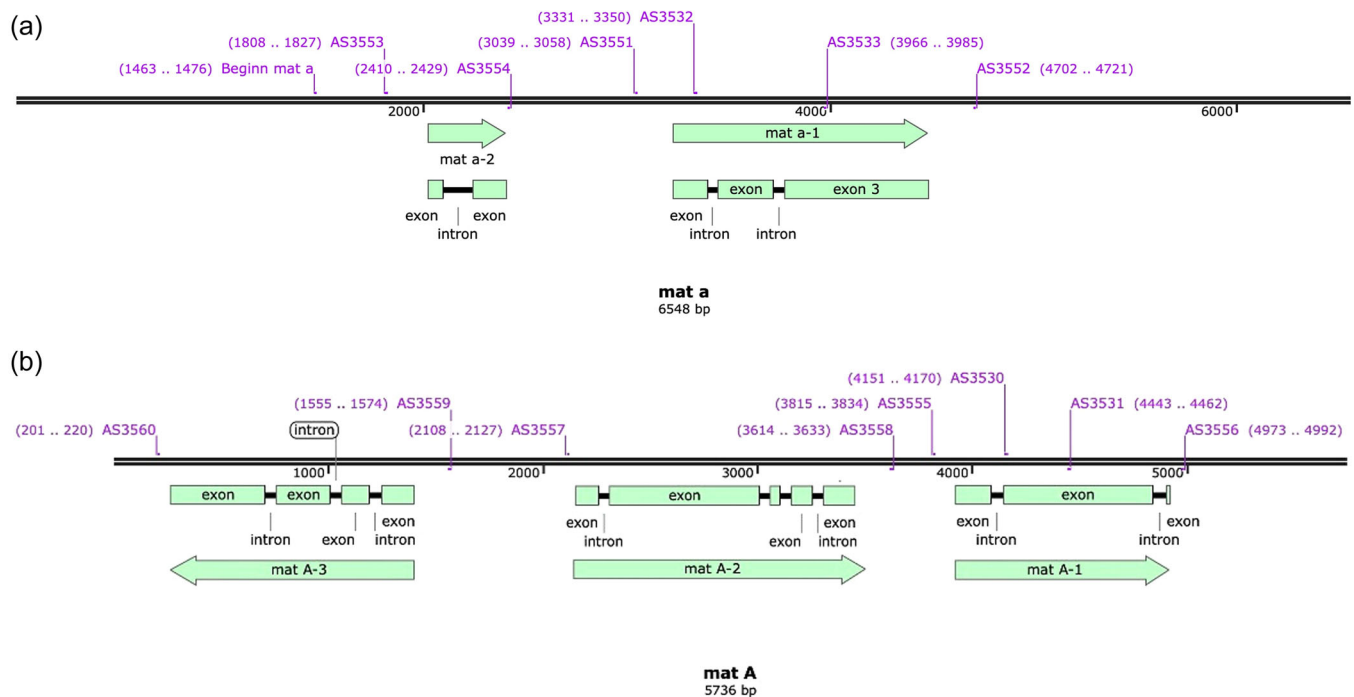
Mating type determination of any given strain of *N. crassa* is based on crossing with a fertile strain of known mating type and can be speeded by maintaining plates of fluffy testers [1] with viable protoperithecia that darken overnight when inoculated with a loop of conidia of the opposite mating type. Here, we describe another approach for the identification of mating type employing newly developed oligonucleotides and PCR amplification.

The DNA sequences for the *N. crassa* mating type genes *matA-1*, *matA-2*, and *matA-3* are found in GenBank accession M33876.1 and for mating type *mata-1* and *mata-2* genes in accession M54787.1. We used *N. crassa* wild-type mycelia with different mating type loci. Mycelium was grown in 3 mL vogels minimal medium (VMM) [10] containing sucrose for 2 days at 28°C and shaking at 150 rpm. The mycelium was washed once with water and dried using filter paper. DNA was isolated using the Quick-DNA Fungal/Bacterial Miniprep. Kit (Zymo Research) according to the manufacturers' recommendation. The oligonucleotides are given in Table 1. PCR was done using Dream Taq Green DNA Polymerase (Thermo Scientific) and 30 ng of fungal DNA in a 15 µL volume employing the oligonucleotides shown in Table 1. PCR conditions were 3'

**Abbreviations:** ORF, open reading frame; PCR, polymerase chain reaction; TE, Tris-HCl/EDTA; VMM, vogels minimal medium.

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**FIGURE 1** Organization of mating type loci in *Neurospora crassa* and localization of oligonucleotides. (a) mating type “a”; (b) mating type “A.” Introns are shown as lines and open reading frames as open boxes. Gene orientation is shown by a green arrow. Oligonucleotide positions are indicated.

**TABLE 1** Oligonucleotides employed in this study.

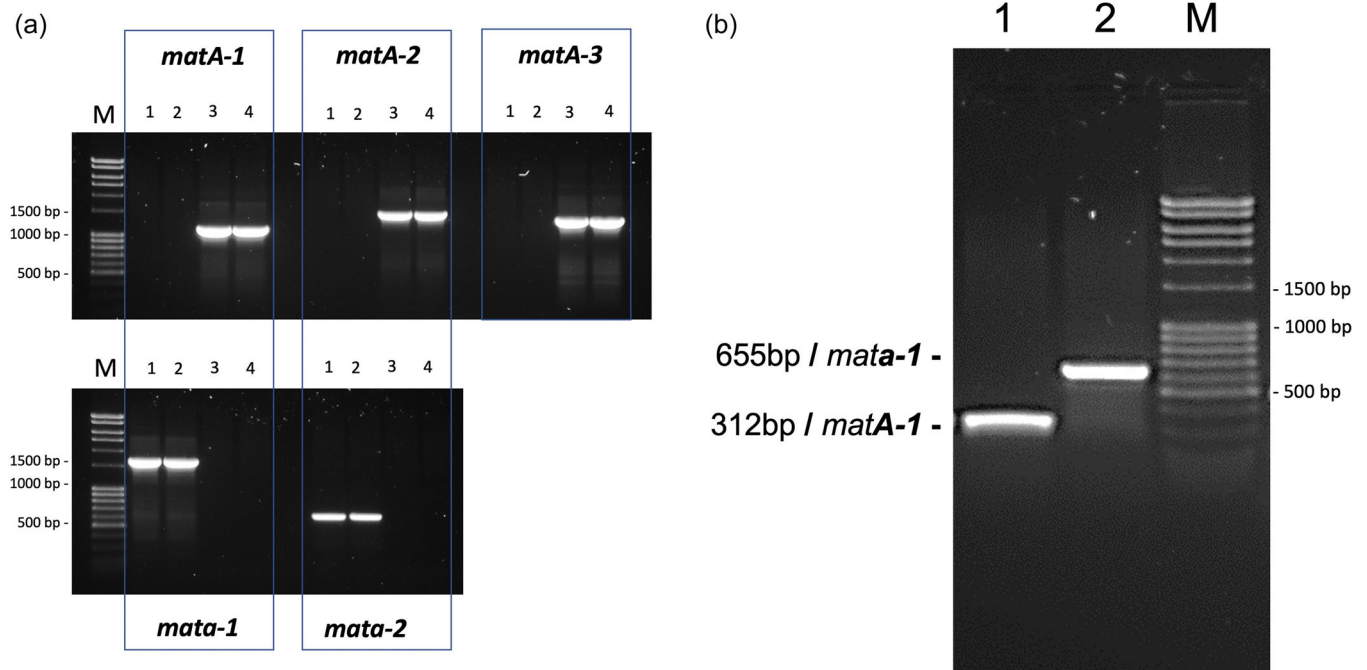
Name	Sequence 5' → 3'	Mating type gene
AS3530	TATCCCCGCTCTTCTCTCA	<i>matA-1</i> ORF upstream
AS3531	G TTCATGGGCTGGAGATTGT	<i>matA-1</i> ORF downstream
AS3532	CATTCCTCTGAGCGACTTCC	<i>matA-1</i> downstream
AS3533	CCCAAAGAAGGTTGCCATTA	<i>matA-1</i> upstream
AS3551	TCTCCCTCCCTTCCAATTCT	ORF <i>matA-1</i> upstream
AS3552	GCTGAACTGCACTGATGGAA	ORF <i>matA-1</i> downstream
AS3553	TATTACGACCGAACTTGGA	ORF <i>matA-2</i> upstream
AS3554	GTCAC TTTGGGGTGATCTTG	ORF <i>matA-2</i> downstream
AS3555	AAACTTCCCACCATCTTTCC	ORF <i>matA-1</i> upstream
AS3556	GCTTTGTATGCCCGTAACAC	ORF <i>matA-1</i> downstream
AS3557	TCAAGCGT TCACTGTTGAGG	ORF <i>matA-2</i> upstream
AS3558	GCGAGGGCTTGATTACATA	ORF <i>matA-2</i> downstream
AS3559	CCCTTTCCTTCTCCAAAC	ORF <i>matA-3</i> upstream
AS3560	GTGGGACAGCGTAGTGAAT	ORF <i>matA-3</i> downstream

Abbreviation: ORF, open reading frame.

95°C; 32× [30" 95°C, 30" 60°C, 30" or 1' 72°C]; 5' 72°C with the exception of oligonucleotides AS3551, AS3552, AS3553 and AS3554 where the annealing temperature was 58°C.

Based on the sequences of the two idiomorphic mating type loci “a” and “A” we designed the

oligonucleotides that would amplify the open reading frames of all five mating type genes (shown in Table 1 and localizations given in Figure 1). PCR amplification was carried out and results are shown in Figure 2a. As shown in the figure, specific bands were obtained for all



**FIGURE 2** (a) Detection of all five mating type genes by polymerase chain reaction (PCR) amplification. Amplification of all five mating type genes from four DNA preparations. Lanes one and two contain DNA from two “a” mycelia and lanes 3 and 4 from two “A” mycelia. “M” indicates an extended 100 bp DNA size marker. *matA-1* amplicon is 1178 bp; *matA-2* is 1576 bp; *matA-3* IS 1211 bp; *mata-1* is 1573 bp and *mata-2* is 600 bp. (b) Multiprimer PCR for simplified mating type determination. Here two pairs of primers, AS3530 and AS3531 (*mat A1*) and AS3532 and AS3533 (*mat a1*) were used in the same PCR reaction. Lane 1—PCR from “A” mycelium; lane 2—PCR from “a” mycelium. “M” indicates an extended 100 bp DNA size marker.

five mating genes in the respective “a” or “A” mycelia. To further simplify the process of identification of a mating type we then made use of multiprimer PCRs where two other pair of oligonucleotides for both mating types were used in a single PCR reaction (Figure 2b): Here a 312 bp fragment of *matA-1* or a 655 bp fragment of *mata-1* was produced. These can be distinguished by their respective sizes. This method can easily be employed for large screens of newly isolated strains from crosses where boiling or microwaving a loopful of conidia in Tris-HCl/EDTA (TE) buffer can provide template [11–13] for PCR determination of mating type within a few hours.

#### AUTHOR CONTRIBUTIONS

**Anke Schäfer:** Formal analysis; investigation; methodology. **Frank Kempken:** Formal analysis; project administration; software; validation; writing—original draft; writing—review and editing.

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#### CONFLICT OF INTEREST STATEMENT

The authors have declared no conflict of interest.

#### DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no data sets were generated or analyzed during the current study.

#### ORCID

Frank Kempken  <http://orcid.org/0000-0002-7853-8009>

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