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McrA and McrB restriction phenotypes of some *E.coli* strains and implications for gene cloning

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ABSTRACT

The McrA and McrB (*m*odified *c*ytosine *r*estriction) systems of *E. coli* interfere with incoming DNA containing methylcytosine. DNA from many organisms, including all mammalian and plant DNA, is expected to be sensitive, and this could interfere with cloning experiments. The McrA and B phenotypes of a few strains have been reported previously (1-4). The Mcr phenotypes of 94 strains, primarily derived from *E. coli* K12, are tabulated here. We briefly review some evidence suggesting that McrB restriction of mouse-modified DNA does occur *in vivo* and does in fact interfere with cloning of specific mouse sequences.

INTRODUCTION

Introduction of foreign DNA into a wild type bacterial cell frequently leads to restriction: the newly introduced DNA is inactivated and eventually degraded. Susceptibility to restriction can be the result of either the absence or the presence of modified bases in DNA (5, 6). Most researchers engaged in primary cloning of foreign sequences into *E. coli* K12 carefully to avoid restriction by the familiar *Eco*K endonuclease, which cleaves only when its site is unmethylated. This nuclease, encoded by the *hsdRMS* genes of K12, is inactivated by mutation in many common host strains used in cloning experiments.

Recently, several workers found that *E. coli* K12 also restricts DNA specifically when particular nucleotide sequences are methylated (1, 7-11). It had been known for a long time that *E. coli* restricts DNA with an unusual cytosine modification (5-hydroxymethylation of cytosine residues, conferring sensitivity to the RglA and RglB (*r*estricts *g*lucose*l*ess phage) systems; 6, 12, 13; see below). It is now clear that methylation of cytosine, a more common modification, can also confer sensitivity to restriction, apparently by the same systems that restrict DNA containing hydroxymethylcytosine. The two site-specific systems McrA and McrB that restrict DNA containing methylated cytosine at particular sequences (1) are controlled by the same genetic loci that govern Rgl restriction (EAR, R. Trimarchi and HR, in preparation). We will here refer to the restriction phenotypes as McrA and McrB, since the mnemonic more accurately describes the observations made so far: some sequences containing either type of modified cytosine are specifically restricted. A third site-specific, methylation-dependent restriction locus, *mrr*, has recently been identified; DNA containing N^6methyladenine at particular sequences is sensitive to Mrr (10).

Mcr and Mrr restriction present potential problems in cloning genomic DNA. Many organisms methylate their DNA, usually at cytosine residues in eukaryotes (14) and at cytosine or adenine residues, or both, in prokaryotes (14). Avoiding this restriction in cloning experiments has been problematic, since the status of the *mcr*A, *mcr*B and *mrr* restriction loci has been reported for very few laboratory strains (1-4, 10). As it turns out, there is considerable variability among laboratory derivatives of K12. Only two common strains, HB101 and RR1, are known to be defective for *mrr* function (10). In Table 1 we summarize the McrA and McrB phenotypes of those strains that have been tested, briefly noting genotypic characters of particular interest (such as the status of *Eco*K restriction and the presence of mutations in *rec*A, *rec*BCD, *sbc*, *lon*, *hfl*A, and *sup*F). We have not surveyed these strains for Mrr function. The genotypes given here are not complete, and the original source or the reference given should be consulted for this.

ASSAY METHODS

The strains above have been tested in a variety of ways by different laboratories; some strains, as noted, have been tested in more than one laboratory. There are three testing methods shown:

T-even phages: The two Mcr restriction systems described here were originally characterized (6, 12, 13) as specific for 5-hydroxymethylcytosine (HMC)-containing T-even phages. These phages contain HMC if a mutation (gt mutation) eliminates the glucosyltransferase(s) that normally modifies the HMC and shields it from restriction. The two systems are distinguishable because T6gt is not restricted by McrB (formerly RglB) but is restricted by McrA (formerly RglA); T2gt and T4gt are restricted by both. Wild type T2 (which glucosylates only 75% of its HMC residues) is slightly restricted by McrB only; however, the effect is small, and T2 is difficult to use as a test for McrB activity. The tests with the gt phages are easy to perform and easy to read. The plating efficiency of a sensitive phage on a restricting host is typically 10^{-6} with respect to a non-restricting host (except for T2; see above), and qualitative results can be obtained quickly by cross-streak tests. The results obtained so far using this test always agree with the results of the tests described below, except that in a few cases (see footnote d, above) the degree of restriction of T-even phages is much less than expected and the result cannot be read by cross-streak. This test also has the disadvantage that the presence or absence of McrB cannot be easily determined if McrA is present.

Modified plasmids and λ: McrA and McrB also restrict DNA carrying methylcytosine, in a site-specific manner (1). Such modification is indicated by appending the name of the modification to the name of the replicon: e.g., λ.HpaII is λ DNA modified at its *Hpa*II sites (C ^{me}CGG) by the methylase (M.HpaII) associated with the *Hpa*II restriction system. Plasmids used were pBR322 (36) and pXAd (42). Phage used were λ_{vir} (at NEB) and a λgt10 (43) clone carrying a 2.15 kb mouse genomic insert (in San Francisco). McrA restricts M.HpaII-modified DNA, but not DNA modified by any other cytosine methylase that has been examined. McrB restricts DNA modified by M.HaeII (RGCGCY), M.AluI (AG ^{me}CT), or M.MspI (^{me}CCGG), as well as plasmids carrying genes for any of 11 additional methylases (1; see below) but not DNA modified by M.HpaII. Modification can be carried out *in vitro*, the phage DNA packaged and restriction tested with the resulting infective particles; or the phage

Table 1

McrA and McrB Phenotypes of 94 *E. coli* strains

Strain	Observer[a]	Test used	Mcr phenotype[b] A	Mcr phenotype[b] B	Reference, source, comments
1100	HR	T-evens	+	+	(15) I.R. Lehman; Hsd$^+$ *end*A1
AB266	HR	T-evens	+	+	(15) Hsd$^+$
AB1157	ER	pBR322.HpaII	+	?	(15) Hsd$^+$
AT2459	HR	T-evens	-	+	A. Taylor Hsd$^+$
BNN93[c]	DW	λ.HpaII, λ.AluI	-	-	(16) *hsd*R
BNN102[c]	DW	λ.HpaII, λ.AluI	-	-	(16) *hsd*R *hfl*A; sometimes called C600.Hfl
C235	HR	T-evens	-	+	(17) Hsd$^+$
C600[d]	NM	T-evens	-	+	(15) Hsd$^+$
	HR	T-evens			
	ER	pBR322.HpaII, pM.HaeII			
	RB	pM.PvuII[e]			
CES200	DW	λ.HpaII, λ.AluI;	-	+	(21); *hsd*R *rec*B21 *rec*C22
	NM	T-evens			*sbc*B15; T6^r
CH734	ER	pBR322.HpaII	+	?	(22)
CH1332	ER	pBR322.HpaII	-	?	(22)
CH1371	ER	pBR322.HpaII	-	?	(22)
CPB1293[f]	JE	T4gt, T6gt	-	+	Hsd$^+$
CPB1321	JE	T4gt, T6gt; pM.Eco47II	-	-	JE; *hsd*R2 *mcr*B1 derivative of CPB1293
CR63	ER	T-evens	-	+	(15) Hsd$^+$
CSR603	RB	pM.PvuII[e]	?	+	(23) Maxicell strain
χ2813	ER	T-evens; λ.HpaII, λ.MspI	-	-	R. Curtiss via P. Wolk; *hsd*R2; *rec*A56 recombinant of K802
DH1	DH	pBR322.HpaII; pXAd.HpaII	+	+	(15) *hsd*R17; *rec*A1; descendant of MM294
	RB	pM.PvuII[e]			
DH3	DH	pBR322.HpaII; pXAd.HpaII	-	(+)	(9) *hsd*R17 *rec*A1; isolated from DH1 by selection with pXAd.HpaII

Strain	Observer[a]	Test used	Mcr phenotype[b] A	B	Reference, source, comments
DH5	DH JE	pBR322.HpaII; pM.Eco47II	+	+	DH; *hsd*R17 *rec*A1; high efficiency of transformation; derived from DH1
DM800[d]	ER	λ.HpaII, λ.MspI; T-evens	-	+	B. Bachmann; Hsd$^+$ Δ(*top*A-*cys*B)
ED8641	ER HR	λ.HpaII, λ.MspI; T-evens	-	+	NM; *hsd*R514 *rec*A56 derivative of K803
ED8654[d]	NM, HR ER JE	T-evens; T-evens λ.HpaII, λ.MspI; pM.Eco47II	-	+	(24) *hsd*R514 *sup*F58 derivative of K803; LE392 is a subline of this strain
ED8739	ER	λ.HpaII, λ.MspI, T-evens	-	-	NM; *hsd*S3 *sup*F; from K803
ED8767	NM DW ER	T-evens; λ.HpaII, λ.AluI λ.HpaII, λ.HaeII	-	-	(25) *hsd*S3 *sup*F *rec*A13; derivative of ED8739
ER1370	ER	T-evens; pM.HaeII	+	+	(1) Hsd$^+$; derivative of JC1552 via NK7254
ER1381	ER	T-evens; pBR322.HpaII pM.HaeII	+	+	(1) *hsd*R2 recombinant of ER1370
ER1378	ER	T-evens; pBR322.HpaII pM.HaeII	+	-	(1) *hsd*R2 *mcr*B1 recombinant of ER1370
ER1398	ER	T-evens; λ.HpaII, pM.HaeII	+	-	(1) *hsd*R2 *mcr*B1 recombinant of MM294
ER1414	ER	pM.HaeII	(+)	+	ER; *hsd*R2 *mcr*B1 recombinant of W3110
ER1451	ER	T-evens; pM.HpaII, pM.HaeII	-	-	(1) *hsd*R2 or R17 *mcr*B1 recombinant of JM107
ER1458	ER	T-evens; λ.HaeII	-	-	ER; *hsd*R2 *mcr*B1 recombinant of Y1084
ER1562	ER	T-evens; λ.HpaII, λ.MspI	-	-	ER; *mcr*A1272::Tn10 recombinant of ER1398
ER1563	ER	T-evens; λ.HpaII	-	+	ER; *mcr*A1272::Tn10 recombinant of MM294
ER1564	ER	T-evens; λ.HpaII	-	+	ER; *mcr*A1272::Tn10 recombinant of ER1381

Strain	Observer[a]	Test used	Mcr phenotype[b]		Reference, source, comments
			A	B	
ER1565	ER	T-evens; λ.HpaII, λ.MspI	-	-	ER; *mcr*A1272::Tn10 recombinant of ER1378
FS1585	NM	T2gt, T6gt	-	+	(26) *rec*D *sup*F; Hsd$^+$ C600 background
GM161	RB	pM.PvuII[c]	?	+	M.G. Marinus; *dam*
GM271			(-)	(-)	M.G. Marinus; *hsd*R2 *mcr*B1 *dcm*6; parent of GM2163
GM272	RB	pM.PvuII[c]	?	+	M.G. Marinus; *dam dcm*
GM2163	ER	pBR322.HpaII, pM.HaeII	-	-	M.G. Marinus; *hsd*R2 *mcr*B1 *dam*13::Tn9 *dcm*-6
GW1002	ER	pBR322.HpaII	-	?	G. Walker; Hsd$^+$
H680	ER	T6gt	-	?	P.G. de Haan via B. Bachmann; Hsd$^+$
HB101	ER	pBR322.HpaII, pM.HaeII	+	-[g]	(27) *hsd*S20 *mrr*; carries the *hsd*–*mcr*B region from *E. coli* B and phenotypically R$^-$M$^-$ for both *Eco*K and *Eco*B
	RB	pM.PvuII[c]			
	JE	pM.Eco47II			
Hfr3000 YA149	ER	λ.HpaII, λ.MspI; T-evens	-	+	(15) B. Bachmann; Hsd$^+$; derivative of Hfr Hayes
Hfr4000	ER	λ.HpaII, λ.MspI; T-evens	+	+	(15) Ancestral strain; Hsd$^+$; aka AB257, HfrP3
Hfr Cavalli	ER	λ.HpaII, λ.MspI; T-evens	+	-	(15) Ancestral strain; Hsd$^+$; presumed origin of *mcr*B1 allele
HfrH *thi*$^-$	HR	T-evens	-	+	S.E. Luria; Hsd$^+$
HfrP4X6	HR	T-evens	+	+	(15) S.E. Luria; Hsd$^+$
JH132	ER	T4gt, T6gt	-	-	J. Heitman; Mrr$^-{}_B$ HsdR$^-$M$^-{}_B$ McrB$_B$ derivative of K802
JK268	ER	T6gt	-	?	R.W. Simons; Hsd$^+$
JM83	RB	pM.PvuII[c]	?	+	(28) Hsd$^+$ Δ(*lac-pro*AB) (φ80 Δ(*lac*Z)M15)
JM101	ER	pBR322.HpaII	+	?	(28) Hsd$^+$ Mrr$^+$ Δ(*lac-pro*AB) /F'*lac*I^{q}Δ*lac*Z)M15
JM107	ER	pBR322.HpaII, pM.HaeII;	-	+	(28) *hsd*R17 Δ(*lac-pro*AB) /F'*lac*I^{q}Δ*lac*Z)M1;
	RB	pM.PvuII[c]			derivative of DH1

Strain	Observer[a]	Test used	Mcr phenotype[b] A	Mcr phenotype[b] B	Reference, source, comments
JM107MA2	RB	pM.PvuII;	-	-	(7) Spontaneous mutant
	ER	pM.HaeII; T-evens			permissive for pM.PvuII; see also ER1451
JM109			(-)	(+)	(28) Mrr$^+$; *rec*A1 derivative of JM107
5K	NM	T2gt, T6gt	-	+	(29) *hsd*R514 derivative of C600
K12	ER	pBR322.HpaII,	+	+	(15) Ancestral strain; Hsd$^+$
		pM.HaeII; T-evens			
K802					See WA802
K803					See WA803
K10	ER	λ.HpaII, λ.MspI;	+	-	(15) Hsd$^+$; ancestral strain;
		T-evens			descendant of Hfr Cavalli.
KMBL1164	ER	T6gt	-	?	(30) Hsd$^+$
LE392[d]	DW	λ.HpaII, λ.AluI;	-	+	(31) Mrr$^+$ *hsd*R514;
	ER	λ.HpaII, λ.MspI;			$CaCl_2^r$ derivative of ED8654
		T-evens			
MC1040			(+)	(+)	
MC1061	NM	T2gt, T6gt;	-	-	(32) *hsd*R Δ(*lac*)X74
	ER	pM.HaeII			
MM294	ER	T-evens;	+	+	(15) *hsd*R17 mutant of 1100
		pM.HpaII, pM.HaeII			
	RB	pM.PvuII[e]			
NM477	NM	T2gt, T6gt	-	-	(33) *hsd*MS Δ5 derivative of C600
NM494	NM	T2gt, T6gt	-	-	NM; pop101 Δ*hsd*S *hfl*
NM514	DW	λ.HpaII, λ.AluI;	-	+	NM; pop101 *hsd*R *hfl*A
	NM	T2gt, T6gt			
NM519	NM	T-evens	+ and/or +		NM; *hsd*R *rec*BC *sbc*A T6^r
NM538	NM	T-evens	-	+	(34) *sup*F *hsd*R
NM539			(-)	(+)	(34) *sup*F *hsd*R (P2cox3)
NM554	NM	T2gt, T6gt;	-	-	NM; *rec*A13
	ER	λ.HpaII, λ.MspI			derivative of MC1061
NM621	NM	T-evens	-	-	NM; T6^r; *rec*D *hsd*R
	DW	λ.HpaII, λ.AlwI			derivative of C600
PA309	HR	T-evens	+	+	(15) Hsd$^+$

Strain	Observer[a]	Test used	Mcr phenotype[b]		Reference, source, comments
			A	B	
PC0950	ER	pM.HaeII	?	+	P.G. de Haan via B. Bachmann; Hsd$^+$
Q358	NM	T-evens	-	+	(35) *hsd*R *sup*E
Q359			(-)	(+)	(35) P2 lysogen of Q358
RL88	ER	T-evens	-	-	B. Bachmann; Hsd$^+$ λ^r Δ(*ton*B-*cys*B)
RR1	ER	pBR322.HpaII, pM.HaeII	+	-[g]	(36) *hsd*S20 *mrr*; HB101 *rec*A$^+$
	RB	pM.PvuII[e]			
SK5022	ER	λ.HpaII, λ.MspI; T-evens	-	+	B. Bachmann; Hsd$^+$
W6[h]	ER	T-evens	+	+	(15) Ancestral strain; Hsd$^+$ F$^+$
W3110	ER	λ.HpaII, λ.MspI, pM.HaeII; T-evens	+	+	(15) Hsd$^+$ *sup*^{o}; ancestral strain
	RB	pM.PvuII[e]			
W4597	HR	T-evens	+	+	S.E. Luria; Hsd$^+$
WA704			(-)	(-)	(37) Hsd$^+$ parent of WA802 (K802) and WA803 (K803)
WA802	DW, ER	λ.HpaII, λ.AluI; pM.HpaII, pM.HaeII;	-	-	(37) aka K802; *hsd*R2 mutant of WA704
	RB	pM.PvuII[e]			
	NM	T2gt, T6gt			
	HR				
WA803	NM	T2gt, T6gt;	-	-	(37) aka K803; *hsd*S3 mutant of WA704
	HR				
	ER	λ.HpaII, λ.MspI			
WW3352	ER	λ.HpaII, λ.MspI; T-evens	-	+	B. Bachmann; Hsd$^+$ Δ(*trp-ton*B)
X149	HR	T-evens	-	+	(17) Hsd$^+$
Y10	ER	λ.HpaII, λ.MspI; T-evens	+	+	(15) Ancestral strain; Hsd$^+$
Y53	ER	λ.HpaII, λ.MspI; T-evens	+	+	(15) Ancestral strain; Hsd$^+$
Y70	ER	λ.HpaII, λ.MspI; T-evens	-	+	(15) Ancestral strain; parent of C600; Hsd$^+$

Strain	Observer[a]	Test used	Mcr phenotype[b] A	B	Reference, source, comments
Y1084	ER	pBR322.HpaII, λ.MspI, λ.HaeII	-	+	(38) and R. Young; Hsd$^+$ *lon sup*F Δ(*lac*IpoZYA)U169; plasmidless parent of Y1090
Y1088[d]	ER	λ.MspI, λ.HaeII; T-evens	-	+	(38) *hsd*R514 *sup*F Δ(*lac*IpoZYA)U169 (pMC9); descendant of LE392
Y1090			(-)	(+)	(38) contains pMC9; see Y1084
Non-K12 strains					
E. coli B	HR	T-evens	+	+[i]	S.E. Luria; Hsd$^+$
E. coli B/r	RB	pM.PvuII[e]	?	+[j]	(39); Hsd$^+$
E. coli C	NM	T6gt	-	(-)	(40) T2^r, T4^r; has no homology to K12 in *hsd–mcr*B region (41)

[a] DW: David Westaway; DH: Douglas Hanahan; ER: Elisabeth Raleigh; HR: Helen Revel; JE: Jeff Elhai; NM: Noreen Murray; RB: Robert Blumenthal

[b] () type inferred from ancestral or descendant type

[c] BNN93 is sometimes called C600 R$^-$. It is best to use a distinctive isolation name so that it is not confused with the original. Appended terms (as in C600 R$^-$ and C600.hfl) tend to get lost with time and to lead to confusion.

[d] These strains show much reduced restriction of T2gt and T4gt under conditions used, but restrict pM.HaeII and λ.MspI normally. This phenomenon is under investigation.

[e] It has recently been shown that some bacteria modify cytosine at the N^4 position (18, 19), and this methylase may be one of these (20; RMB, unpublished).

[f] Recovered from stab of W3110 *lacI*^{q}L8/pTac11 (from J. Brosius); lacks pTac11.

[g] The wild type *E. coli*B *rgl*B restriction locus, which is carried by this strain, confers a very weak restriction phenotype when tested with T-even phage (6). However, we have found no detectable McrB-dependent restriction of methylated DNA in this strain. We may not have a methylase of the proper specificity to observe such restriction.

[h] Isolate tested was λ^r

[i] The wild type *E. coli*B *rgl*B restriction locus, which is carried by this strain, confers a very weak restriction phenotype when tested with T-even phage (6). This strain has not been tested for restriction of methylated DNA.

[j] We do not know the reason for the difference between rejection of pM.PvuII in this strain and its acceptance in HB101 and RR1, which should carry the same allele.

can be modified *in vivo*, by growing the phage on a strain carrying the cloned modification methylase. λ.HpaII, λ.MspI and λ.HaeII were prepared *in vivo* at NEB; λ.HpaII and λ.AluI were prepared *in vitro* at UCSF. Similarly, plasmid DNA can be modified *in vitro* and then tested by transformation. For McrA, pXAd (42) was particularly useful, since it is a large (39 kb) plasmid with a high GC content and elicited a strong restriction response (efficiency of transformation 10^{-4} when methylated

with M.HpaII). Tests with DNA methylated *in vitro* are more laborious than the T-even tests. They are also harder to read, since plating efficiency on a restricting host is typically 0.1-0.01, rarely 10^{-3}, with respect to a non-restricting host (pXAd.HpaII, used as an assay for McrA, is an exception to this rule). This is at least 1000-fold less sensitive than the T-even tests, and means that assays must be done quantitatively. However, such tests are most directly relevant to investigators interested in cloning DNA with various modifications into *E. coli*, and McrA and McrB phenotypes are easily separated.

Cloned modification methylase genes: As might be expected, restricting hosts do not accept cloned modification methylase genes that confer sensitivity to restriction, especially if the methylase is expressed well. Consequently, another test for Mcr restriction is to transform with such a clone, and compare transformation efficiency with that of the original (permissive) host. McrA, by definition, restricts the cloned M.HpaII gene; McrB, by definition, restricts the cloned M.HaeII gene. Such a clone is designated, e.g. pM.HpaII. McrB also restricts plasmids carrying 13 other cloned methylase genes (1), most of them originally isolated in an McrA$^+$ McrB$^-$ host. Performing these tests on many strains simultaneously is laborious, but the results are relatively easy to read, since the difference between restricting and non-restricting strains is typically 10^3–10^5, and transformation efficiency can be determined qualitatively.

EFFECT OF RESTRICTION ON CLONING EXPERIMENTS

Specificity of restriction The specificities of the two Mcr systems are clearly different, but the precise recognition sequences are not known. McrB restricts plasmids carrying any one of 14 different cloned prokaryotic modification methylase genes (1), and the consensus sequence G ^{me}C has been suggested. The murine modification methylase (which confers the modification ^{me}CG) has also been shown to confer sensitivity to McrB (2; see below); this is consistent with the propoposed consensus sequence, since about one in four CG sequences will be preceded by a G (depending on base composition of the DNA). McrA is not known to restrict DNA carrying any methylation other than *Hpa*II modification, but the sample of methylase clones available is biased, since most were cloned using an McrA$^+$ host. It has not been determined whether the murine modification methylase confers sensitivity to McrA.

Degree of restriction in cloning genomic DNA Three lines of evidence suggest that mouse DNA is specifically restricted by McrB in cloning experiments. We have no evidence that really addresses the role of McrA, since many cloning strains are already McrA$^-$.

First, one of us (RB) modified pBR322 DNA *in vitro* with purified mouse modification methylase and demonstrated that McrB$^+$ hosts (JM107, C600) yielded fewer transformants per microgram with this methylated vector than they did with the same amount of unmethylated vector. The number of transformants decreased progressively as the degree of methylation increased (2). The maximum reduction was by a factor of forty, but since it was not clear that all potential sites had been modified in the most highly methylated sample, this is a minimum estimate of the degree of restriction possible. In contrast, matched McrB$^-$ hosts (JM107MA2, WA802) showed a much smaller decline in transforma-

tion efficiency as the degree of methylation increased. All strains were McrA$^-$. In this experiment the strains compared were isogenic (JM107, JM107MA2) or nearly so (C600, WA802).

The second line of evidence suggests that mouse modification occurring *in vivo* also confers McrB-sensitivity on an otherwise innocuous sequence. One of us (DH) found that pBR322 that had been propagated in mouse cells transformed DH1 with an efficiency per microgram of 0.1–0.01 compared with pBR322 recovered from *E. coli* K-12 (9, 44). Mixing experiments showed that the presence of mouse DNA in the transformation mixture did not affect acceptance of *E. coli* K-12-modified pBR322. DH3 was isolated from DH1 as a mutant derivative permissive for DNA methylated by M.HpaII (in retrospect, we identify this as an McrA$^-$ mutant), but this strain still showed reduced recovery of mouse-modified pBR322. Since DH3 and DH1 are isogenic, one can conclude that either McrB or another unidentified function present in this background contributes significantly to this restriction. McrA could still contribute to restriction in the wild type situation, since no *mcr*B mutant was tested. This experiment and that above demonstrate that the restriction depends on methylation, not on sequence organization or information, since pBR322 by itself is perfectly acceptable to the strains used.

Finally, two actual cloning experiments provide evidence for reduced recovery of specific mouse sequences in McrB$^+$ hosts compared with recovery in McrB$^-$ hosts. In the first experiment, one of us (DW) found that the proportion of clones positive for the specific 2.15 kb fragment was low (0/360,000) in a library plated on the McrB$^+$ host, NM514; but it was high (or reasonable, 10/300,000) in a library plated on the McrB$^-$ host, BNN102 (45). The same mixture of *in vitro*-packaged ligation products was used in both cases; however, the two hosts were not isogenic, and there could be contributions from other factors. It was shown that the clones, once obtained, plated with equal efficiency on the two hosts, so again the effect was not the result of other interfering factors, such as inverted repeat sequences (46) or expression of toxic products.

The second experiment was similar. Two of us (ADR and PWJR) examined recovery of two particular mouse genomic loci using hybridization probes obtained from cDNA clones, GR1 and 1.6U. One of these sequences (GR1) had been sought exhaustively in existing amplified cosmid and phage libraries (from F.G. Grosveld, P.F.R. Little, and W.J. Brammar) which had been used successfully to obtain other chromosomal clones (F.G. Grosveld, pers. comm.; P.F.R. Little, pers. comm.; 47). This sequence had not been recovered (in all, $\leq 2 \times 10^{-7}$ positive clones per clone examined). A new unamplified packaged λ library was constructed, and the packaging mixture was examined in three ways. The overall titer on McrB$^+$ (CES200, LE392) and McrB$^-$ (NM621) strains was determined and was found to be two- to three-fold lower on the McrB$^+$ strains. Since these strains are not an isogenic series, these small effects could be due to factors other than McrB action. Next, 3×10^5 plaques each grown on CES200 and NM621 were probed with the GR1 cDNA clone. The McrB$^-$ host, NM621, yielded at least one positive clone, while the McrB$^+$ host, CES200, did not. Last, the same filters were washed and probed with the other cDNA clone, 1.6U (Reith and Rigby, in preparation); four positive clones were obtained on NM621 and none on CES200. The clones themselves were shown to plate with approximately equal efficiency on all three hosts (in contrast to the original packaged particles); in fact,

titers were highest on CES200. No rearrangement or loss of DNA occurred in any of the five clones after propagation in CES200. Again, therefore, the clones themselves do not seem to be at a selective disadvantage of once constructed.

Although not conclusive, these data suggest that the lack of McrB restriction in NM621 facilitates recovery of some sequences. The cumulation of data from the four experiments discussed in this section makes it overwhelmingly probable that McrB action can significantly reduce the representation of specific sequences in shotgun libraries of mouse DNA. Of course, other factors may also interfere with recovery of particular sequences.

By analogy, modified DNA from other sources should also be restricted; the magnitude of the restriction would depend on the fraction of cytosine residues methylated in the sequence of interest, and the sequence specificity of the methylation. McrB restriction should act on DNA from mammals other than the mouse, since most or all mammals methylate CG dinucleotides, at least in some chromosomal regions and in some tissues (14). The DNA of plants may carry methyl groups on up to 25% of C residues (14) and should also be restricted by McrB. The DNA of lower eukaryotes and of prokaryotes frequently carries methyladenine or methylcytosine or both, although two important experimental organisms reportedly carry neither: *Saccharomyces cerevisiae* (48) and *Drosophila melanogaster* (49).

Restriction of cDNA libraries Normally, cDNA libraries should not suffer Mcr restriction, since no methylating activity is usually included in the procedure for preparing cDNA. The exception to this rule is when the cDNA is specifically methylated following synthesis, to protect sites internal to the cDNA from endonuclease digestion later in the protocol. For example, the cDNA is methylated with M.*Eco*RI; *Eco*RI linkers are then ligated to the ends of the protected fragments; these fragments, with protected *Eco*RI sites internally and unprotected sites at the ends, are then digested with *Eco*RI to provide cohesive ends for cloning purposes (31). In principle, McrA, McrB or Mrr might interfere with such experiments, depending upon the methylase used. Linkers commonly used are those carrying *Eco*RI, *Bam*HI or *Hind*III sites. Methylation by M.*Eco*RI (GA ^{me}ATTC) should cause no problems. M.*Bam*H1 (GGAT ^{me}CC) caused a slight induction of DNA repair functions in wild type but not McrB$^-$ cells (10), suggesting that weak restriction may occur. M.*Alu*I (**AG ^{me}CT**), which is used to protect *Hind*III (**AAGCTT**) sites, definitely causes McrB sensitivity, and DNA methylated with M.*Alu*I was restricted 1000-fold by an McrB$^+$ host (50); but, surprisingly, no depression in overall plating efficiency was seen when a primary cDNA λ library (with *Alu*I-methylation only on the inserts) was plated on an McrB$^+$ host (50). This result might depend on the distribution of *Alu*I sites on the insert DNA or on the fraction of *Alu*I sites that are also McrB targets. In any event, a judicious choice of host strains should significantly reduce the potential for loss of sequences of interest.

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