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Uniform Nomenclature for Bacterial Plasmids: a Proposal

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INTRODUCTION

During the rapid growth in plasmid research that has taken place in recent years, a great deal of variability has developed in the use of basic terminology in publications in this area. In particular, many terms have multiple meanings, and there is often a profusion of terms for a particular entity or concept. The resulting confusion is aggravated by the lack of a systematic designation and referencing code for plasmids. In consequence, a given plasmid may be referred to by several different designations; a given designation may refer to several plasmids; and many plasmids are described without being given any identifying designation at all. A similar state of confusion exists for the designation of plasmid-carried genes.

There has been a growing awareness of this problem for some years, and at a joint Japan-U.S. meeting on bacterial plasmids (Honolulu, November 1972) the problem was briefly discussed, and there was found to be a general consensus that it would be worthwhile to attempt to develop a uniform system of plasmid nomenclature. The result of this would be, it was hoped, to promote increased clarity in communicating scientific results and thus to permit readers not directly involved in plasmid research, as well as those involved, to comprehend the published material with greater facility.

At the Honolulu meeting, we were named as a working group with the task of preparing a proposal on nomenclature that, after consultation with the group at large, as well as with other plasmid workers who did not happen to be at the meeting, might be published to serve as a set of guidelines in much the same manner as was done for microbial genetics in general (23).

We met in March 1973 and prepared a draft proposal that was distributed in November 1973 to more than 200 scientists, with a request for comments and suggestions. More than 50 responses were received, most containing helpful ideas, either on the entire proposal or on specific items. Subsequently, the draft proposal was presented and discussed during the American Society for Microbiology Conference on Extrachromosomal Elements in Bacteria, held in January 1974. We met after this conference to consider the written and verbal responses to the draft proposal and to incorporate these in a second draft. Two additional meetings of the group were held in November 1974 and March 1975 to consider and incorporate input from various additional sources during the production of the present document. The proposal as it appears here has been considerably modified from the 1973 draft and reflects the broadest consensus that we have been able to obtain. Our hope is that it is specific without being inflexible and that it strikes a balance between

the need for full and accurate description on the one hand and brevity for recording purposes on the other.

The major intentions are (i) to clarify obscurities and resolve ambiguities in the present usage of terminology in the hope of encouraging the adoption of one basic series of clearly defined terms in place of multiple and often confusing synonyms, and (ii) to recommend uniform systems of designations for plasmids and for plasmid-carried genes in the hope of avoiding multiple names for a single genetic element and vice versa.

It cannot be stated too strongly that the proposal is intended as a set of guidelines rather than as a set of regulations. Although we believe that a broad consensus on nomenclature can ultimately evolve through usage, we appreciate the difficulty of obtaining widespread agreement among scientists working in this complex and rapidly evolving field. It may be useful to note in this connection that, although initial acceptance of the 1966 nomenclature proposal by Demerec et al. (23) was less than complete, its major conventions have now gained wide acceptance and have clearly led to clarification and standardization of the general nomenclature currently used in bacterial genetics.

This proposal contains two main parts, one on terminology and one on designations for plasmids and genes. The section on terminology is restricted to a list of some 30 terms which we believed to be particularly problematical and in serious need of clarification. The section on plasmids and gene symbols is considerably more important in that it attempts to provide a systematic and unambiguous reference system for coping with the large number of plasmids and plasmid-borne hereditary determinants already described in the literature and the still larger number that are certain to appear in the future.

We consider this proposal as basically an amplification in one area of the recommendations by Demerec et al. (23) on bacterial genetics nomenclature, and we have attempted to build upon these recommendations whenever possible. Throughout its preparation, therefore, we have utilized established conventions wherever possible; we have endeavored to make use of terms and abbreviations that were already in use as long as they were clear and appropriately descriptive; and when we have found it necessary to introduce new terms and abbreviations, we have tried to make them as descriptive as possible of the phenomenon being considered. Finally, we have attempted to make our definitions, categories, and concepts broad enough to provide sufficient flexibility to encompass new discovereries.

TERMINOLOGY

(i) Plasmid (synonym: extrachromosomal genetic element). *A plasmid is a replicon that is stably inherited (i.e., readily maintained without specific selection) in an extrachromosomal state. Naturally occurring plasmids of prokaryotes are generally dispensable.*

The term "plasmid" was introduced by Lederberg (48) as a generic term for extrachromosomal genetic elements, and it is proposed here in that sense. Thus, it replaces "episome" in the generic sense (but see below, item ii). Bacterial plasmids appear to constitute a well-differentiated taxon. Typical examples are the F plasmid, colicinogenic plasmids, antibiotic resistance plasmids, toxinogenic plasmids, etc. In eukaryotic systems, the genomes of mitochondria and plastids (but not the organelles themselves) may be considered to be plasmids. It should be recognized that extrachromosomal nucleic acid molecules are not necessarily plasmids; the definition implies genetic homogeneity, constant monomeric unit size, and the ability to replicate independently of the chromosome. Thus, the heterogeneous circular deoxyribonucleic acid (DNA) molecules of *Bacillus megaterium* (9) are not necessarily plasmids, nor are abortive transducing fragments or other abortive exogenotes. Genetic elements that are composed of multiples of a monomeric plasmid unit (such as certain R [63] and Col plasmids [6]) and others that are composites of two or more plasmids (28, 54) are themselves plasmids so long as they are stable in the extrachromosomal state.

The dividing line between plasmids and phages (or viruses) is not sharp—for example, the P1 prophage and other similar prophages are typical plasmids (44) even though the products of their vegetative growth are typical bacteriophages; the replicative forms of coliphages f1, fd, M13, and their relatives are stably inherited in an extrachromosomal state that is characterized by the continued production of infective phage particles (41). Nevertheless, typical plasmids and phages (or viruses) are well differentiated; the in-between types could constitute evolutionary intermediates.

(ii) Episome. *An episome is a genetic element that can replicate in either of two alternative states: integrated into or independent of the host chromosome (45).*

This definition was introduced to cover the behavior of F, coliphage λ, and ColE1 (which

was then thought to integrate into the *Escherichia coli* chromosome). However, it soon came to be used as a generic term for all bacterial extrachromosomal elements. This latter usage has, in the long run, turned out to be confusing or ambiguous for two reasons. First, because the episome definition crossed the taxonomic boundary between plasmids and temperate phages, it tended to obscure that boundary and the very basic differences between these two classes of genetic elements. Second, because the definition included the requirement that the element be able to integrate reversibly into the host chromosome, it was too restrictive and imposed a considerable intellectual burden on the early plasmid workers who needed a term to describe the elements they were discovering. In consequence, it was sometimes necessary to perform elaborate intellectual contortions to "prove" that a particular extrachromosomal element was an episome. For example, when conjugative R plasmids (see items iii and ix below) were first identified (2, 60), their similarity to F was clear enough; therefore, they were assumed to be episomes. But they could not be shown to integrate. Satisfaction was obtained by the finding that they could **mobilize** (see item xiii) chromosomal genes (71), even though this was hardly proof of integration. The situation with the ColI plasmids was similar (12, 61). Other "episomes" could not by any stretch of the imagination be shown to integrate—in particular, the evidence upon which ColE1 was originally classed as an episome was shown to be spurious (13), and the integration of ColE1 has, in fact, never been observed. These various experimental discomforts eventually resulted in the resurrection of the more general term "plasmid" (13, 53, 55) after 11 years of interment.

Since "episome" does not define a taxonomic entity but is instead descriptive of the behavior of some members of at least two very different taxa, we recommend that it not be used as the taxonomic label for self-replicating extrachromosomal elements in bacteria, i.e., as a synonym for plasmid (33, 56).

(iii) Conjugative plasmid. *A conjugative plasmid is a plasmid that can bring about the transfer of DNA by conjugation (see also items xii and xiii). Examples include the F plasmid of E. coli; the ColIb, V, and B plasmids, and many of the R plasmids of gram-negative bacteria. With the exception of F (see item vi), naturally occurring plasmids identified on the basis of their ability to mediate conjugation should be referred to simply as conjugative plasmids rather than labeled with some other specific term.*

There are several synonyms for "conjugative plasmid" currently in use. We suggest that some of these be avoided and others deemphasized because of ambiguity and other reasons. Thus, "transmissible plasmid," which has been defined as "capable of mediating conjugation, literally means "capable of being transmitted." "Transferon" and "conjugon" are neologisms for which there appears to be no special need. Furthermore, they do not readily permit the construction of antonyms. "Sex factor" and "fertility factor" are ambiguous in that there is often uncertainty about whether transfer of the factor itself is inferred or that of other genetic material in the cell, especially chromosomal genes, or both. These forms also do not readily lend themselves to the construction of antonyms. "Factor" is better abandoned when the properties of the object become better known. The general use of other terms such as "conjugal fertility factor," "conjugation factor," "autotransfer factor," and "infectious plasmid" is discouraged for one or more of the above reasons. (However, it is recognized that the need for variety requires the use of synonyms, so that there are times when some of these terms may be useful.)

(iv) Nonconjugative plasmid. *A nonconjugative plasmid is a plasmid that cannot bring about the transfer of DNA by conjugation.*

The term **defective conjugative plasmid** has been used in reference to naturally occurring nonconjugative plasmids that appear to be genetically related to conjugative ones (17, 52). This usage is confusing; naturally occurring nonconjugative plasmids should be referred to as defective conjugative plasmids only if they have some demonstrable remnant of a conjugative system.

(v) Cryptic plasmid. *A cryptic plasmid is a plasmid to which no phenotypic traits have been ascribed.*

Strictly speaking, the presence of extrachromosomal circular DNA is in itself a phenotype. However, this situation is operationally sufficiently far removed from the usual sense of phenotype that it does not flaw the definition. Cryptic plasmids are not to be confused with cryptic prophages; the latter are defective prophages that do not express immunity (27).

(vi) F plasmid. *F plasmid is the prototype "fertility factor" responsible for conjugation in the K-12 strains. This term was used by Lederberg et al. (49), by Cavalli-Sforza et al. (10), and by Hayes (32) in their early studies of bacterial mating. The letter F refers specifically to this plasmid and thus should not be used to refer to other naturally occurring conjugative plasmids.*

(vii) F′ plasmid. *An F′ plasmid is an F derivative incorporating a segment of the bacterial chromosome.*

This term supercedes "F′ factor." An organism in which an F′ plasmid has arisen (66) and which carries a chromosomal deletion corresponding to the segment incorporated by the plasmid is termed a **primary F′-containing strain**, and an organism into which an F′ plasmid has been transferred is termed a **secondary F′-containing strain** (7). The differentiation into primary and secondary is intended to emphasize a situation (the former) in which the plasmid generated may be essential to the growth of the particular organism in which it arose.

A nomenclature system for F′ plasmids is in existence (50) which suggests that new F′ plasmids be given serial numbers. It is our recommendation that F′ plasmids be identified by a pair of initials as well as by a number (see the section, Plasmid Designations). The published F′ numbering system recommends designating derivatives of a particular F′ plasmid by a suffix number. We suggest instead that all derivative plasmids be given new plasmid numbers and that suffixes be avoided for reasons given in the section, Plasmid Designations. See also the discussion of this point in Demerec et al. (23).

Note that the definition of F′ given in Demerec et al. is inconsistent with the above definition in that it defines F′ as a strain carrying an F′ plasmid. In practice, this definition has not been widely adopted. Inasmuch as the symbol F′ has generally (and in our view, appropriately) been applied to the plasmid rather than to the strain, it is preferable to use F′$^+$ or F′-containing to refer to the state of harboring an F′ plasmid. These usages are consistent with the distinction between F and F$^+$ originally made by Demerec et al. (23), in which the former refers to the plasmid and the latter to strains carrying it.

By analogy, the prime symbol appended to any plasmid designation could be used informally to signify incorporation of a segment of bacterial chromosome into that plasmid. This should not, however, generate numbering systems for "prime" plasmids separate from those for other plasmid derivatives (see section, Plasmid Designations).

(viii) Hfr. *Hfr is the state of harboring a conjugative plasmid that is integrated into the chromosome and consequently is able to promote oriented chromosomal transfer to suitable recipients.*

In general, Hfr strains transfer certain chromosomal segments at a greater frequency than donor strains carrying the same plasmid in the autonomous state. However, it is our view that the basic features of the Hfr state are integration and oriented transfer, and so we have omitted from the definition any requirement involving frequency. In addition, we have framed the definition to include integration and oriented chromosomal transfer involving conjugative plasmids other than F so as to emphasize the fundamental similarity of these plasmids and of the conjugative process they bring about.

(ix) Resistance plasmid (R plasmid). *An R plasmid is a plasmid that carries genetic information for resistance to antibiotics and/or other antibacterial drugs. "R plasmid" supercedes "R factor" (see above, item iii) and "R (or r) determinant" (in the sense of a nonconjugative plasmid carrying resistance genes).*

We have had reservations about formalizing the elevation to taxonomic rank of certain characters carried by plasmids merely because they constitute salient phenotypes (see section, Plasmid Designations). However, since two of them as such and at least to define them as clearly as possible. It should be noted that plasmids initially identified as R or Col, etc., are frequently found later to carry additionally other types of genes, and so their designations ultimately become quite arbitrary.
tionally other types of genes, and so their designations ultimately become quite arbitrary.

This being the case, it seems logical to frame the definition of "resistance plasmid" so that it conforms at least with the general clinical significance of plasmid-linked resistance. Thus, the letter "R" (or "r") in reference to plasmids implies naturally occurring resistance to antibiotics and other antibacterial drugs rather than resistance in general (such as to radiation, bacteriocins, phages, etc.). We recognize that the line between antibacterial drugs and other antibacterial substances may not always be sharp; however, we believe that generalization to all resistance would destroy whatever usefulness the term may have.

In connection with this definition it is recommended that "determinant" (as in "r determinant") not be used to refer to an entire plasmid or to a polyfunctional plasmid segment, since a determinant is a gene or a contiguous set of genes specifying a *single* phenotypic character.

Finally, it is intended that "R plasmid" be used in an informal sense rather than to define a formal taxon. (Other informal classes of plasmids are outlined in the section, Plasmid Designations.)

(x) Resistance donor (R donor). *A resistance donor is a (bacterial) strain that is capable of transferring resistance genes, usually by*

conjugation, to a suitable recipient.

Many natural isolates of gram-negative bacteria are capable of transferring resistance genes to other organisms. Some of these carry a single conjugative plasmid in which the resistance genes are incorporated. These have been referred to as class I R factors (4) and as plasmid cointegrates (14). Others carry several plasmids, including one or more conjugative plasmids and one or more nonconjugative plasmids that determine resistance and are *mobilized* (see item xiii) by the former. These have been referred to as class II R factors (4) and as plasmid aggregates (14). In the absence of information on the plasmid content of such naturally occurring strains, they should be referred to simply as resistance donors, with the implication that plasmids are involved. Although conjugation is the usual mode of resistance transfer, especially in gram-negative bacteria, spontaneous transduction appears to be the rule in *Staphylococcus aureus* (58); a strain capable of transferring an R plasmid by spontaneous transduction should also be considered a resistance donor.

The term "RTF" has been used to refer to the agent responsible for transfer of resistance genes by conjugation in strains that we define here as R donors. For reasons given below (see item xv) we suggest that the use of "RTF" be avoided. In cases where a plasmid separates into conjugative and nonconjugative components, these should be referred to simply as such.

(xi) Col plasmid. *A Col plasmid is any plasmid that carries genetic information for the production of a colicin. This term supercedes "Col factor" (see item iii).*

(xii) Bacterial conjugation. *Bacterial conjugation is the process of genetic exchange between bacteria, dependent on cellular contact, in which genetic material is transferred from one organism (the **donor**) to another (the **recipient**).*

We suggest that "donor" and "recipient" be used to specify bacterial mating types and that "male" and "female" generally be avoided because they refer only to the presence or absence of F; a strain carrying *any* conjugative plasmid can express the donor phenotype.

(xiii) Mobilization. *Mobilization is the process by which a conjugative plasmid brings about the transfer of DNA to which it is not stably and covalently linked (whether or not transient linkage is established during such transfer is still unclear and in any case irrelevant to this definition).*

Examples of mobilization are the transfer by autonomous F or other conjugative plasmids of chromosomal segments or of other plasmids such as ColE1 (29) and nonconjugative R plasmids (3). Where a conjugative and a nonconjugative plasmid are joined to form a stable **cointegrate** (see item xxv), the transfer of the latter by the former should not be referred to as mobilization, nor should complementation between *tra*$^-$ (see item xv) or other defective conjugative plasmids. It should be noted that this is a purely operational definition and implies nothing about mechanism; there may well be several mechanisms involved.

It should be noted that a particular plasmid can legitimately be referred to as non-mobilizable or non-transferable only in reference to those individual conjugative plasmids with which it has been tested.

(xiv) Transconjugant. *A transconjugant is a bacterial cell that has received genetic material from another bacterium by conjugation. A transconjugant should be referred to as a recombinant only if the transferred genetic material has been inserted into a preexisting replicon in the recipient. If the transferred material is perpetuated per se as a plasmid, then the cell is ordinarily referred to as a **plasmid transconjugant**. If such material is not a plasmid and does not become incorporated into a resident replicon, it will fail to replicate and will be lost through dilution. In this case, the bacterium will ordinarily be referred to as an **abortive transconjugant**.*

We suggest "transconjugant" here because it conveys the sense of transfer. Other alternatives such as "conjugant" (analogous to transformant), "conjugatant" (analogous to transductant), and "exconjugant" (currently in use) imply merely that the cell has participated in conjugation, either as donor or recipient.

The term **transcipient** is currently in wide use, but has at least two different meanings: (i) the consequence of conjugational transfer of DNA, or (ii) the result of DNA transfer by any means. In parallel with **transformant** and **transductant**, the respective consequences of transformation and transduction, we suggest an equivalent term (i.e., "transconjugant") for a recipient of genetic information via conjugation. Thus, we recommend that "transcipient" be used in the nonspecific sense, i.e., as in ii above (although we find it difficult to imagine that this usage will be common).

(xv) Transfer genes. *Transfer genes are those genes carried by a conjugative plasmid that are responsible for the donor phenotype. The recommended abbreviation for such genes is tra. Other genes commonly carried by conjugative plasmids but not essential for fertility (e.g., phage inhibition, incompatibility, etc.) should not be referred to as tra genes.*

The transfer genes of a conjugative R plasmid

have been referred to collectively as a resistance-transfer factor, or RTF. A conjugative plasmid capable of transferring a nonconjugative R plasmid has also been referred to as an RTF. Because of this ambiguity and because the designation RTF implies that there is an exclusive class of conjugative plasmids capable of transferring resistance genes, we urge retirement of "RTF."

(xvi) Superinfection inhibition. *Superinfection inhibition is an operational umbrella term referring to interference with the entry or establishment of an entering plasmid by a resident plasmid. This term supercedes "superinfection immunity" which, by analogy with prophage immunity, should be specifically restricted to inhibition of replication. Among the mechanisms that may be involved in superinfection inhibition are entry exclusion (see below), DNA restriction, incompatibility, and the inhibition of transcription or translation.*

(xvii) Entry exclusion. *Entry exclusion is interference by a resident plasmid with the entry of genetic material via conjugation (56).*

This phenomenon has also been referred to as surface exclusion (1). Our preference for "entry exclusion" is based upon the fact that this is what is usually measured experimentally; "surface exclusion" is too specific in its implication of mechanism. Moreover, there is historical precedent for the use of "entry exclusion" (56).

(xviii) Segregation. *Segregation is the separation of independent, usually homologous genetic elements (e.g., replicas) during cell division. Failure of normal segregation to take place should be referred to as* ***defective segregation****. Distribution of two or more (usually nonhomologous) genetic elements to the same progeny cell has usually been referred to as cosegregation (literally: "separation together"). Since this usage has an uncomfortable aura of self-contradiction about it, we suggest that it be replaced by* ***co-distribution****.*

"Segregation" has been used rather loosely to refer to a variety of phenomena. Some of these usages are contradictory to others, and some would better be supplanted by other terms. We propose that "segregation" be confined to the two usages listed below, and that the usages listed in (iii) be abandoned: (i) separation of homologous genetic elements during meiosis in diploid cells; and (ii) separation of sister chromatids during mitosis and, by extension, separation of any two independent genetic elements during cell division in prokaryotes. (iii) With respect to plasmids, "segregation" has been used improperly in at least three ways: (a) to refer to separation of a plasmid into two or more independent replicons. This phenomenon should be referred to simply as **separation**, which is the better term in our view, since it does not imply distribution to progeny cells. (b) "Segregation" has been used to refer to loss of genetic material where the lost material is not recoverable as part of a functional genetic unit. This loss is more accurately referred to as "deletion"—it is presumably a process analogous to chromosomal deletion and its greater frequency and greater relative extent in plasmids is presumably a consequence of the fact that most of the plasmid genome is nonessential. (c) "Segregation" has also been used to refer to loss of an entire plasmid. This is a contradiction of definition (ii) above; to reconcile this contradiction, one ought properly to speak of loss of an entire plasmid as the result of defective segregation rather than as a segregation event. Thus, mutants in which an entire plasmid is lost at greater than normal frequency are termed "Seg$^-$ mutants."

(xix) Plasmid incompatibility. *Plasmid incompatibility is the inability of two different plasmids to coexist stably in the same host cell in the absence of continued selection pressure.*

One may speak of incompatibility only when it is certain that entry of the second plasmid has taken place, and where DNA restriction is not involved.

Groups of plasmids that are mutually incompatible with one another have been variously referred to as incompatibility or as compatibility classes or types. Since the members of such a group are mutually incompatible, it seems more logical to refer to it as an incompatibility group, class, or type.

(xx) Conjugative (or donor) pili. *Conjugative pili are conjugation-specific hairlike appendages of bacteria (8).*

Conjugative pili have also been referred to as "fimbriae" (30). However, the former term is preferable on phonetic grounds and, moreover, has some historical precedence.

(xxi) Fertility inhibition. *Fertility inhibition is inhibition by one plasmid of conjugative pilus synthesis or of conjugation mediated by another plasmid when both are present in the same cell (see Appendix 1 for suggested phenotypic notations).*

Most plasmids that exhibit fertility inhibition also repress synthesis of their own conjugative pili.

(xxii) Donor-specific phages. *Donor-specific phages are those that infect only strains carrying conjugative plasmids; in all cases so far known, the role of the plasmid involves entry of the phage genome (although not necessarily via a conjugative pilus).*

These phages have heretofore been referred to as male-specific or pilus-specific phages, and it is suggested that these terms be abandoned

as general references—the former for the reason given in item xii, the latter for the reason that penetration by means other than attachment to conjugative pili is possible among such phages. There is, of course, no objection to the term "pilus specific" if attachment of phage to pili has been demonstrated.

(xxiii) Homogenic. *Two genetic elements are homogenic with respect to one another if they are descended from a common ancestor by a known sequence of steps.*

For example, all mutant derivatives of a particular genetic element should be considered homogenic to one another; descendants that contain gross rearrangements of gene sequence should likewise be considered homogenic so long as no new genetic material has been added (see item xxiv). Where a deletion has occurred, the element carrying the deletion is still homogenic to its parent. An acceptable synonym is **congenic**; "isogenic" is in common use in the sense in which "homogenic" is defined above; however, as it literally means "identical," we think it is sufficiently misleading in that context to warrant the recommendation that it should be used only in reference to direct replicas of a given genome.

(xxiv) Heterogenic. *Two genetic elements are heterogenic with respect to one another if they are not known to have common ancestry.*

The intention here is to provide an operational rule of thumb to facilitate the handling of similar genetic elements when ancestry cannot be documented. Thus, coliphages ϕx174 and S13 are heterogenic, as are coliphages f1 and M13, f2 and MS2, staphylococcal plasmids pI524 and pI258, enteric plasmids R1 and R100, etc., even though members of these pairs are quite similar to one another.

A recombinant between two heterogenic elements is considered homogenic to each of its parents with respect to that portion of its genome derived therefrom. Thus, the F plasmid is homogenic to F *gal*, coliphage λ is homogenic to λ *gal*, and F *gal* and λ *gal* may be homogenic to each other with respect to the *gal* region carried by each but are obviously heterogenic with respect to the F and λ portions.

(xxv) Cointegrate. *A cointegrate is a naturally occurring genetic element composed of two or more complete replicons in covalent linear continuity where the component replicons are known to be capable of physically independent replication. (See reference 14.)*

This term is intended to imply that the cointegrate has an individually separate replicator region corresponding to each of its component replicons. It thus refers to chromosomes with integrated prophages or plasmids (including the chromosomes of Hfr strains) as well as to certain composite plasmids. It does not refer to plasmids that have simply acquired one or more new genes (these are heterogenic recombinant plasmids until proved otherwise), nor does it apply to plasmids that are made up of two or more regions with distinct functions.

Two interesting recently described examples of cointegrates are certain naturally occurring R plasmids that can replicate from at least two alternative origins and can reversibly separate into two (or more) individual plasmids (16, 54), and an in vitro-constructed composite of enteric plasmids pSC101 and ColE1 that uses one or the other of its component replicons according to whether external conditions favor one or the other (73).

Generally, two sets of terms are in use referring to the formation and breakdown of cointegrates. **Integration** and **excision** are used primarily to refer to interactions of smaller replicons with the chromosome, whereas **cointegration** and **separation** would be preferable terms for the corresponding events involving two or more replicons of similar sizes.

(xxvi) Plasmid chimera, or chimeric plasmid. *A plasmid chimera is a recombinant plasmid derived from two parental genetic elements obtained from organisms that are ordinarily unable to exchange genetic information. Plasmids constructed in vitro or in vivo and containing DNA from organisms that can ordinarily exchange genetic information should be referred to as* ***hybrid plasmids****. A general term such as* ***composite plasmid*** *may be used to define a stable recombinant plasmid constructed in vitro that contains DNA from organisms that may or may not be able to exchange genetic information ordinarily. "Composite plasmid" is a general term, and as such refers to elements that may or may not be composed of two or more separate replicons.*

(xxvii) Translocation (or transposition) sequence. *A translocating sequence is a well-defined genetic element, usually of constant size, that translocates intact from one genetic locus to another.* ***Insertion sequences*** *are translocation sequences that have been identified on the basis of the polar mutations that they cause at the site of insertion.*

Historically, the first such elements identified in bacteria were termed "insertion sequences" (51, 70). These, of which four have been described to date (IS1, IS2, IS3, and IS4), are characterized as different on the bases of length and nucleotide sequence. So far, they have not been found to carry identifiable genetic markers.

Subsequently, it has been found that certain

plasmid-carried resistance genes undergo translocation (38, 40, 46). Since these are genetically marked, it has been possible to observe the translocation event experimentally. The translocation sequences were found to have inverted repeat nucleotide sequences homologous to the previously described insertion sequences, and so it has been inferred that the latter cause mutation by translocating. Hence, we suggest that the class designation be "translocation sequences" and that "insertion sequences" be considered a subclass.

Translocation sequences have been referred to as "transposons" (38) and as "translocons" (40). We question the utility of coining a neologism for this type of genetic element.

Translocation sequences have been abbreviated "Tn" and given letters to indicate their gene locus (e.g., TnA has been used to indicate a translocation sequence carrying ampicillin resistance [40]). Since "A" refers to the ampicillin resistance phenotype, it would be preferable to use the accepted phenotypic notation, Ap, so that the recommended notation would be TnAp. Inasmuch as more than one such element may exist, it is suggested that unique identifications be appended, referring to the genome where the translocation sequence was first identified (e.g., RP4TnAp; however, more informal notations may be used in context, so long as the reference is clear; see the sections, Plasmid Gene Abbreviations and Molecular Rearrangements).

(xxviii) Plasmid copy number. *The plasmid copy number is the number of molecules of a specific plasmid per genome equivalent or per host cell (synonym: plasmid multiplicity).*

Terms such as **multicopy plasmid** and **oligocopy plasmid** may be useful in reference to plasmids normally present in many or few copies per genome or cell, respectively. Since there is a continuum of plasmid copy numbers, these terms are of limited utility and should be used only in a general descriptive sense. The terms "stringent" and "relaxed" replication control should not be used as synonyms for oligocopy and multicopy plasmids, respectively (see item xxix). An organism carrying two or more different plasmids should be referred to as a di-, tri-, tetra-, etc., or multiplasmid organism or strain.

(xxix) Relaxed and stringent control of plasmid replication. *There are at least four possible parameters by which plasmid replication control may be characterized: (i) ability or inability to replicate in the absence of chromosome replication; (ii) plasmid multiplicity and its variability; (iii) continuity or punctuality of plasmid replication with respect to the cell division cycle; and (iv) randomness or regularity of plasmid replication with respect to choice of molecule.*

The analysis of plasmid replication control is a rapidly developing area; although no plasmid has so far been fully characterized with respect to all four of these parameters, the terms "relaxed" and "stringent" plasmid replication control have been used variously to refer to one or more of the four (14, 26, 62). Ultimately, it may turn out that these terms are not very useful; however, we believe that it is necessary at least to suggest uniform interim definitions so that when they are used they will have a specific meaning.

Therefore, we suggest that criterion i above should be the one upon which the definitions are based, because it is relatively easy to apply and would appear to define a dichotomy rather than a continuum. Hence:

(xxixa) Relaxed control of plasmid replication. *Relaxed control should be used in reference to plasmid replication that is not obligatorily coupled to chromosome replication.*

(xxixb) Stringent control of plasmid replication. *Stringent control should be used in reference to plasmid replication that is obligatorily coupled to chromosome replication.*

Even here, it must be emphasized that the terms are useful only as informal phenotypic descriptions of the behavior of a particular plasmid in a particular host; they should not be applied without qualification.

PLASMID DESIGNATIONS

In general, published designations of plasmids should be retained except where they are duplicated or ambiguous (see below). Therefore, our recommendations are primarily for the naming of new and derivative plasmids. The basis of these recommendations is the use of uniquely identified numerical series analogous to the numerical seriation of strains recommended by Demerec et al. (23) (recommendation 9). To distinguish plasmid designations from bacterial strain designations, we suggest the letter "p" as a prefix, in addition to the two letters referring to the naming laboratory. Thus, pXY1234 = plasmid no. 1234 from the collection of the investigator using the identifying initials XY.

Any genotypic modification of a plasmid would engender both a new strain number and a new plasmid number; all derivative plasmids (mutants, deletions, recombinants, etc.) generated by a particular laboratory as well as all naturally occurring ones isolated by that laboratory should be assigned to a single series. It is urged that this rule be applied to derivatives of plasmids whose designations do not presently

conform to this recommendation, as well as to those whose designations do conform.

The addition of hyphenated or other suffixes to plasmid numbers to indicate derivation is undesirable for several reasons: it inevitably generates duplications; it endows plasmid numbers with generic significance; it generates complications such as how to designate recombinants, etc.; and it makes the numbers for derivative plasmids increasingly unwieldy.

Changes in plasmid number would not be made after simple transfer unless there were some reason to suspect genotypic change accompanying the transfer, for example, transduction by a phage whose genome is smaller than the plasmid.

Some investigators may wish to use letters other than "p" to signify "plasmid," e.g., "r" for "resistance plasmid." This is acceptable so long as it is clear. We would urge, however, that duplication of numbers in a given series be assiduously avoided, i.e., a laboratory should not have both pXY1001 and rXY1001. There is no reason, however, why the same initials should not be used for the plasmid series and for the bacterial strain series of the same laboratory. In the event that duplications arise in the assignment of names, numbers, etc., it is recommended that these be handled by the standard taxonomic practice, i.e., the first publication gets priority and the subsequent one(s) must be changed so as to eliminate the duplication.

It is extremely important to keep careful histories of plasmids. As hidden molecular rearrangements may take place from time to time, one would like to be able to trace pedigrees in order to pinpoint, where possible, the occurrence of such changes. In this connection, it should be emphasized that it is highly desirable in publications to quote the original reference for the source of a plasmid or bacterial strain and its derivation, not merely the worker from whom the culture was obtained.

Plasmid-containing cultures received from other laboratories should be treated as follows. (i) If the plasmids are prototypes with well-established names or stock numbers, the latter should be retained and used in publications. (ii) If they have stock numbers that conform to the above recommendation, these should likewise be retained. (iii) If they have numbers that do not conform, they should be given conforming numbers in consultation with the sending laboratory and in publications both old and new numbers should be listed. (iv) Numbers given to derivative plasmids should conform to the seriation of the laboratory that produces the derivatives. (v) In general, when using this system of seriation, it will be useful in textual material to indicate parenthetically the salient features of a plasmid or strain so designated. Thus, "pXY1234 (a 40-megadalton conjugative R plasmid) was introduced...," etc.

One of the problems that stimulated this series of recommendations was the duplication of plasmid designations due to the parallel growth of different series bearing the same numbers and the same prefix letter (usually R). It is urged that the laboratories that have initiated these series remedy the situation by changing them, where possible, to conform to these recommendations. To this end, the prefix "p" followed by identifying initials should be appended to the extant plasmid designation, retaining the original number: thus, R124 might become pXY124. Of utmost importance in recording such changes is a list of synonyms in the published tabulations of strains.

For general understanding and readability in context, informal plasmid designations have been useful, generally referring to some salient feature of the plasmid, e.g.: antigen plasmid, bacteriocinogenic plasmid, chloramphenicol resistance plasmid (not "chloramphenicol plasmid"), degradative plasmid, enterotoxinogenic plasmid, hemolysin plasmid, mutator plasmid, pigment-inducing plasmid, resistance plasmid, toxinogenic plasmid, tumorigenic plasmid, and virulence plasmid.

Although such informal designations are helpful and their use should be continued, they should not be used as taxonomic classes and, especially, should never be used in place of formal numerical designations. Abbreviations for these phenotypes should be kept as simple as possible—preferably a capital and two lower case letters, e.g., Deg, Ent, Tox, etc., and should be treated in context as trivial names with clear reference to unique numerical designations.

STRAIN DESIGNATIONS

This recommendation refers to the designation of plasmid-carrying strains only, and to the tabulation of such strains for publication. Strain numbers for plasmid-carrying strains should be formulated as recommended by Demerec et al. (recommendation 9, see Appendix 3). Since a strain number constitutes a complete genotype, the introduction of a plasmid engenders a new strain number for the resulting host-plasmid complex as does any genotypic modification of an existing host-plasmid complex. In listings of derivative strains, ancestral strain numbers may be indicated as part of the

genotype. This practice greatly simplifies complex genotypes and serves also to clarify pedigrees. Plasmids and prophages should each be enclosed in a separate parenthesis and, where a strain has been cured of plasmid or prophage, this information should be included in the genotype. A series of K-12 derivatives and their corresponding genotypes might be listed as follows:

K-12	*E. coli* prototroph
C600	K-12(F^-)(λ^-) *thr leu tonA lacY thi*
XY1000	C600(F'155)(pXY1234)
XY1001	XY1000(pXY1234$^-$)

In the last strain, if the listing were "C600-(F'155)," there would be no indication that the strain had once carried, and been cured of, pXY1234.

Complex strain lists will often be greatly simplified by listing host strains and plasmids separately, with the implicit understanding that the construction of plasmid-carrying derivatives is without difficulty unless so stated.

It should be noted that there is a natural tendency for designations of particularly important strains (and also of plasmids) to become generic types. Some obvious examples of this are the various (now generic) *E. coli* prototypes—the B, C, W, and K-12 strains and the F plasmid, and at least one generation of K-12 substrains—CR34, C600, W1485, etc. At some stage in this process, such designations, which were, after all, originally stock numbers used to designate specific strains, gradually lose their specificity and become used generically. This can sometimes give rise to ambiguity; thus the notation K-12 is often used in place of K-12(F^-) or K-12(λ^-) so that one is unsure which is meant. This is a generally undesirable practice but one that is often difficult to inhibit, especially when the formally complete designations are unwieldy. If strains are listed as in the above example, always with ready reference to complete genotypes, such ambiguities are automatically eliminated.

PLASMID GENE ABBREVIATIONS

Phenotypes

The plasmid itself, strictly speaking, has no phenotype; rather, the plasmid genes are phenotypically expressed by the strain carrying the plasmid. Thus, the word "phenotype" should be taken to mean the phenotype of the bacterial cell carrying the plasmid.

Plasmid genes should be designated phenotypically according to convenience and in a manner designed to avoid ambiguity. Therefore, in general, we recommend a capital plus a lower case letter (or two where necessary). A list of recommended phenotypic abbreviations for the commoner plasmid-carried genes is appended (Appendix 1). This type of notation should serve, in some cases, to distinguish plasmid-carried genes from chromosomal loci giving the same phenotype, since the latter most usually have three-letter abbreviations (e.g., Sm^r versus Str^r for plasmid and chromosomal streptomycin resistance phenotypes), and it will also improve the clarity of listed plasmid phenotypes, since a plasmid carrying resistance to ampicillin, kanamycin, streptomycin, and sulfonamide will be listed as "Ap Km Sm Su" instead of "AKSSu," as has usually been done. Although it seems useful to distinguish phenotypically plasmid-linked from chromosomal resistance genes because both are common and because they usually have different modes of action, it is not our intention to set up a rigid distinction between chromosomal and plasmid genes in general by assigning a different type of phenotypic abbreviation to each. Such a distinction would appear to serve no useful general purpose and, in fact, a glance at Appendix 1 will reveal many three-letter plasmid phenotypes.

In view of recent evidence suggesting that all or part of the biosynthetic pathways for certain antibiotics may be plasmid-linked, the question arises of how to distinguish phenotypic notations for synthesis of a particular antibiotic from those for resistance to the same compound. Although we do not suggest any such specific notations, we suggest that synthesis be denoted by an "S" prefixed to the usual phenotypic notation for resistance, e.g., Skm would denote kanamycin synthesis.

There is one conceptual difference between our recommendation for phenotypic notations and that of Demerec et al. (23). This difference stems from the fact that a variable and often rather large portion of the plasmid genome is nonessential for the existence of the plasmid as an autonomous replicon (the larger the plasmid, the larger the nonessential regions). Consequently, there is great variability among closely related plasmids in the carriage of the various nonessential genes that determine the phenotype of the plasmid-carrying organisms. Therefore, we suggest that listing of plasmid genes by phenotype should be taken to signify positive expression of the phenotype involved. This is in contrast to the usual convention (23), which is to list only those alleles that differ by mutation from the wild type. In the plasmid notation, however, the absence of a phenotypic

TABLE 1. *Example of plasmid recombinant notation*

Plasmid	Phenotype	Genotype	Derivation
R100	Tc Cm Sm Su Tra	*tet⁺cat⁺aadA⁺sul⁺tra⁺*	Naturally occurring
R6	Tc Cm Sm Su Km-Nm Tra	*tet⁺cat⁺aadA⁺sul⁺aphC⁺tra⁺*	Naturally occurring
pXY550	Sm^s Tra^-	*aadA2 traE8*	Sm^s, Tra^- mutant of R100
pXY560	Rep(Ts) Tra^-	*repB7 traB4*	Rep(Ts), Tra^- mutant of R6
pXY570	Sm^r Rep^+ Tra^+	550[*traB⁺-traI⁺repB⁺*]:560[*traJ⁺-traE⁺aadA⁺aphC⁺*]*traK⁺tet⁺cat⁺sul⁺*	Recombinant, pXY550 × pXY560

abbreviation implies absence of the expressed phenotype regardless of mechanism. An exception to this would be in a listing of derivatives of a given plasmid for which the entire phenotype (and/or genotype) need be given only for the first listing of the plasmid; in listing derivatives, then, one need only identify those loci at which there are differences from the prototype (see Table 1). It is recommended that this procedure be followed as a rule; simply referring to an earlier publication for a complete plasmid phenotype (or genotype) is discouraged. In certain cases it may be desirable to include the abbreviation for an absent gene for clarity or emphasis. In such cases a (−) superscript is necessary. A (−) superscript may also be used to indicate a sensitive or negative derivative of a plasmid originally carrying the gene in its positively expressed state. The use of subscripts should be avoided, since it creates difficulties in typesetting and so adds to journal publication costs.

In some cases it may be useful to employ other phenotypic modifiers. This should be done according to the recommendations of Demerec et al. (23), as shown in the following example (note that whereas single-character modifiers such as r, s, +, −, etc., appear as superscripts, multiple-character modifiers are best given in parentheses, as shown): Km(Ts) Ap Tc^- Tra(Sus) would be the phenotype of an R plasmid that is thermosensitive for kanamycin resistance, expresses ampicillin resistance, is suppressor sensitive for conjugational transfer (Sus means responsive to the action of a translational suppressor), and is a tetracycline-sensitive derivative of a plasmid originally carrying tetracycline resistance. Note that the modifiers are not separated from the phenotypes they modify, but the notations are separated by spaces.

New plasmid characteristics should be abbreviated briefly and descriptively; e.g., Fb might be used for a fibrinolysin marker. New phenotypic abbreviations should be chosen carefully so as to avoid confusion with genetic or biochemical notations already in use. For example, Na would be better than Nad for nalidixic acid resistance because of the biochemical meaning of the latter.

Analogue series. The existence of series of similar but nonidentical genetic traits poses a special problem for plasmid nomenclature because of the large numbers of genes involved, because of the especial importance of some of these genes in plasmid biology, and because plasmids carrying analogous genes may readily coexist in the same cytoplasm, giving rise to recombinants, translocations, etc. A formal solution for this problem in nomenclature is recommended in the section, Genotypes, below. For phenotypes, a purely operational approach would appear to suffice. The phenotypic abbreviation for a gene should reflect the property by which the gene was identified. Thus, if a β-lactamase gene was identified by ampicillin resistance, its phenotype is Ap; if it was identified by penicillin G resistance, its phenotype is Pc. In cases where it is necessary to differentiate, for example, two or more similar resistance genes specifying different cross-resistance patterns, compound phenotypic abbreviations may be useful. For example, Sm would refer to streptomycin resistance, Sm-Sp to streptomycin-spectinomycin resistance. In the latter case, the implication would be that one knows that both resistances are specified by a single gene.

Phenotypic abbreviations usually refer to a single gene or trait; however, any phenotype as given need not be complete (e.g., additional cross-resistances, known or unknown, may exist).

Among the analogue series, certain groups of plasmid traits present special problems and need to be dealt with individually. These are series of traits where specificity is an essential aspect of the phenotype. Three such series are the determinants of bacteriocinogeny, incompatibility specificity, and DNA restriction. We make specific recommendations for these three, the essence of which is the inclusion in the phenotypic notation of an indication of specificity. It is hoped that this principle will also be used for other analogue series where specificity is an essential aspect of the phenotype.

Bacteriocinogeny. The established nomenclature for colicins (and other bacteriocins) is problematic from the standpoint of this proposal for two reasons. First, the usual abbreviation, e.g., ColB, which was originally intended to refer to the entire plasmid that encodes colicin B production, is also used to refer to the phenotype associated with colicin B production. Second, the phenotypic notation, ColB, may cause confusion if translated directly to a genotypic notation (see below), inasmuch as the genotypic notation *colB* would conventionally signify "the B cistron of the *col* locus" and not "the production of colicin B."

To make the best of a difficult situation, we suggest the following: (i) that the established nomenclature for the bacteriocins themselves be retained (we offer no suggestion for the eventuality, for colicins, that all 26 letters get used up); and (ii) that the phenotypes and genotypes for bacteriocinogeny be abbreviated in conformity with established practice (see Appendix 1). It is our recommendation, therefore, that the established abbreviations (ColB, etc.) be retained as designations for the entire plasmid and that the phenotypic and genotypic abbreviations be different so as to eliminate the existing ambiguity.

Incompatibility specificity. The phenotypic notation for incompatibility should consist of "Inc" plus an indicator of specificity. "Inc" is favored over "Com" since members of the same group are mutually incompatible. A list of known incompatibility types is given in Appendix 2. The existing incompatibility type designations as listed in Appendix 2 are well established and should be retained. New classes should be designated by "Inc" plus an arabic numeral. For the enteric plasmids, this numerical series should start with Inc26 since there are 25 established classes at the time of this writing.

Assignment of incompatibility classes should be made in recognition of the fact that all plasmids that can be maintained in a given host species are capable of interacting with one another in vivo. For example, incompatibility classes should be assigned to nonconjugative plasmids from enteric species with reference to those assigned to conjugative plasmids from these organisms. In general, the assignment of a new incompatibility type should not be made without a test against a complete set of the existing appropriate prototypes.

Restriction-modification. Genetic notations for restriction-modification systems have been complicated, confusing, and nonuniform. We should like to propose for phenotypic notations a modification of those commonly in use (5) (Table 2).

Antibiotic-inactivating enzymes. An extremely important group of plasmid-linked traits consists of the antibiotic-inactivating enzymes. These, in general, occur in a number of similar but nonidentical forms. On one hand, it is rarely possible to say that two different plasmids encode the very same enzyme, and, on the other hand, it is rarely possible to subdivide analogous enzymes having the same mode of action into unambiguous groups (see below). Therefore, we recommend that no indicator of specificity be appended to the phenotypic notation for (enzymatic) antibiotic resistance (see below for a recommendation on genotypic notation for these traits).

Genotypes

In general, we suggest that recommendations 1, 2, and 3 of Demerec et al. (23; see Appendix 3) be followed for the designation of loci, genes, and alleles. We have implicitly adopted recommendations 4 (which has to do with the designation of plasmids as a whole) and 5 (which suggests that genotypes of plasmid markers be assigned according to the same rules as those of chromosomal markers).

In specifying plasmid genotypes in general, the absence of any notation for a gene should be taken to indicate the wild-type allele. In this, we follow the standard practice for chromosome genotype notation. In some cases, it may be desired to include a notation emphasizing that a gene is present. Here, a superscript should be used: (+) for the wild type,

MOLECULAR REARRANGEMENTS

Plasmid recombinants. Recombinants between homogenic plasmids should be considered as simple derivatives and designated

TABLE 2. *Genetic notations for restriction-modification systems*

Recognition system	Notation in common use	New recommendation
Phage P1	r_{P1}^-	Res$^-$(P1)[a]
	m_{P1}^-	Mod$^-$(P1)
Plasmid RII[b]	r_{RII}^-	Res$^-$(RII)
	$r_{RII}^- m_{RII}^-$	Res$^-$ Mod$^-$(RII)

[a] An indicator of specificity should appear as a phenotype modifier, but only when necessary for clarity.

[b] Note that "RII" does not refer to a particular plasmid but rather to a restriction-modification system that recognizes and acts upon a particular nucleotide sequence-specific site.

and (−) for a mutation where an allele number has not been assigned. As recommended by Demerec et al. (23), the designation of an allele says nothing about the phenotype corresponding to that allele. Thus, modifiers such as ts (for thermosensitive), etc., should not be included in formal genotype specifications but may be included informally in context for descriptive purposes.

The assignment of genotypes to members of analogue series of plasmid genes involves a special problem that is not generally encountered in dealing with other genomes and that requires a specific solution if the literature on plasmids is to be decipherable. This problem has been stated clearly by Demerec et al. (23) who, however, despaired of its solution. We quote here their passage on hybrid strains:

> The system described above for designating mutant loci and mutation sites presents no problems as long as all strains are derived from a single wild type. As discussed under Recommendation 1, a locus is considered mutant if it differs from the corresponding locus in the arbitrarily chosen wild-type strain.
>
> Thus, a series of mutant loci have been designated within strains derived from *E. coli* K-12, another series within strains derived from *E. coli* B, still another within strains derived from *Salmonella typhimurium,* and so on. But what is the genotype of a hybrid strain, arising from a cross between wild-type *E. coli* K-12 and wild-type *E. coli* B? Some of its loci will be derived from one wild type, and some from the other. If K-12 were considered as the reference strain, the loci inherited from B would be mutant, and vice versa. Furthermore, the genotype of the hybrid could not be written until it was known from which parent each locus was derived.
>
> Should it be possible to determine from which parent a particular wild-type locus was derived, a symbol could be devised to convey this information. Most loci, however, are likely to remain unidentified. In some situations, e.g. when many new strains are to be derived from a particular hybrid, it will be best to designate the hybrid itself as a new prototype strain comparable to a wild type.

Thus, the problem of distinguishing among analogous genes arises only where different genomes may interact directly with one another—which is very much the case here, inasmuch as different plasmids, related or unrelated, may occupy the same host cell at the same time and complement one another or recombine to produce hybrids. To deal with this situation, a definitive means of differentiating among analogous genes carried by different plasmids is required.

Ideally, one would like to give a single genotypic designation to all analogous loci that are alleles and different ones to all that are not. However, it seems to us useless on theoretical grounds to attempt to make such distinctions, for, among naturally occurring analogues, there is really no dividing line between allelic genes and similar but nonallelic ones. Nevertheless, in conducting genetic analyses, one must have a way of distinguishing, for example, the *traA* cistron of F from that of pXY1234.

Our recommendation, then, is that, where possible, analogous genes should be given the same genotypic symbol, but in genetic analyses, the specific, formal genotypic notation of record should include an identification of the plasmid of origin (e.g., *FtraA, pXY1234traA*). Because of the unwieldiness of these notations, they should be used in context only where necessary to make the distinctions.

With respect to antibiotic-inactivating and other plasmid-coded enzymes, we recommend that the genotypic notation be solely a reflection of the mechanism of action of the enzyme (see Appendix 1). Where there is a series of similar but nonidentical enzymes having the same mode of action, such as the β-lactamases, their genetic determinants should all carry the same genotype, and individuals should be identified by reference to their plasmid of origin, as recommended above. Where a classification of such enzymes has been developed (for example, on the basis of substrate specificity [61a, 65a]), this should not be reflected in the genotypes (or, for that matter, in the phenotypes), but should be simply indicated in context as required, e.g., ". . . the RP4*bla* gene, encoding a type II β-lactamase . . ." etc. Where a plasmid specifies several enzymes constituting all or part of a biochemical pathway, the phenotypic and genotypic notation will generally reflect the function of the overall pathway, for example, *tol* and *raf* for toluene degradation and raffinose fermentation, respectively.

As a rule of thumb, unless one is dealing with mutations and direct plasmid interactions requiring the use of genotypes, it will be much simpler to refer to plasmid genes by their phenotypes.

accordingly (see section, Plasmid Designations). Composite or recombinant plasmids containing covalently joined segments from two or more heterogenic genetic elements, however, require more elaborate designations as follows. First, they should always be given new sequential numbers as noted above (see section, Terminology, item xxiv). Second, in specifying the genotypes of such hybrids, it is necessary to identify the origin, insofar as it is known, of every gene. It is suggested that this be done with square brackets, as shown in Table 1. The hyphens between *traB*$^+$ and *traI*$^+$ and between *traJ*$^+$ and *traE*$^+$ signify the inclusion in each case of a block of genes between the two limiting loci according to the known map order of the *tra* operon. The notations outside the brackets need merely be sufficient to identify the plasmids in context. Genes of uncertain parentage in such hybrids should be listed outside the last bracket.

Kilobase (kb) coordinates are physical map distances in thousands of nucleotide pairs from an arbitrarily determined point of origin. For circular genomes, kilobase distances increase in the clockwise direction, based on an arbitrary orientation of the map. Kilobase coordinates are determined from contour length measurements in conjunction with the identification of fixed topographical features of the genome map. Where kilobase coordinates (68, 69) are available from molecular mapping data, these can also be included, e.g., F[20-67 kb *traA6*]:R100[67/60'-19'/20 kb *tetA2*] would indicate an F-R100 recombinant in which the region from 20 to 67 kb is from F, and the region from 67 through 0 to 20 kb has been replaced by a segment from R100 with coordinates of 60' through 0' to 19'. The junction points are at 67/60' and 19'/20 (the primed numbers refer to locations on the original R100 genome and the unprimed numbers refer to locations on the original F). The plasmid carries the mutations *traA6* in the F region and *tetA2* in the R100 region.

Deletions, insertions, transpositions, translocations, and inversions. These molecular rearrangements constitute structural changes in genotype. Lacking evidence to the contrary, it is safest to assume that such events are unique and therefore to assign to each occurrence a unique genotypic notation. Of especial importance in plasmid genetics are two basic classes of such rearrangements: insertions (including transpositions and translocations) and deletions. In recommending specific notations for these, we expand the established convention of using the Greek delta to designate deletions by suggesting the Greek omega as a designation for insertions.

Complete genotypic notations for deletions and insertions would include numerical seriation and reference to the parent plasmid as follows.

Deletions are conventionally identified by a Greek delta. Where only one gene is known to be affected, an allele number is given, e.g., Δ*blaZ7* specifies that allele no. 7 in the *blaZ* gene is a deletion. Where more than one gene is affected, the deletion is specified by a delta and a number followed by a list of deleted genes separated by hyphens and enclosed in square brackets. Alternatively, this may be done by specifying the first and last genes of a deleted region; however, the circularity of plasmids may give rise to ambiguity here, and this possibility should be borne in mind. If the map is known, an en bloc deletion may be specified by its first and last genes in clockwise order or from left to right, separated by a hyphen. The delta indicates a single en bloc deletion. If the plasmid has suffered more than one deletion, each should be indicated separately. If available, the kilobase coordinates of deletions and other salient features of the heteroduplex map may be specified as part of the genotype (68, 69). Three examples of plasmids with molecular rearrangements and their corresponding genotypes are:

pXY9109 FΔ*traA3*[63.1-64.0 kb]
pXY1112 pI258Δ7[*cad-asa*]
pXY2101 pSC101Ω4[0 kb:K-12*hisA* 1.5 kb]

The first is a Tra$^-$ F plasmid carrying the *trA3* allele, which is a deletion encompassing the region from 63.1 to 64.0 kbs. The second is a derivative of *S. aureus* plasmid pI258 with deletion no. 7 spanning the region from *cad* to *asa*. The third is a pSC101 derivative with an insertion at kb 0 of a 1.5-kb segment carrying the *E. coli* K-12 *hisA* locus. We have not included any recommendation for translocation sequences and related genetic elements since the study of these is so new that such a recommendation would be premature.

It is hoped that these guidelines for designating molecular rearrangements will be useful for other naturally occurring rearrangements as well as for artificially constructed recombinant DNA molecules.

APPENDIX 1

Suggested Designations for Plasmid-Carried Genes[a]

Phenotype[b]	Genotype[b]	Effect
Ac	*acr*	Acridine resistance
Ak[c]	*aacA*[c, d]	Amikacin resistance (aminoglycoside 6′-*N*-acetyltransferase)
Ak	*aacC*	Amikacin resistance (aminoglycoside 3-*N*-acetyltransferase)
Ak	*aadC*	Amikacin resistance (aminoglycoside 4″-adenylyltransferase)
Ap [A, Amp]	bla *[pen, amp]*	Ampicillin resistance (β-lactamase)
Asa [AsO_4^{2-}]	*asa*	Arsenate resistance
Asi [AsO_3^{2-}]	*asi*	Arsenite resistance
Bi	*bis*	Bismuth ion resistance
Bt	*aphA*[f]	Butirosin resistance (aminoglycoside 3′-phosphotransferase)
Bt	*aacA*	Butirosin resistance (aminoglycoside 6′-*N*-acetyltransferase)
Bt	*aacB*	Butirosin resistance (aminoglycoside 2′-*N*-acetyltransferase)
Cam	*cam*	Biodegradation of camphor
Cb	*bla*[e]	Carbenicillin resistance (β-lactamase)
Cd	*cad*	Cadmium ion resistance
Ce	*bla*	Cephalexin resistance (β-lactamase)
Icx	*icx [clx, imm]*	Immunity to colicin "X"
Caa [ColA]	*caa*	Production of colicin A
Cba [ColB]	*cba*	Production of colicin B
Cda [ColD]	*cda*	Production of colicin D
Ce1 [ColEl]	*cea*	Production of colicin E1
Ce2 [ColE2]	*ceb*	Production of colicin E2
Ce3 [ColE3]	*cec*	Production of colicin E3
Cia (ColIa)	*cia*	Production of colicin Ia
Cib (ColIb)	*cib*	Production of colicin Ib
Cka (ColK)	*cka*	Production of colicin K
Cva (ColV)	*cva*	Production of colicin V
Cxa (ColX)	*cxa*	Production of colicin X
Cm (C)	*cat [cam, cml]*	Chloramphenicol resistance (chloramphenicol acetyltransferase)
Co	*cob*	Cobaltous ion resistance
Cp	*bla*	Cephalosporin resistance (β-lactamase)
Cr	*bla*	Cephaloridine resistance (β-lactamase)
Cx	*bla*	Cloxacillin resistance (β-lactamase)
Dm	*aacA*	3′,4′-Dideoxykanamycin B resistance (aminoglycoside 6′-*N*′-acetyltransferase)
Dm	*aacB*	3′,4′-Dideoxykanamycin B resistance (aminoglycoside 2′-*N*-acetyltransferase)
Dm	*aacC*	3′,4′-Dideoxykanamycin B resistance (aminoglycoside 3-*N*-acetyltransferase)
Dm	*aadB*[g]	3′,4′-Dideoxykanamycin B resistance (aminoglycoside 2″-adenylyltransferase)
Eb	*Ebr*	Ethidium bromide resistance
Eex [Sex, Sfx]	*eex [traS, sex]*	Entry exclusion
Em	*erm [ero, ery]*	Erythromycin resistance (ribosomal ribonucleic acid methylase)
Ent	*ent*	Enterotoxin production
Ext	*ext*	Exfoliative toxin production
Fa	*fus*	Fusidic acid resistance
Fi^+(X) [fi^+]	*fin*	Inhibition of conjugational transfer mediated by plasmid "X"
Fi^-(X) [fi^-]		Lack of inhibition of conjugational transfer mediated by plasmid "X"
Gm [G, Gk]	*aacA [gat]*	Gentamicin resistance (aminoglycoside 6′-*N*-acetyltransferase)
Gm	*aacB*	Gentamicin resistance (aminoglycoside 2′-*N*-acetyltransferase)
Gm	*aacC*	Gentamicin resistance (aminoglycoside 3-*N*-acetyltransferase)
Gm[G, Gk]	*aadB*	Gentamicin resistance (aminoglycoside 2″-adenylyltransferase)
Hg [Hg^{2+}]	*mer*	Mercuric ion resistance (mercuric reductase)
Hly		Hemolysin production
Hys		Hydrogen sulfide production
Inc [Com, Mc, Mcr]		Determination of incompatibility specificity
Km [K]	*aphA [kan]*	Kanamycin resistance (aminoglycoside 3′-phosphotransferase)
Km [K]	*aacA [kat, kan]*	Kanamycin resistance (aminoglycoside 6′-*N*-acetyltransferase)

APPENDIX 1 (*continued*)

Phenotype[b]	Genotype[b]	Effect
Km [K]	*aacB*	Kanamycin resistance (aminoglycoside 3-*N*-acetyltransferase)
Km [K]	*aacC*	Kanamycin resistance (aminoglycoside 3'-*N*-acetyltransferase)
Km [K]	*aadB*	Kanamycin resistance (aminoglycoside 2''-*N*-adenylyltransferase)
Km [K]	*aadC*	Kanamycin resistance (aminoglycoside 4''-adenylyltransferase)
Lv	*aphA [lpt]*	Lividomycin resistance (aminoglycoside 3'-phosphotransferase)
Lv	*aacB*	Lividomycin resistance (aminoglycoside 2'-*N*-acetyltransferase)
Lv	*aacC*	Lividomycin resistance (aminoglycoside 3-*N*-acetyltransferase)
Mc	*bla*	Methicillin resistance (β-lactamase)
Mod	*hsm*	Modification of DNA by methylase activity
Mod	*hss*	Modification of DNA requiring site specificity protein for expression of methylase activity
Mut		Mutator activity
Nah	*nah*	Biodegradation of naphthalene
Nic	*nic*	Nickelous ion resistance
Nif		Nitrogen fixation
Nm [N]	*aphA [npt, neo]*	Neomycin resistance (aminoglycoside 3'-phosphotransferase)
Nm [N]	*aacA [neo]*	Neomycin resistance (aminoglycoside 6'-*N*-acetyltransferase)
Nm [N]	*aacB [neo]*	Neomycin resistance (aminoglycoside 2'-*N*-acetyltransferase)
Nm [N]	*aacC [neo]*	Neomycin resistance (aminoglycoside 3-*N*-acetyltransferase)
Oct	*oct*	Biodegradation of octanol
Ox	*bla*	Oxacillin resistance (β-lactamase)
Pc [P]	*bla [pen, amp]*	Penicillin resistance (β-lactamase)
Phi		Interference with phage production
Phi	*pif*	Interference by F with T3 and T7 propagation
Phi		Interference by R plasmids with coliphage λ and coliphage T1 propagation
Phi		Interference by ColI with T5 propagation
Pil	*tra*	Pilus synthesis or structure
Pm	*aphA*	Paramomycin resistance (aminoglycoside 3'-phosphotransferase)
Pm	*aacB*	Paramomycin resistance (aminoglycoside 2'-*N*-acetyltransferase)
Pm	*aacC*	Paramomycin resistance (aminoglycoside 3-*N*-acetyltransferase)
Raf		Raffinose fermentation
Rep	*rep*	Replication
Rep [ts]	*[seg][tsr]*	Thermosensitive
Res	*hsr*	Restriction of DNA by endonuclease activity
Res	*hss*	Restriction of DNA requiring site specificity protein for expression of endonuclease activity
Rm	*aphA*	Ribostamycin resistance (aminoglycoside 3'-phosphotransferase)
Rm	*aphB*	Ribostamycin resistance (aminoglycoside 5''-phosphotransferase)
Rm	*aacB*	Ribostamycin resistance (aminoglycoside 2'-*N*-acetyltransferase)
Rm	*aacC*	Ribostamycin resistance (aminoglycoside 3-*N*-acetyltransferase)
Sal	*sal*	Biodegradation of salicylates
San		Surface antigen production (e.g., K88)
Sb	*ant*	Antimony ion resistance
Seg	*seg*	Plasmid segregation
Sm [S]	*aphC [spt, str]*	Streptomycin resistance (aminoglycoside-3''-phosphotransferase)
Sm [S]	*aadA [sas, str]*	Streptomycin resistance (aminoglycoside 3''-adenylyltransferase)
Sp	*aadA*	Spectinomycin resistance (aminoglycoside 3''-adenylyltransferase)
Su [Sa]	*sul*	Sulfonamide resistance
Tc [T]	*tet*	Tetracycline resistance
Tm	*aacA*	Tobramycin resistance (aminoglycoside 6'-*N*-acetyltransferase)

APPENDIX 1 (*continued*)

Phenotype[b]	genotype[b]	Effect
Tm	*aacB*	Tobramycin resistance (aminoglycoside 2′-*N*-acetyltransferase)
Tm	*aacC*	Tobramycin resistance (aminoglycoside 3-*N*-acetyltransferase)
Tm	*aadB*	Tobramycin resistance (aminoglycoside 2″-adenylyltransferase)
Tm	*aadC*	Tobramycin resistance (aminoglycoside 4″-adenylyltransferase)
Tol	*tol*	Biodegradation of toluene
Tp	*dfr*	Trimethoprim resistance (dihydrofolate reductase)
Tra	*tra [fer]*	Mediating conjugation
Uv	*[uvr]*	Resistance to ultraviolet

[a] Abbreviations for chromosomal genes often found on plasmids are not given. The reader is therefore referred to the articles by Taylor and Trotter (72), Sanderson (65), and Hopwood (42).

[b] Entries in square brackets refer to published synonyms which, it is hoped, will be superceded by the main entries. Where the gene(s) responsible for a given phenotype has not been defined, no genotype symbol is given.

[c] It is suggested that resistance phenotypes refer to the substance(s) to which the marker confers resistance and that, where appropriate, genotypes constitute an abbreviation of the inactivating enzyme. It should be noted that this convention will, in a general way, distinguish typical plasmid resistance genes from the usual types of chromosomal resistance markers. It should be reiterated that, in the absence of information on the nature of the plasmid-specified, drug-inactivating enzyme, only the phenotype designation should be used.

[d] There have been described seven seemingly different aminoglycoside acetyltransferases, each of which inactivates a unique spectrum of aminoglycoside antibiotics by acetylation at the 3, 2′, or 6′ position on the antibiotic molecule. Although the genes coding for each of these could be given a specific cistron designations from *A* to *G*, we beiieve this might be premature since some of the enzymes acetylating the same position on the antibiotic molecule might be related to each other by simple mutational alteration of a common ancestor gene. We therefore suggest that genes specifying 6′-*N*-acetyltransferases be designated *aacA*, genes specifying 2′-*N*-acetyltransferases be designated *aacB*, and genes specifying 3-*N*-acetyltransferases be designated *aacC*.

[e] In keeping with recommendations in the section, Plasmid Gene Abbreviations, we have given all β-lactamases the same genotypic symbol, since they all have the same mechanism of action.

[f] There have been described three different aminoglycoside phosphotransferases, each of which inactivates a unique spectrum of aminoglycoside antibiotics by phosphorylation at the 3′, 5″, or 3″ position, and we recommend that the genes specifying these enzymes be designated *aphA*, *aphB*, and *aphC*, respectively.

[g] There have been described three different aminoglycoside adenylyltransferases, each of which inactivates a unique spectrum of aminoglycoside antibiotics by adenylylation at the 3″, 2″, or 4″ position, and we recommend that the genes specifying these enzymes be designated *aadA*, *aadB*, and *aadC*, respectively.

APPENDIX 2

Plasmid Incompatibility Groups[a]

Plasmids of enteric bacteria

Published group designations (including synonyms)	Suggested phenotypic notations	Plasmid prototypes	Reference
FI	IncFI	F, R386(HH)[b]	24
FII	IncFII	R100, R1	35
FIII	IncFIII	ColB-K98	35
FIV	IncFIV	R124(HH)	35
FV	IncFV	Fo*lac*	18
Com5	Inc5	R27(IP)[b]	Y. Chabbert, personal communication
M, Com7[c]	IncM, Inc7[c]	R446b(HH) R69(IP)	11, 37
Com9	Inc9	R71(IP)	11
Com10	Inc10	R72(IP)	67
Com11	Inc11	R147(IP)	67
A	IncA	RA1(HH)	34
B	IncB	TP113, TP125	31
C, Com6[c]	IncC, Inc6[c]	R40a(IP), R55(IP)	20, 67
H	IncH	R27(HH) (=TP117)	22, 31
Iα, ComI, II[c]	IncIα, IncI, IncII[c]	ColIb-P9, Δ, R144(HH)	11, 31, 36
I2	IncI2	TP114	31
Iγ	IncIγ	R621a(HH) ColIb-IM1420	36, 43
J	IncJ	R391(HH)	15
L	IncL	R471a(HH)	39
N, Com2[c]	IncN, Inc2[c]	N3, R15	19
O	IncO	R[16], R724(HH)	22, 25
P, Com4[c]	IncP, Inc4[c]	RP4	21
S	IncS	R478(HH)	39
T	IncT	Rts1	15
W	IncW	S-a, R7K	34

[a] Although this list is based on all of the data available at the time of this writing, it must be regarded as provisional because work on the interrelationships among the listed plasmids has not been completed.

[b] (HH) and (IP) indicate that the plasmids so designated belong to the Hammersmith Hospital and Institut Pasteur series, respectively. These series have overlapping numbers that have not yet been reconciled (see Section, Plasmid Designations, above).

[c] It has been necessary to list synonyms in some cases, because these exist in the literature, and general agreement on a single designation has not yet been reached. In some cases, the indication of synonyms is provisional, since the prototype plasmids have not all been fully tested for compatibility with all of the other prototypes listed.

Plasmids of *Staphylococcus aureus*

Group	Phenotype	Plasmid prototypes	Reference
1	Inc1	pI524, pI258	59
2	Inc2	pII147	59
3	Inc3	pT127	64
4	Inc4	pS177	64
5	Inc5	pC221	64
6	Inc6	pK545	64
7	Inc7	pUB101[a]	47; R, Novick, unpublished data

[a] pUB101 is a plasmid carrying genes for penicillinase and fusidic acid resistance present in strain FAR4 (47).

APPENDIX 3

Summary of Recommendations of Demerec et al.

1. Each locus of a given wild-type strain is designated by a three-letter, lower-case italicized symbol.

2. Different loci, any one of which may mutate to produce the same gross phenotypic change, are distinguished from each other by adding an italicized capital letter immediately following the three-letter lower-case symbol.

3. A mutation site should be designated by placing a serial isolation number after the locus symbol. If it is not known in which of several loci governing related functions the mutation has occurred, the capital letter is replaced by a hyphen.

4. Plasmids and episomes should be designated by symbols which are clearly distinguishable from symbols used for genetic loci.

5. Mutant loci and mutational sites on plasmids and episomes should be designated by symbols of the same kind as those used for loci and sites on the chromosomes.

6. The description of a strain carrying an episome should include a statement concerning the state and/or location of the episome. The symbols F^-, F^+, F′ and Hfr should be used only to designate the sex factor states as outlined above, and not to convey information concerning the phenotypic properties of mating activity.

7. Genotype symbols which have already been published and which conform to the system recommended above should not be changed. Genotype symbols which do not conform to the above system should be changed accordingly, and the change should be noted when the new symbol is first published.

8. Phenotypic traits should be described in words, or by the use of abbreviations which are defined the first time they appear in a given paper. The abbreviations should be clearly distinguishable from genotype symbols.

9. Strains should be designated by simple serial numbers. To avoid duplications, different laboratories should use different letter prefixes. Strain designations should not be italicized.

10. Strain designations which have already been published and which conform to Recommendation 9 should not be changed. Strain designations which do not conform to Recommendation 9 should be changed accordingly, and the change should be noted when the new designation is first published.

11. When a strain is first mentioned in publication its genotype should be described, and relevant phenotypic information should be

given. The genotype includes a list of all mutant loci and/or mutation sites, a list of episomes and/or plasmids, and information concerning the state and location of any episome.

(Appendix 3 of the proposal on nomenclature for bacterial genetics by Demerec et al. [23]. This summary is quoted here for reference; it should be noted that in the present proposal the term "episome" [recommendations 4, 5, and 6] is updated [see text], and the abbreviation F' [recommendation 6] is redefined [see text].)

APPENDIX 4

Summary of Recommendations on Plasmid Nomenclature

1. Each newly described plasmid and each newly isolated genotypic modification of a known plasmid is given a unique numerical designation of the form pXY1234. Published plasmid designations may be retained even if they do not conform to this recommendation unless they are ambiguous or have been duplicated, in which case they should be changed and the change noted in publications.

2. Bacterial strain numbers refer to strains as they exist, including plasmid content.

3. Any change in the plasmid content of a bacterial strain, including genotypic alterations of preexisting plasmids, gives rise to a new strain number.

4. The phenotypic notation for a plasmid gene should consist of a capital and a lowercase letter (or two if necessary) and should reflect the phenotypic trait for which the gene is responsible.

5. In the initial listing of a plasmid phenotype or genotype, all genes known to be present should be specified, even those that are present in their wild-type configuration. Phenotypic designations may require an indicator of specificity.

6. Genotypic notations for plasmid genes should be in the form recommended by Demerec et al. (23) for bacterial genes (recommendations 1, 2, and 3). The complete genotypic identification of a plasmid gene, however, includes as a prefix the name of the plasmid on which the gene was found.

7. Plasmid recombinants should be given new plasmid numbers. Where the plasmids involved are heterogenic, the complete genotype of the recombinant should specify the contributions of each parent by enclosing the appropriate symbols within square brackets and should indicate also those genes whose parentage is uncertain by listing the appropriate symbols outside of the brackets.

8. Plasmid deletions should be indicated by a Greek delta and identified by unique serial numeration and a list of the deleted genes.

9. Plasmid insertions, transpositions, and translocations should be indicated by a Greek omega and identified by unique serial numeration and a list of the translocated genes. The physical location of the inserted material should be specified where known, as should its origin.

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