PCR-based markers for genetic mapping in *Neurospora crassa*.

Moshi Kotierk and Myron L. Smith, Carleton University, Ottawa-Carleton Institute of Biology, 1125 Colonel By Drive, Ottawa, Ontario, K1S 5B6, Canada

Fungal Genetics Newsletter 51:26-26

Eighteen PCR-based markers are described for use in mapping mutations in Oak Ridge background strains of *Neurospora crassa*. These markers are located on each of the seven linkage groups and in the mitochondrial genome to enable course-scale linkage mapping. Following mapping to a linkage group, additional markers can be developed in the co-segregating region for fine-scale mapping of mutations. As with the *N. crassa* RFLP map (Metzenberg and Grotelueschen, 1993; Nelson and Perkins, 2000), the addition of PCR-based markers by members of the *Neurospora* community will enhance this marker set for mapping purposes.

Map-based or positional cloning is necessary to locate, identify and characterize genes that are associated with spontaneous or induced mutations. Methods available for locating mutations in *Neurospora crassa* involve co-segregation analysis using phenotypic (e.g. auxotrophic) markers. For example, multiply marked centromere tester strains of *N. crassa* are available for this purpose through the Fungal Genetics Stock Center (e.g. Perkins, 1972; Metzenberg *et al.*, 1984). Often, however, co-segregation analysis with a set of phenotypic markers will not provide adequate resolution for the fine-scale linkage mapping necessary to clone a trait of interest and may require subsequent crosses to achieve further resolution.

In this note, PCR-based markers are described for locating mutations generated in Oak Ridge background strains. The method takes advantage of abundant sequence differences between Oak Ridge and Mauriceville genetic backgrounds, and the recently completed *N. crassa* genome sequence of the Oak Ridge standard strain 74-OR23-1VA (Galagan *et al.*, 2003). The PCR-based markers are distributed throughout the seven *N. crassa* linkage groups and the mitochondrial DNA (Figure 1). Mapping of a mutation involves four steps. 1) Crossing an Oak Ridge-background *mat-a* strain bearing the mutation of interest with the Mauriceville-background strain, FGSC# 2225. 2) Locating the mutation to linkage group by identifying PCR-based polymorphism(s) that co-segregate with the mutation. 3) Generation of additional markers in the region of interest to carry out high-resolution mapping of the mutation. 4) Identification of the mutated gene by, for example, complementation with co-segregating open reading frames (ORFs) based on the *N. crassa* genome sequence.

In Table 1, the primer sequences, location and PCR conditions are described for each of three types of polymorphisms identified. *A*mplified *P*roduct *L*ength *P*olymorphisms (APLPs) are evident when PCR products are of different sizes. Also used is presence/absence of PCR product in the Oak Ridge and Mauriceville strains, respectively. Finally, *R*estriction *F*ragment *L*ength *P*olymorphisms (RFLPs) are used to identify internal sequence differences in PCR products that are the same size.

Once the mutation is located to linkage group, mapping can be refined with additional markers in the co-segregating region using the *N. crassa* genome database as a reference to design primer pairs. We found the most efficient way to find PCR-based polymorphisms was to use primers placed within adjacent ORFs to obtain amplification products of non-coding, intergenic sequences. Polymorphisms in these non-coding regions are present in most cases between the Oak Ridge and Mauriceville strains.

As an example of the use of these markers, we examined their segregation with respect to a Supercontig 12 PCR-marker. Supercontig 12 was anchored to linkage group I and linkage group VI since it bore two and three markers from each linkage group, respectively. The segregation pattern for the Supercontig 12 marker indicates that it is located on linkage group I, between our *met-6* and *arg-13* markers. These 18 and other markers added by the *Neurospora* community will provide a valuable resource for the positional cloning of traits of interest.

Acknowledgments. Funding for this research was provided by a Discovery Grant to MLS from the Natural Sciences and Engineering Research Council of Canada and from the Mississaugas of the New Credit First Nation to MK. Dr. John Vierula kindly provided primers for the *nmt-1* marker.

Table 1: Details of *N. crassa* PCR-based polymorphisms, including marker location, primer sequences, PCR conditions and polymorphism type.

Linkage Group, arm and region[1]	Primer sequence (5' > 3')	Annealing Temp. / Extension time (min:secs)	Polymorphism[2] Type	OR	M
LG IL Fr	FrP1: CGAGGCAGAGTAGGAAGAAGCAAA FrP2: ATGAGTGGTTGGAGGGAAGGTATG	60 ˚C 1:00	+/-	1012	-
LG IR *met-6*	Met6P1: TAAGGACGAAGGAGAGGTTCTGGA Met6P2: AAGAGGTGATGATGGGTGATGGTG	60 ˚C 2:10	+/-	2106	-
LG IR *arg-13*	Arg13P2: GCTCGCACCGCGCTTCAGC Arg13P3: GCTGTAGTGTAACTACTCACGG	57 ˚C 2:30	RFLP *Xma*I	1760, 115	1875
LG IIL *un-24*	6JP6: GTGCGGGCTTAACCGCTG 6JP11: CTCCGGATGAGGTTGCCG	60 ˚C 1:40	RFLP *Pvu*I	785, 767	1552
LG IIR *nmt-1*	NMT1-F: GCGCAACATGTCTACCGA NMT1-2R: ACCGCAGCAAGCCACATT	57 ˚C 1:30	RFLP *Sal*I	883, 290	1173
LG IIIL *acr-2*	Acr2P1: ATGGAGAAGGTCTTCGCAAACTGG Acr2P2: ATGGTGAGATGGAGAACTGGTTGG	68 ˚C 1:45	APLP	1706	1200
LG IIIR *ro-11*	Ro11P1: TGCCGCAGAAGTTGTTCAATCTGG Ro11P2: GACAACAGTGAGAAAGGTGGTGGA	64 ˚C 1:45	APLP	1669	3000
LG IVR pyr-1	Pyr1P1: ACCATCACCAGAATCAAGTCCGAG Pyr1P2: TACCGCGCAACTAGGATAACCTTC	64 ˚C 2:30	RFLP *MseI*	1300, 1000	1900, 400
LG IVR *pyr-2*	Pyr2P1: TGTGTGAACTTGACCTTACGGGTG Pyr2P2: GCCTTGAGGAAGTCAGCCTTGTAA	52 ˚C 1:30	RFLP *Bgl*II	627, 482	1109
LG VR 4622	P04622P3: AAGCACGCGTTCTCCGTTCG P04622P4: GGGGCAGTTGGGTGGTAGGG	64 ˚C 4:00	+/-	3805	-
LG VR *pyr-6*	Pyr6P1: TGTAGTTTAGCTCGGCTTGGTGTC Pyr6P2: GATAGAGCAGCATGAGCCATGGAA	64 ˚C 2:30	RFLP *Fok*I	926, 747, 242, 156	600, 747, 300, 242, 156

LG VIL *nit-6*	Nit6P2: GTAGAGGTGGGCAGGAGGG Nit6P3: CTAGCGACATCAATCCCCGG	60 ˚C 2:00	RFLP *Xho*I	1356, 326	1682
LG VIL 5603	P05603-P1: CTAAACCGGTTGACTGACTCCCAA P05603-P2: AGTGGACATTGAAGGCATGCTACG	64 ˚C 2:00	RFLP *Hae*III	629, 586, 330, 284, 76, 49	~600, ~600, 330, ~200, 76, 49
LG VIR nuo21.3c	Nuo213cP1: GGAGAACCAGAAGCGTAACGGTAG Nuo213cP2: TAACAAACCTACCCGCAACAGGAG	64 ˚C 2:20	RFLP *KpnI*	1069, 1013	2082
LG VII L 9565	P09565-P1: CTTCTCTGTTGCCGTCAAACCTTG P09565-P2: ATGGGAATAAGGCCCTTGATAGCG	64 ˚C 2:00	RFLP *Mse*I	693, 453, 345, 319, 148	693, ~400, 345, 319, ~100
LG VIIL 06045	P06045-P1: GAACAGCTAGCATTCCGCTCCTTA P06045-P2: AAACTCAGGTTCTCCCTCACAAGG	60 ˚C 1:50	RFLP *Hha*I	699, 587, 332, 145	600, 587, 332, 145, 100
LG VIIR *arg-10*	Arg10P1: TCGAGGAGGATATCTCCAAGGTGT Arg10P2: CTTGGTTGTCGGTAGGTAGCTGTT	60 ˚C 1:30	RFLP *Hin*dIII	1106, 331	1437
Mitochon-drial Pmt	mtP1: CGTATTCTAGGGAAAGATGCTCTCCC mtP2: CGCAGTAATACCTTATGGACCGTCA	66 ˚C 2:00	APLP	1831	700

[1] Marker region, primer names and sequences are based on nearby mapped loci or the Broad Institute's NC locus designation (Version 1) for assembly version 3.
[2] +/- = Presence / absence of a PCR product; RFLP = restriction fragment length polymorphism with given restriction enzyme; APLP = amplified product length polymorphism. Approximate fragment sizes (bp) are given for each under OR (Oak Ridge) and M (Mauriceville). The expected restriction fragment patterns, based on the genome sequence, were obtained in our Oak Ridge background strain for all but the *pyr-1* marker. For the *pyr*-1 marker, the Mauriceville background strain had the expected Oak Ridge pattern.

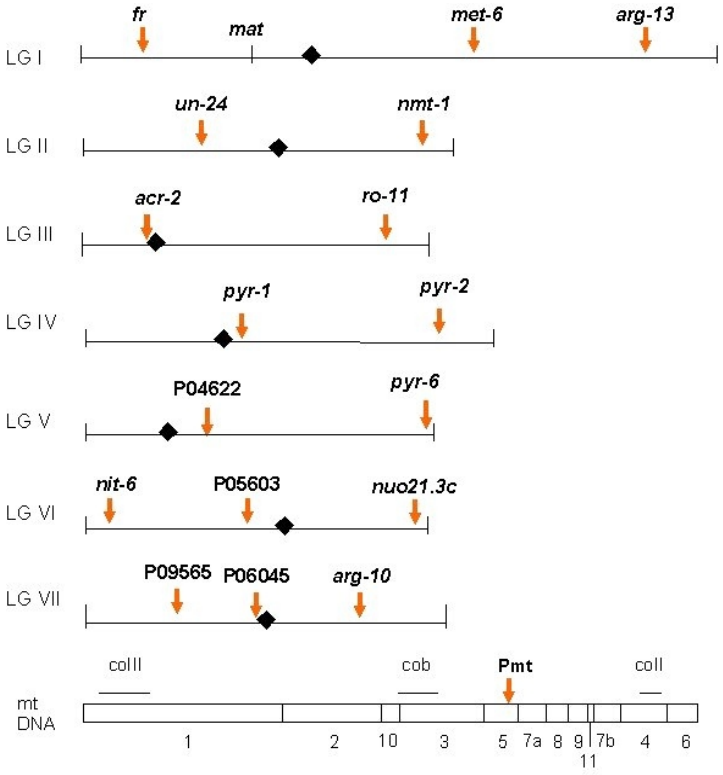

Figure 1: The position of PCR-based markers on each of the seven linkage groups and the mitochondrial DNA are indicated by arrows. The nuclear maps are based on Perkins *et al.*, (2001). The numbers beneath the mitochondrial map indicate Oak Ridge *Eco*RI fragments as designated in Taylor and Smolich, (1985). The mitochondrial map is not on the same scale as the nuclear maps. The mitochondrial genes indicated are as in Perkins *et al.*, (2001).

References.

Galagan *et al.* 2003. The genome sequence of the filamentous fungus *Neurospora crassa*. *Nature* **422:** 859-868.

Metzenberg, R. L., J. N. Stevens, E. U. Selker and E. Morzycka-Wroblewska. 1984. A method for finding the genetic map position of cloned DNA fragments. *Neurospora Newsl.* **31:** 35-39.

Metzenberg, R. L. and J. Grotelueschen. 1993. Restriction polymorphism maps of *Neurospora crassa*: update. *Fungal Genet. Newsl.* **40:** 130-138.

Nelson, M. A. and D. D. Perkins. 2000. Restriction polymorphism maps of *Neurospora crassa:* 2000 update. *Fungal Genet. Newsl.* **47:** 25-39.

Perkins, D. D. 1972. Special purpose *Neurospora* stocks. *Neurospora Newsl.* **19:** 30-32.

Perkins, D. D. , A. Radford and M. S. Sachs. 2001. *The Neurospora Compendium: Chromosomal Loci.* Academic Press, New York .

Taylor, J. W. and B. D. Smolich. 1985. Molecular cloning and physical mapping of the *Neurospora crassa* 74-OR23-1A mitochondrial genome. *Current Genet.* **9:** 597-603.