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Quantitative evaluation of *Escherichia coli* host strains for tolerance to cytosine methylation in plasmid and phage recombinants

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ABSTRACT

Many strains of *E.coli* K12 restrict DNA containing cytosine methylation such as that present in plant and animal genomes. Such restriction can severely inhibit the efficiency of cloning genomic DNAs. We have quantitatively evaluated a total of 39 *E.coli* strains for their tolerance to cytosine methylation in phage and plasmid cloning systems. Quantitative estimations of relative tolerance to methylation for these strains are presented, together with the evaluation of the most promising strains in practical recombinant cloning situations. Host strains are recommended for different recombinant cloning requirements. These data also provide a rational basis for future construction of 'ideal' hosts combining optimal methylation tolerance with additional advantageous mutations.

INTRODUCTION

The phenomenon of restriction of DNA containing 5-methylcytosine (5mC) was independently discovered by 3 groups during attempts to clone bacterial and phage methylase genes (1,2,3). They showed that the genes formerly thought to restrict only DNA containing hydroxymethylcytosine (present in non-glucosylated T-even phage (4)) also recognize 5mC. These products which restrict 5mC-containing DNA are encoded by the *rglA* and *rglB* genes (restriction of glucoseless phage). A more recent and descriptive nomenclature for these genes is *mcr* (modified cytosine restriction) (3). The designations *rgl* and *mcr* can be considered interchangeable (2,3). The genes for two other restriction activities, *hsd* and *mrr*, are adjacent to the *mcrB* gene in wild type *E. coli* K12. The *hsd* gene encodes the EcoK type I restriction activity while *mrr* restricts DNA containing N6-methylated adenines in certain sequence contexts (5).

We have previously quantitated the effects of the McrB (RglB) methylcytosine specific nuclease activity which is present in many strains of *E.coli* K12 used as hosts for recombinant cloning. We demonstrated a drastic reduction in the efficiency of recovery of recombinants containing methylated genomic inserts in both plasmid and phage vector systems (6). Similar observations of restriction of genomic DNAs in conventional mcr^+ host strains have been reported by others (7,8,9). Here we report the results of quantitative assessments of methylation tolerance in an extensive series of host strains for plasmid and phage vectors. In our previous study (6), we found that one of the $mcrA^-mcrB^-$ strains investigated retained partial *mcrB* function. We show here that this strain is not unique in that many mcr^- strains show limited tolerance to cytosine methylation.

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MATERIALS & METHODS

The *Petunia hybrida* genomic library was constructed as described previously (6) with a nuclear preparation of plant genomic DNA with minimal chloroplast contamination. The 16 to 20 kilobase pair (kb) fraction from a Sau3A partial digest was ligated into BamHI cut lambda 2001 arms (Stratagene) and packaged with Packagene (Promega). The control, recombinant-derived insert (pK BamHI) was supplied by Stratagene with the lambda arms. The partial genomic library was from the mouse cell line LTA-5 which had been transfected with human DNA (provided by T. de Kretser and L. Devereux). A partial Sau3A-digest of DNA was half end filled with dGTP and dATP using Klenow enzyme, ligated with XhoI-cut dephosphorylated lambda GEM-11 half filled arms (Promega) and packaged with the recommended extract (Packagene). Plating bacteria for phage were grown overnight in LB from a plate stock, diluted $^1/_{10}$ in fresh LB with 0.2% maltose and grown to mid exponential phase (a Klett increment of 80 to 100). After centrifugation, the bacteria were suspended in $^1/_2$ volume of ice cold 10mM $MgSO_4$. 0.1 ml of the plating bacteria were incubated for 20 minutes at 37°C with $< 10\mu l$ of phage, and plated in 3ml of 0.7% agar in LB with 0.2% maltose and 10mM $MgSO_4$ onto fresh plates of 1.5% agar in LB.

Strains NW1 and NW2 were constructed by T4GT7 transduction of the *mcrB* (*rglB*) genes from TC600 and TC410 respectively into the *mcrA*$^-$ *mcrB*$^+$ *mrr*$^+$ *serB*$^-$ strain AT2459 (2). The Δ (*mrr-hsdRMS-mcrB*) genotype of TC410 and NW2 (see Table I) was verified by Southern hybridization experiments which employed pBg3 (10) and pBN99 (Noyer-Weidner, unpublished) as probes. pBg3 comprises the entire *mrr* gene (11) and large parts of the *hsd* region (10). pBN99 contains the entire *mcrB* region. While both plasmids gave signals with digests of DNA prepared from the TC410 parental strain DS410, no signal was obtained with TC410 or NW2 DNA. The genotype of TC410 and NW2 was further confirmed by appropriate genetic tests.

The Bluescribe plasmid (pBS M13+)(Stratagene) was methylated *in vitro* with the human placental DNA (cytosine-5) methyltransferase (12) as described previously (6). Bacterial transformation was by the high efficiency Hanahan method (13) with 10^7 to 10^8 transformants obtained per μg plasmid DNA with the strains showing the higher transformation efficiencies.

Purification of the 1.8kb KpnI fraction from human DNA and ligation into the KpnI site of pBS plasmid was as described in ref.6. Random transformants were restreaked on fresh LB-amp plates and grown overnight. Background frequency of transformants from KpnI cut, alkaline phosphatase-treated plasmid ligated without insert was 3% to 18% with different strains (highest in the strains with the lowest transformation efficiencies). Colony streaks were scored for L1 recombinant colonies (6) using the insert from plasmid pBK(1.8)26, a clone of the 5′ 1.8kb KpnI fragment of the human L1 repeat (14).

RESULTS

The status of the *mcr* (*rgl*) genes in the *E.coli* strains tested are given in Table I, together with other salient features of their genotypes. The list includes DS410/TC410 and C600B/TC600, the two pairs of *mcrA*$^-$*mcrB*$^+$/*mcrA*$^-$*mcrB*$^-$ strains used in our previous study (6). We have also tested a number of conventional host strains, most of which are wild type for either or both the *mcrA* and *mcrB* genes, together with a collection of many of the *mcrA*$^-$ *mcrB*$^-$ strains currently available.

With phage recombinants, the primary test of tolerance to cytosine methylation was the relative plating efficiencies of phage with highly methylated or unmethylated inserts, as

Table I

Strain	Mcr(Rgl) A	B	Genotype;(Reference)	Source[#]
DS410	–	+	*minA minB hsd*$^+$ (2,6)	N-W
TC410	–	–	Δ(*mrr-hsdRMS-mcrB*) derivative of DS410 (2,5)	N-W
C600B*	–	+	*hsdR* derivative of C600 (2,6)	N-W
TC600	–	–	*mcrB* derivative of C600B (2,6)	N-W
C600	–	+	*hsd*$^+$ (22)	NM
C600Hf1[@]	–	–	*hsdR hfl* (6)	Promega
LE392	–	+	*hsdR* (7)	Promega
AT2459	–	+	*hsd*$^+$ *serB mrr*$^+$ (7)	N-W
NW1	–	–	*hsd*$^-$ *mcrB*$^-$ derivative of AT2459 by transduction from TC600	N-W
NW2	–	–	Δ(*mrr-hsdRMS-mcrB*) derivative of AT2459 by transduction from TC410	N-W
DH5a	+	+	*hsdR*17 *recA*1; high efficiency transformation strain (13)	BRL
DB1316	+	–	*recD*1014 *hsdR*2 (15)	BB
MB406	–	+	*recB*21 *recC*22 *sbcB*15 *sbcC hsdR hflA hflB*	Promega
KW251	–	–	*recD*1014 *hsdR*2 *mcrA mcrB*1	Promega
ED8767	–	–	*hsdS*3 *supF recA*13 (17)	KHC
MC1061	–	–	*hsdR* Δ(lac)X74 (23)	LS
K802	–	–	*hsdR*2 (24)	NM & ER
K803	–	–	*hsdS*3 (24)	N-W
χ2813	–	–	*hsdR*2 *recA*56 (3)	ER
GM2163	–	–	*hsdR*2 *mcrB*1 *dam*13::Tn9 *dcm*-6 (3)	ER
NM477	–	–	*hsdMS* 5 derivative of C600 (7)	NM
NM496	–	–	*hsdS* (7)	NM
NM554	–	–	*recA*13 derivative of MC1061 (7)	NM
NM621	–	–	*hsdR mcrB recD* (from C600) (16)	NM
DL538	–	–	*sbcC* derivative of NM621 (16)	DL
JH76			*mrr*-2, probably *mcrA*$^-$*B*$^-$ (5)	JH
JH132	–	–	*mrr*$^-$ *hsdS* by transduction of hsd region from HB101 to K802	JH
ER1372	–	+	derivative ER1370	ER
ER1380	+	+	" "	ER
ER1645	–	+	*mcrA*1272 *serB*28	ER
ER1647	–	–	Δ(*mrr-hsdRMS-mcrB*) *recD* derivative of ER1645	ER
ER1648	–	–	as ER1647 but recD$^+$	ER
Strain C	–	–	non-K12 strain (7)	NM
NM664	–	–	Spontaneous *mrr*$^-$ *mcrB*$^-$ *hsd*$^-$ derivative of C600 (DW9)	NM
NM670	–	+	*mcrB*$^+$ *hsd*$^+$ *dnaC*t.s. *tet*^{R} derivative of K802. Parent of strains below.	NM
NM673	–	–	*mcrB hsdR dnaC*$^+$ by T4 from K802	NM
NM674	–	–	as NM673 by P1 from NM621	NM
NM675	–	–	as NM673 by P1 from NW2, *mrr*$^-$	NM
NM676	–	–	as NM673 by P1 from NM664	NM

Sources of strains. N-W; M. Noyer-Weidner; NM: Noreen Murray; KHC: K.H. Choo; ER: Elisabeth Raleigh; BB: Barbara Bachmann; LS: laboratory strain; DL: David Leach; JH: Joseph Heitman.
* Strain C600B, because it is *hsd*$^-$, must be a derivative of the original C600 which is *hsd*$^+$. C600B is referred to as C600 in ref. 5.
@ Strain C600Hfl was apparently constructed from an *hsd*$^-$ derivative of C600. Since *mcrB* is adjacent to *hsd*, the *hsd*$^-$ parent of C600Hfl may also have been coincidently *mcrB*$^-$.

Table II Phage titre relative to TC410

Strain	Unamplified Petunia Library	Unamplified Control Insert	Phage MTR	Amplified Petunia Library
DS410	0.6	83	0.007	105
TC410	100	100	1	100
C600B	0.7	108	0.006	99
TC600	2.3	120	0.019	72
C600	<0.7	153	<0.005	170
C600Hfl	49	83	0.59	70
LE392	2.3	140	0.017	266
AT2459	0.5	134	0.004	101
NW1	71	142	0.50	110
NW2	179	127	1.41	127
KW251	48	133	0.36	119
MB406	1.0	103	0.01	102
MC1061	72	148	0.49	63
K802	45	101	0.45	93
K803	31	87	0.36	108
NM477	19	134	0.14	90
NM496	42	115	0.37	130
NM621	34	70	0.49	69
DL538	29	78	0.37	36
JH76	10	65	0.15	56
JH132	97	124	0.78	144
ER1645	0.5	179	0.003	111
ER1647	248	176	1.41	111
ER1648	231	159	1.45	92
Strain C	32	37	0.86	159
NM644	130	128	1.02	118
NM670	2	78	0.03	96
NM673	36	64	0.56	87
NM674	44	92	0.48	113
NM675	102	80	1.28	106
NM676	110	102	1.07	117

Titres with the three phage types are all normalized to the titres obtained in the same experiment with strain TC410 (set as 100). The Methylation Tolerance Ratio (MTR) is the corrected titre with the unamplified Petunia library divided by the corrected titre of the unamplified lambda recombinants containing the control, unmethylated insert.

used in ref. 6. Genomic DNA from the plant *Petunia hybrida* with an average 26.7% 5mC (6) was cloned into lambda 2001 and packaged as described in Materials and Methods. In parallel, the recombinant-derived and hence unmethylated insert was similarly ligated into the phage vector and packaged. The titre of unamplified phage was determined in each strain. Using the protocol described in Material and Methods, phage titres proved highly reproducible between experiments. All results have been normalized to the titres obtained with strain TC410, the most methylation tolerant strain from our previous study (6). To provide a simple numerical comparison of tolerance to cytosine methylation in the different strains, the corrected value for phage titre for the unamplified library was divided by the corrected value for the unamplified phage with control insert to give the phage Methylation Tolerance Ratio (MTR). The titre was also determined for each strain with a previously amplified *Petunia* genomic library. Amplification had been performed

Table III Effect of Host Strain on Titres of Mouse Genomic Library

Host Strain	pfu/ml	Relative Titres
LE392	0.49×10^4	(1)
KW251	2.8×10^4	5.8
JH132	5.0×10^4	10.3
NW2	6.1×10^4	12.4
DL538	5.9×10^4	12.1
ER1647	9.1×10^4	18.5
ER1648	8.3×10^4	16.9

A partial genomic library in lambda GEM-11 constructed using DNA from a mouse cell line (LTA-5) transfected with human DNA was titred on a selection of the most promising host strains for phage recombinants. The data are averages from 3 or 4 experiments per strain with quadruplicate plates per experiment. In the third column, titres are expressed relative to the phage titre in the conventional host strain LE392. We have since found a similar relationship between these strains using 'complete' human and mouse libraries (not illustrated).

using strain C600Hfl which had been found empirically to give higher titres with the plant library than several other strains and which subsequently proved to be *mcrA*$^-$*mcrB*$^-$ (6, and see below). The titre for the amplified *Petunia* library with each strain was also corrected to the titre with strain TC410.

The data presented in Table II show that conventional host strains used in phage cloning such as LE392, C600 and MB406 have very low MTRs of <0.004 up to 0.01. It also shows that strains judged to be *rgl*$^-$ by classic microbiological criteria may retain significant 5mC-restricting activity. For instance, the 'leaky' *mcrB*$^-$ strain TC600 (6) shows a low MTR value of 0.019, only 3 times that of its *mcrB*$^-$ parental strain. Most of the *mcrA*$^-$ *mcrB*$^-$ strains tested show lower MTR values than TC410. Exceptions are strains NW2 which has the Δ (*mrr-hsdRMS-mcrB*) deletion from TC410 transduced into a non-minicell producing background (AT2459) and strain NM675 which has the NW2 (and hence TC410) deletion introduced into the K802 background. Strains ER1647 and ER1648 which are derived from the *mcrA*$^-$ strain ER1645 by a deletion through the *mrr-hsd-mcrB* region both show high MTR values. ER1647 has the additional potential advantage of being *recD*$^-$ and hence should allow more efficient replication of genomic inserts containing inverted repetitions (15). A complete deletion of the *mcrB* region thus seems advantageous for maximizing the titre with the unamplified plant genomic library. NW2, ER1647, and ER1648 are all potentially good host strains with all showing high MTR values and high titres with the unamplified Petunia library but with the caveat that the latter 2 strains, in our experience, tend to give a higher proportion of very small plaques with mammalian genomic libraries. The best strains for recovering clones from regions containing inverted repeats should be those deficient in exonuclease I (*sbcC*$^-$) as is strain DL538 (16) and possibly in exonuclease V (the *recBCD* enzyme) (15) as is strain KW251. However strain DL538, like its parental strain NM621, and also strain KW251, have MTR values less than 0.4, more than 3 fold lower than the most methylation tolerant strains.

To assess the relative contributions of different *mcrB* alleles and also of the genetic background of these different strains, we utilized a number of novel strains constructed by N. Murray containing 4 different *mcrB* alleles introduced into the same background. The *mcrB-hsd* region of strains K802, NM621, NW2 and NM664 were independently transduced into strain NM670, a *mcrB*$^+$ *hsd*$^+$ *dnaC*t.s. derivative of K802 (Table I). With all 4 of these *mcrB*$^-$ derivatives of NM670, the phage MTR was found to be effectively

Table IV Rank order plasmid transformation efficiency

Strain	Unmethylated pBS	6% 5mC pBS	Plasmid MTR	pBN16 (McrB)	pM.HpaII (McrA)
DS410	4	−2	0.003	< −1	5
TC410	4	4	1.0	4	4
C600B	5	0	0.003	−2	nd[$]
TC600	5	1	0.006	2	5
C600Hfl	4	4	0.80	4	nd
AT2459	1	< −3	0.02	−4	1
NW1	1	−3	0.02	−4	0
NW2	1	1	1.41	1	1
ER1372	3	0	0.013	3	−1
ER1380	3	−1	0.006	−1	0
DH5a	5	1	0.02	nd	−2
DB1316	0	−2	0.10	1	−2
MB406	4	1	0.03	−1	nd
ED8767	4	3	0.36	3	2
MC1061	4	3	0.20	2	1
K802	5	5	0.84	5	nd
K803	5	5	0.93	5	nd
$_{\chi}$2813	2	1	0.62	2	nd
GM2163	3	3	0.90	2	nd
NM477	3	2	0.63	3	4
NM496	2	2	1.15	2	3
NM554	−1	−1	0.39	0	nd
NM621	2	2	0.78	2	3
DL538	2	1	0.41	1	2
JH132	2	2	0.56	2	2
ER1645	3	1	0.02	−3	2
ER1647	3	3	0.89	3	3
ER1648	3	3	0.86	3	3

Plasmid DNA (pBS) was methylated *in vitro* with human DNA methyltransferase to a level of 6% 5mC. Each host strain was transformed by the Hanahan protocol with unmethylated pBS, methylated pBS, and bacterial and phage methylase genes (the plasmids pBN16 and pM.HpaII). The methylated sites produced by these two methylases are restricted by the Mcr(Rgl) B and A activities respectively. All data have been normalized to the transformation frequencies obtained in the same experiment with strain TC410 initially set as 100. The Methylation Tolerance Ratio (MTR) for plasmid transformation was calculated by dividing the corrected transformation efficiency with methylated pBS by the corrected transformation efficiency with unmethylated pBS plasmid. Because of the wide variation in transformation efficiencies of the different strains, the values so calculated have been tabulated as their nearest half log increment to facilitate ease of comparison. Hence the rank order for strain TC410 was $2 \times \log_{10} 100 = 4$. A comparable transformation ranking with the methylase plasmids pBN16 and pM.HpaII to that obtained with unmethylated pBS implies that that strain is minus for the Mcr (Rgl) B and A genes respectively.
[$] nd: not determined.

identical to that of the strain from which its *mcrB* allele had been derived (Table II). Hence the relative titres of the phage recombinants containing the highly methylated *Petunia* DNA seem to be an inherent property of the *mcrB* allele present in each of these strains. However it is not possible to totally rule out the possibility that *Petunia* DNA has some other other form of modification other than 5mC which might cause restriction by *mrr* or by some other as yet uncharacterized system present in *E. coli*.

For cloning mammalian DNAs with generally lower methylation levels, the increment in phage titres is not so dramatic as it is in the plant library. An immediate increase in

Table V Percentage Positive for L1 Inserts

Strain	Exp.1,	Exp.2	Average
TC410	26.8,	15.2	21.0
DL538	12.7,	31.7	22.2
ER1647	37.1,	24.3	30.7
ER1648	35.9,	30.0	33.0
K803	32.2,	33.6	32.9

In each experiment, >100 random transformants obtained in each strain with the ligation of the 1.8kb KpnI fraction of human DNA into pBS were regrown as short streaks on large plates. Colony lifts were tested for the presence of L1 inserts by hybridizing with an appropriate ^{32}P-labelled probe.

titre of at least 10 fold can be obtained by using methylation tolerant hosts (Table III). This is numerically similar to that reported previously (8) for a human genomic library. Strain DL538, which does not give as high a titre as NW2, ER1647 and ER1648 with the *Petunia* library, gives equivalent titres to these other strains with the mouse library. However strain KW251 gives lower titres than the other *mcr*$^-$ strains, suggesting that, while the requirements for optimal titre of a mammalian library may be more relaxed than with a plant library, not every *mcr*$^-$ strain can be expected to give equivalent results.

To assess relative methylation tolerance for plasmid cloning, each strain was transformed using the high efficiency Hanahan method (13) with plasmids containing a methylase gene of bacterial or phage origin. These methylate at sites recognized by the McrB (RglB) or the McrA (RglA) nucleases (plasmids pBN16 (2,6) and pM.HpaII (3) respectively). Transformations were also performed using unmethylated pBS plasmid (Bluescribe M13+) or with pBS methylated *in vitro* with the DNA (cytosine-5) methyltransferase from human placenta (12) to a level of 6% 5mC (6). Again, all results were initially normalized to the value obtained with strain TC410 set as 100. However because of the wide variation in intrinsic transformation efficiencies of the different strains with the unmethylated plasmid, the data have been expressed as a logarithmically-based ranking order (see Table IV caption).

Plasmid Methylation Tolerance Ratios were calculated similarly to the phage MTRs with the transformation efficiency with methylated pBS divided by the transformation efficiency with the unmethylated pBS (both corrected relative to the transformation frequencies obtained in the same experiment with the methylated and unmethylated plasmid in strain TC410). The calculated MTR values for plasmid and phage cloning are notably different in a number of strains such as NW1 and, to a lesser extent, MC1061. This discrepancy may possibly reflect the time required for the different types of vectors to replicate twice and hence escape the possibility of *mcr* restriction. The first major variable with respect to the suitability of the different strains as plasmid hosts was the transformation efficiency obtained with unmethylated plasmid. Unlike titres with the phage containing the control insert which varied by less than six fold between all the strains tested, plasmid transformation efficiencies varied over more than 10^3. Strains showing the highest transformation efficiency combined with good methylation tolerance were K803 and K802. Other high transformation efficiency strains such as C600B and DH5a show low plasmid MTRs and hence cannot be recommended for cloning animal or plant genomic fragments. Strain ED8767 which is used as a cosmid host (17) showed relatively poor methylation tolerance combined, in our hands, with a propensity to generate satellite colonies. While we have not as yet tested it in cosmid cloning, these data suggest it might be suboptimal for this purpose.

The commonly available *mcrA*$^-$ *mcrB*$^-$ strain MC1061 cannot be recommended due to its poor MTR value. Also, while this strain allows a good transformation efficiency by the simple $CaCl_2$ method, it does not transform well by the Hanahan protocol (13). The *mcrA*$^+$ *mcrB*$^-$ strain ER1372 and the *mcrA*$^+$ *mcrB*$^+$ strain ER1380 were also tested, showing that a strain which is *mcrA*$^+$ *mcrB*$^-$ has as low an MTR value as that observed with *mcrA*$^-$ *mcrB*$^+$ strains, and that MTR is reduced even further in an *mcrA*$^+$ *mcrB*$^+$ strain.

As a further practical test, five different strains were used in cloning the highly methylated 1.8kb KpnI fragment from near the 5′ end of the human L1 transposon (18). We had previously shown that this fragment had some 18.3% of cytosines methylated *in vivo* (6), more than 4 times the genomic average. The 1.8kb KpnI size fraction from human genomic DNA was ligated into the KpnI site of the pBS plasmid and aliquots of the same ligation used to transform the 6 different strains. More than 100 randomly chosen colonies for each strain from each experiment were scored for the presence of 1.8kb L1 inserts. Because of the background smear of unrelated fragments in the 1.8kb size fraction, random recovery of the 1.8kb L1 clones should give in the order of 30% positive colonies (6). Results from 2 independent experiments are presented in Table V. Strains chosen for this practical test were TC410, DL538, ER1647, ER1648, and K803. Despite TC410 being a minicell producing strain which results in its losing the pBS plasmid readily, this strain was chosen because of its use as a reference in our other experiments. DL538 was chosen because of the presence of the *sbcC* mutation which improves the recovery of plasmid recombinants containing inverted repeats (16). The remaining 3 strains were chosen due to reasonably high MTR values and efficiencies of transformation. K802 was not used due to low plasmid yields in a previous attempt to recover 1.8kb L1 recombinants in pBS (Crowther, unpublished). However this problem was probably plasmid-specific in that we obtained good yields for other plasmids with this host.

In our previous study (6), the 'leaky' *mcrB*$^-$ strain TC600 gave at least 20 fold improvement in the the recovery of clones from this highly methylated fragment from the human L1 element compared to its *mcrB*$^+$ parental strain. However yields with host strains ER1647, ER1648, and K803 improved significantly over those obtained with TC600. The frequency of 1.8kb L1 positive colonies with these strains are equivalent to a random recovery of these highly methylated elements from the 1.8kb KpnI size fraction in which the majority of fragments have a much lower methylation level (6). In accord with the less stringent requirements for methylation tolerance in plasmid cloning, the results with strain DL538 were probably equivalent to the other *mcr*$^-$ strains tested despite a lower yied of L1 recombinants in one experiment (Table V).

Note that the data presented here relates directly only to these strains from the sources nominated in Table I. Supposedly identical strains from different sources can have different genotypes (eg. C600 and C600B—see note to Table I).

DISCUSSION

The use of a host strain tolerant to cytosine methylation is clearly a major requirement for the construction of eukaryotic genomic libraries. For cloning highly methylated genomes such as those of many plant species, obtaining a significant number of recombinants can in itself be the primary consideration. Use of a highly methylation tolerant host allows an immediate improvement in titre of some two orders of magnitude with a plant genomic library compared to that obtained with a conventional host such as C600, LE392 or MB406

(Table II). (Note however that plant DNA preparations containing significant amounts of unmethylated chloroplast DNA are likely to show much smaller increments in total phage titre due to the unrestricted recovery of chloroplast clones in conventional hosts.) Even with mammalian genomic DNA which has a much lower average methylation level than plant DNA, an immediate improvement in titre of at least 10 fold of a phage library can be obtained through the use of *mcr*$^-$ hosts (8, and Table III). This increment is sufficient to turn many partial genomic libraries into complete libraries.

In addition, even when a particular mammalian genome has a relatively low methylation level overall, the choice of host strain will still be important for the recovery of clones from more heavily methylated regions of that genome or from chromosomal segments adjacent to such highly methylated segments. Examples of highly methylated elements likely to be adjacent to structural genes of interest are the human L1 repeats, the most prominent family of long interspersed repetitive elements found in the human genome (18). The 5′ end of apparently 'full length' members of this repeat family have a methylation level >4 times the genomic average (6). Using the best strains from those analysed in this study, apparently random recovery of such highly methylated elements seems to have been achieved.

With the likely exception of clones from regions of high secondary structure, the best host strains for plasmid cloning at the moment are ER1648 and K803, with the latter having a slightly higher transformation efficiency with equal methylation tolerance. Note that the *recD*$^-$ status of ER1647 would not be of any advantage for plasmid cloning. Also strain K802 may be an appropriate host for use with some plasmid vectors but not with pBS in our experience. For phage cloning, NW2, ER1647, ER1648, and also DL538 and JH132 can all be recommended as general phage hosts with the caveat that the latter 2 strains may be suboptimal in methylation tolerance for recombinant cloning of more heavily methylated genomes. However, strain DL538 is the only currently available *mcr*$^-$ host which carries the *sbcC* mutation which acts to enhance the stability of recombinants containing long inverted repeats (16) and may therefore prove the best host for the recovery of certain sequences. While the construction of phage genomic libraries from more heavily methylated genomes may prove to be a problem with this latter strain, equivalent phage titres were obtained with the mouse library. However we have yet to test for biases introduced during phage cloning by interspersed highly methylated elements. Other potentially good host strains include NM664, NM676, and especially NM675. However we have yet to test these strains exhaustively.

None of the strains investigated here combine the properties of the 'ideal' host for either plasmid or phage recombinant cloning in *E. coli*. However, these data provide a rational basis for future strain construction, most particularly the observation that the highest levels of methylation tolerance were observed in *mcrA*$^-$ strains which carried a deletion through the entire *mrr-hsd-mcrB* region. It remains to be elucidated whether the high methylation tolerance of such strains is due to the complete loss of the several genes which determine the McrB nuclease (19,20,21) or whether it is due to the concurrent loss of Mrr activity. This latter possibility would imply either that *Petunia* DNA contains methylated adenine residues or that the Mrr nuclease also effects 5mC-containing DNA. Whatever the reason, the use of Δ (*mrr-hsd-mcrB*) derivatives seems highly recommendable in future strain construction. Beyond their general methylation tolerance, such deletion strains are also suitable in cloning situations in which N6-methyladenine as well as 5mC might be encountered (as with bacterial DNAs).

Because of the complexity of factors which might contribute to methylation tolerance of *E. coli* strains, it still seems advisable that any new strain should be tested quantitatively for its suitability as a host for recombinant cloning. Enquiries about quantitative assessments of new host strains should be addressed to the corresponding author.

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