Improved backcrossed strains giving consistent map distances

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Abstract

Improved backcrossed strains giving consistent map distances

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Backcrossed mutant strains which
produce consistent map distances
and negligible interference.

year or two. So far all these strains were found to be heterokaryon compatible (presumably het C d e).

1) Wild type 74-ORS-6a (deposited as FGSC #4200) is a progeny from the 6th backcross to 74-OR23-1VA of ORS a (FGSC #2490 a), which shows invariant neat round colonies when grown on DNA test agar plus sorbose. It was used for backcrossing when only A mutant strains were available.

2) Morphological mutants in wild type and mutant backcrosses: Since morphological mutants interfere with DNase halo tests, we have recorded any morphologically abnormal variants from all back crosses. Even in small samples (30-100) and also in backcrosses of wild type, these are found not infrequently. Many show a similar phenotype (resembling so) on IR in the so 1:1 in individual asci. One of these mutants has been mapped on IR in the so region. It seemed that the A wild type (74-OR23-1VA, #2489) would be heterokaryotic for so, since it was the common parent. However, when we intercrossed recently obtained A and a progeny from the 7th wild type back cross, we also obtained some asci segregating 1:1 for a morphological mutant (of a slightly different type). The current hypothesis is, therefore, that spontaneous mutants affecting growth rate and morphology are common enough to be produced with low frequency in any strain, and to be found after "purification" in the occasional ascospore progeny of most crosses.

3) Single marker strains: Fig. 1 shows all the markers, as well as uvs and nuh mutants, deposited with FGSC at this time. Markers below the line are deposited only as single mutant strains, except for the case of uvs mutants which also are available with nutritional markers for forced heterokaryons (FGSC #4190-4199). In some cases two different alleles are available when a less leaky or more fertile mutant became available belatedly.

Fig. 1: Map distances of mutants in strains back-crossed to St. Lawrence wild type:
x = several crosses, > 1000 progeny tested;
no superscript, 2 or more crosses, 300-1000 progeny tested;
o = single crosses, totals < 300.

Single marker strains in various linkage groups have the following FGSC #:

LG. I 4000 - 4023
II 4024 - 4042
III 4053 - 4068

LG. IV 4053 - 4068
V 4069 - 4080

LG. VI 4093 - 4110
VII 4081 - 4092

upr and uvs mutants: FGSC #4171-4179 (some backcrossed by A. Schroeder). nuh mutants: 4180-4189.

4) Multiple marker strains for approximate localization of mutants inside each linkage group: Mutants indicated above the line in Fig. 1 have been combined into strains with widely-spaced markers of one linkage group (those for linkage group II are provisional). These strains can be used after crosses to alcoy when linkage group has been found. In such cases, we routinely set up crosses to strains with markers for both possible groups and isolate a sample from the cross that discharges ascospores first. Testing in this sample for the unmapped mutant and the marker closest to the alcoy translocation break reveals which of the two crosses should be analysed in detail.

TABLE I

Backcrossed strains deposited with F.G.S.C.*

FGSC	mt	Genotype	Allele No.	FGSC	mt	Genotype	Allele No.	FGSC	mt	Genotype	Allele No.
Linkage Group I				4036	A	pe		4073	a	al-3	RP-100
4000	A	un-5		4037	a	pe	Y8743m	4074	A	inl	
4001	a	un-5	b39(t)	4038	A	trp-3		4075	a	inl	37401
4002	A	leu-3		4039	a	trp-3	td37.	4076	A	pab-2	
4003	a	leu-3	R156	4040	A	un-15	T54 M50(t)	4077	a	pab-2	H193
4004	A	ad-3B		4041	A	rip-1		4078	A	his-6	
4005	a	ad-3B	2-017-0034	4042	a	rip-1	4M(t)	4079	a	his-6	Y152 M105
4006	A	nic-2		Linkage Group III				4080	a	acu-1	JI48
4007	a	nic-2	43 002	4043	A	acr-2		Linkage Group VII			
4008	A	cr-1		4044	a	acr-2	KH5	4081	A	nic-3	
4009	a	cr-1	B123	4045	A	ad-4		4082	a	nic-3	Y31881
4010	A	thi-1		4046	a	ad-4	44 206,t(F15)	4083	A	thi-3	
4011	a	thi-1	56501	4047	A	leu-1		4084	a	thi-3	18558
4012	A	cyh-1		4048	a	leu-1	33757	4085	A	csp-2	
4013	a	cyh-1	KH52	4049	A	trp-1		4086	a	csp-2	UCLA101
4014	A	al-2	15300	4050	a	trp-1	10575	4087	A	met-7	
4015	a	arg-6	29997	4051	A	dow		4088	a	met-7	4894
4016	A	nic-1		4052	a	dow	P616	4089	A	nt	
4017	a	nic-1	S1413	Linkage Group IV				4090	a	nt	65001
4018	A	arg-13		4053	A	cys-10	(50t)	4091	A	arg-10	
4019	a	arg-13	RU12	4054	a	cys-10	39816	4092	a	arg-10	B317
4020	A	un-18		4055	A	pdx-1		Linkage Group VI			
4021	a	un-18	T 54 M49(t)	4056	a	pdx-1	37803	4093	A	cho-2	
4022	A	R		4057	A	mtr		4094	a	cho-2	47904
4023	a	R	35408	4058	a	mtr	(DRS) <u>15</u>	4095	A	lys-5	37402
Linkage Group II				4059	A	trp-4		4096	a	lys-5	(asco)
4024	A	ro-7		4060	a	trp-4	Y2198	4097	A	lys-5	
4025	a	ro-7	R2470	4061	A	met-2	K43	4098	a	lys-5	DS6-85
4026	A	pi		4062	A	met-2	P159	4099	A	ylo-1	
4027	a	pi	B101	4063	A	pan-1		4100	a	ylo-1	Y30539y
4028	A	cys-3		4064	a	pan-1	5531	4101	A	rib-1	
4029	a	cys-3	P22	4065	A	cot-1		4102	a	rib-1	51602(t)
4030	A	pyr-4		4066	a	cot-1	C102(t)	4103	A	pan-2	
4031	a	pyr-4	36601	4067	A	cys-4		4104	a	pan-2	Y154-M64(B5)
4032	A	acu-5		4068	a	cys-4	K-7	4105	A	pan-2	
4033	a	acu-5	JI18	Linkage Group V				4106	a	pan-2	B36(M,Case)
4034	A	arg-5		4069	A	lys-1		4107	A	trp-2	
4035	a	arg-5	27947	4070	a	lys-1	33933	4108	a	trp-2	75001
				4071	A	cyh-2		4109	A	trp-2	
				4072	a	cyh-2	KH53(r)	4110	a	trp-2	(DRS) <u>41</u>

*Not all of the strains were included in the Eleventh Revision of the Stock List (NN #29) at the time we went to press. However, all will be available shortly. Interested person should write F.G.S.C. for any strain listed in the Table but not in the Eleventh Revision.

FGSC mt Genotype Allele No.

4111 A un-5 leu-3 A nic-2 cyh-1
b39(t) R156 43002 KH52

4112 a cyh-1 al-2 nic-1 arg-13
KH52 15300 1413 RU12

4113 A al-2 nic-1 arg-13 un-18

4114 a al-2 nic-1 arg-13 un-18
15300S 1413 RU12 T54M49(t)

4115 A arg-5 pe fl trp-3

4116 a arg-5 pe fl trp-3
27947 Y8743m L td37

4117 A acr-2 leu-1 trp-1 dow

4118 a acr-2 leu-1 trp-1 dow
KH5 33757 10575 P616

4119 A acr-2 trp-1 dow

4120 a acr-2 trp-1 dow
KH5 10575 P616

4121 A cys-10 pdx-1 pan-1

4122 a cys-10 pdx-1 pan-1
39816 37803 5531

4123 A cys-10 pdx-1 pan-1 uvs-2

4124 a cys-10 pdx-1 pan-1 uvs-2
39816 37803 5531 (no#)

4125 A pdx-1 pan-1 cys-4

4126 a pdx-1 pan-1 cys-4
37803 5531 K7

4127 A pdx-1 pan-1 cys-4 uvs-2

4128 a pdx-1 pan-1 cys-4 uvs-2
37803 5531 K7 (no#)

4129 A lys-1 cyh-2 al-3 inl^t pab-2 his-6

4130 a lys-1 cyh-2 al-3 83201^t + his-6
33933 KH53,r RP100 H193 Y152M105

4131 A lys-1 al-3 inl^t pab-2

33933 RP100 83201(t) H193

4132 a lys-1 cyh-2 al-3 inl^t pab-2

33933 KH53,r RP100 83201(t) H193

4133 A cyh-2 al-3 inl^t pab-2

4134 a cyh-2 al-3 inl^t pab-2
KH53r RP100 83201(t) H193

4135 A lys-1 cyh-2 al-3 inl^t his-6

33933 KH53,r RP100 83201(t) Y152M105

4136 a lys-1 inl his-6

33933 37401 Y152M105

FGSC mt Genotype Allele No.

4137 A cho-2 ylo-1 trp-2

4138 a cho-2 ylo-1 trp-2
47904 Y30539y 75001

4139 A rib-1 pan-2(B2) trp-2

4140 a rib-1 pan-2(B2) trp-2
51602(t) Y153M66 (DRS)41

4141 A nic-3 met-7 arg-10

4142 a nic-3 met-7 arg-10
Y31881 4894 B317

4143 A thi-3 met-7 nt

4144 a thi-3 met-7 nt
18558 4894 65001

4145 a acr-2 leu-1 ylo-1 T1 (III;VI) dow
KH5 33757 Y30539y 1 P616

4146 A leu-3 ad-3B thi-1 cyh-1

R156 2-017-0034 56501 KH52

4147 A leu-3 ad-3B cyh-1

R156 2-017-0034 KH52

4148 a cyh-1 al-2 nic-1

KH52 15300 1413

4149 a cys-3 arg-5

P22 27947

4150 A arg-5 pe fl

27947 Y8743m L

4151 A pdx-1 cot-1 cys-4

4152 a pdx-1 cot-1 cys-4
37803 C102(t) K7

4153 A acr-2 dow; cho-2 ylo-1 trp-2

4154 a acr-2 dow; cho-2 trp-2
KH5 P616 47904 Y30539y 75001

4155 A cot-1; al-3

4156 a cot-1; al-3
C102(t) RP100

4157 A sn cr-1; csp-2

C136 B123 UCLA101

4158 A sn cr-1

4159 a sn cr-1
C136 B123

4160 A sn cr-1; al-3 inl^t

4161 a sn cr-1; al-3 inl^t
C136 B123 RP100 83201(t)

FGSC	mt	Genotype	Allele No.	FGSC	mt	Genotype	Allele No.
4162	A	sn cr-1; pe fl		4184	A	nuh-3	
4163	a	sn cr-1; pe fl		4185	a	nuh-3	FK003
		C136 B123 Y8743m L					
4164	A	sn cr-1; pe fl; al-3 inl ^t		4186	A	nuh-5	
4165	a	sn cr-1; pe fl; al-3 inl ^t		4187	a	nuh-5	FK005
		C136 B123 Y8743m L RP100 83201 (t)		4188	A	nuh-6	
4166	A	cr-1; pe fl; al-3 inl ^t		4189	a	nuh-6	FK006
		B123 Y8743m L RP100 83201(t)		4190	A	acr-2; uvs-2; his-6	
4167	A	cr-1; pe fl				KH5 (no#) Y152M105	
4168	a	cr-1; pe fl		4191	a	pan-1 uvs-2	
		B123 Y8743m L				5531 (no#)	
4169	A	pe fl		4192	A	uvs-3; ylo-1 pan-2 (B5)	
4170	a	pe fl		4193	a	uvs-3; ylo-1 pan-2 (B5)	
		Y8743m L				ALS11 Y30539y Y154M64	
4171	a	upr-1 (no#) (RWT)		4194	A	uvs-3 trp-4 pan-1	
4172	A	uvs-2		4195	a	uvs-3 trp-4 pan-1	
4173	a	uvs-2 (no#) (DRS)				ALS11 Y2198 5531	
4174	A	uvs-3		4196	A	acr-2 uvs-4 leu-1	
4175	a	uvs-3	ALS11			KH5 ALS12 33757	
4176	A	uvs-4		4197	a	uvs-4 leu-1	
4177	a	uvs-4	ALS12			ALS12 33757	
4178	A	uvs-6		4198	A	uvs-6; mtr-1 met-2 pan-1	
4179	a	uvs-6	ALS35			ALS35 (DRS)15 P159 5531	
4180	A	nuh-1		4199	a	uvs-6; nic-3	
4181	a	nuh-1	FK001			ALS35 Y31881	
4182	A	nuh-2		4200	a	wild type (SL)	
4183	a	nuh-2	FK027			(BCVI of ORSa) 74-ORS-6a	

 Strains covering each linkage group have the following numbers:

LG. I	4111 - 4114 and 4146 - 4148	LG. V	4129 - 4136
II	4115 - 4116 and 4169 - 4150	VI	4137 - 4140
III	4117 - 4120 and 4145	VII	4141 - 4144
IV	4121 - 4128 and 4151 - 4152		

Similar strains carrying sn cr-1 for replica testing are now being tested and will be deposited if standard procedures can be devised (those for groups III - VII are ready and available from Montreal directly for anyone willing to try them out).

5) Strains for alcoy follow up and mutagenesis:

Alcoy follow ups: III; VI, several markers: FGSC #4153 and 4154
 IV; V: cot-1 al-3: FGSC #4155 and 4156

Strains for velvet replication: sn cr-1; csp-2: FGSC #4157
sn cr-1 FGSC #4158 and 4159

Various other strains carrying sn, cr-1, pe fl, and al-3 inlt
 in several combinations: FGSC #4160 - 4170

6) Linkage data: To increase efficiency and effectiveness of back crossing, up to three markers of the same linkage group have been back crossed together. Single mutant strains were separated, generally in the 5th back cross, and checked singly in a 6th back cross. The final multiply-marked strains were then built up from the latter strains. Extensive coupling and repulsion linkage data are therefore available for various intervals. In general, recombination values did not change significantly during backcrossing and are shown in Fig. 1 as single average values (with a rough indication of sample size as a measure of reliability). For the few cases with inconsistent values a range of averages is indicated. Surprisingly, practically no interference was observed (possibly expected for the large intervals involved) and in some cases ordering of markers was difficult when two mutants were very close and the third at considerable distance (so that double and single cross overs were almost equally rare). In short, patterns of recombination seem to be identical to those of Aspergillus nidulans, but thorough statistical analysis will be needed before definite conclusions can be reached. - - - Department of Biology, McGill University, 1205, Ave. Docteur Penfield, Montreal, H3A 1B1 Canada.