

LAB STRAIN COLLECTION

- **Lab strain collections:** The lab strains are stored in three, separate collections.

MST = strains derived from Typhimurium LT2 or LT7

TYT = all pathogenic strains of Salmonella and other bacteria, including Typhi strains and Typhimruium 14028 strains

EM = *E. coli* strains and strains of all other non-pathogenic bacteria other than *Salmonella*

- **How strains are stored.** All strains in the strain collection should be frozen immediately upon entry into the strain book and on the computer. Older strains were frozen in DMSO (detectable by the odor of the frozen cultures) but now strains are frozen in a final concentration of 2.5 M glycerol.
- **Which of my strains should be stored in the lab strain collection?** Strains should be placed in the lab strain collection only if it is clear that they are correct and will be used by others or in a publication. Avoid putting multiple independent isolates of the same strain or uncharacterized mutations in the strain collection. Keep individual collections for these strains. AS SOON AS it is clear that a strain is correct and of general utility, it should be frozen in the the lab strain collection.
- **What about other strains?** Freeze all strains obtained from other labs AS SOON AS they arrive. Do not confirm the phenotypes first.
- **How to add a strain to the lab strain collection.**
 1. Determine which strain collection should be used based upon the criteria above. The strain should be double-checked to make sure it has the correct phenotype and that it is phage-free.
 2. Grow the strain overnight in liquid medium (include an appropriate selective antibiotic if the strain contains a plasmid or a chromosomal duplication). Add 1 ml of the overnight culture and 1 ml of 5 M glycerol to a freezer vial. Write the strain number (determined as described in #3 below) on the top of the freezer vial and the side of the freezer vial. Place the vial in the -70°C freezer. If the strain will be stored in a collection with boxes placed on their side, first place the vial right-side-up in the -70°C freezer for 1-2 hours to allow it to freeze, then put it into the appropriate slot in the freezer box.
 3. Enter the strain into the strain book and on the Strainfinder list on the computer.

- **How to enter a strain into the strain book.**

The strain notebooks are the ultimate resource for information about strains. These notebooks should be complete and legible. It is important to take care of this resource – if you see a loose page fix it, if you note a problem with a strain add a comment to the strain book (and tell Maloy).

1. DO NOT add strains to the strain book unless you have stored them in the freezer. Use the next unassigned number in the lab strain book to enter a number for each new strain. Use a pencil to write in the strain book.
2. Indicate the species/serovar of the strain in the strain book.
3. Write the COMPLETE GENOTYPE. Include the phenotype if the genotype is not known precisely or if it describes an important attribute of the strain (for example, antibiotic resistance).

It is important to use correct genetic nomenclature. DO NOT write genotypes by referring to other strains. Use multiple lines if necessary.

4. Indicate how the strain was constructed followed by the strain designations of the parents of the new strain under donor and recipient. If the strain was obtained from outside of our lab, indicate the alias (as AKA followed by the original strain designation).

Tdx = Transduction
Tsf = Transformation
Epor = Electroporation
Mate = Mating
Mut = Mutagenesis

5. Allele numbers should be assigned to new mutations using the list in the binder labeled “Strain collections”. Allele numbers assigned to this lab have been obtained from Ken Sanderson at the Salmonella Genetic Stock Centre. After using an allele number, ALWAYS cross that number off of the allele list so someone else doesn’t reuse the same number. If you need new strain numbers, see Maloy.
6. Enter the strains original number from a personal strain collection so that mistakes in freezing or recording may be corrected. Include the comment “see strain letters” and the date of the letter if there is an important comment about the strain in the strain letters binder.

- **How to enter a strain onto the computer.**

1. DO NOT add strains to the computer unless you have stored them in the freezer and entered them into the strain book. The designation on the computer should follow the same format as the strain book.
2. The computer strain list available online via Salmonella.org under “Maloy Lab Strainfinder”. Follow the directions to enter a strain on the computer. Always check with April Stanley before entering strains. Include the complete genotype/phenotype, indicate the method of construction, the donor and recipient should be indicated by “donor / recipient”. The computer should include all of the information in the strain book. It is essential to use uniform genetic nomenclature for effective strain searches on the computer.
3. Strain numbers are assigned in sequential, numerical order.

- **How to properly describe genes and mutations.**

1. **Genotypes.** Each gene is assigned a three-letter designation, usually an abbreviation for the pathway or the phenotype of mutants. When the genotype is indicated, the three-letter designation is written in lower case. Different genes that affect the same pathway are distinguished by a capital letter following the three-letter designation.
2. **Allele numbers.** Each mutation in the pathway is consecutively assigned a unique allele number. A separate series of allele numbers is used for each three-letter locus designation. If there is no capital letter designating a specific gene, insert a dash before the allele number. For example, *put-900* or *putA601*.

3. **Phenotypes.** The phenotype is usually indicated with the same three-letter designation as the genotype but phenotypes start with capital letters. For antibiotic resistance markers, use a three letter designation. On the computer indicate resistance with (R) and sensitivity with (S).

Amp = Ampicillin	Tet = Tetracycline
Azt = Azetidine carboxylic acid	Zeo = Zeomycin
Cam = Chloramphenicol	
DHP = Dehydroproline	XG = X-gal
Gen = Gentamicin	XP = X-phosphate
Kan = Kanamycin	
Neo = Neomycin	GFP = Green florescent protein
Spc = Spectinomycin	RFP = Red florescent protein
Str = Streptomycin	

4. **Conditional alleles.** Conditional alleles indicated by the genotype including allele number followed by the two letter designation for the conditional phenotypes shown in parenthesis. Note that because this is a phenotype it begins with a capital letter.

(Ts) = Temperature sensitive mutation	(Op) = Opal mutation
(Cs) = Cold sensitive mutation	(Oc) = Ochre mutation
(Am) = Amber mutation	

5. **Transposon insertions.** Transposable elements can insert in known genes or in a site on the chromosome where no gene is yet known. When an insertion is in a known gene, the mutation is given a three-letter designation, gene designation, and allele number as described above, followed by a double colon then the type of insertion element. DO NOT leave blank spaces between the letters or numbers and the colon.

When a transposon insertion is not in a known gene, it is named according to the map position of the insertion on the chromosome. Such insertions are named with a three-letter symbol starting with z. The second and third letters indicate the approximate map position in minutes: the second letter corresponds to 10-minute intervals of the genetic map numbered clockwise from minute 0 (a = 0-9; b = 10-19; c = 20-29, etc.); the third letter corresponds to minutes within any 10-minute segment (a = 0; b = 1; c = 2; etc). For example, a Tn10 insertion located near *pyrC* at 23 minutes is designated *zcd::Tn10*. Allele numbers are assigned sequentially to such insertions regardless of the letters appearing in the second and third positions, so if more refined mapping data suggests a new three-letter symbol, the allele number of the insertion mutation is retained. This nomenclature uses *zaa* (0 min) to *zjj* (99 min). Insertion mutations on extrachromosomal elements are designated with *zz*, followed by a letter denoting the element used. For example, *zzf* is used for insertion mutations on an F⁺ plasmid. Insertions with an unknown location are designated *zxx*.

<i>zaa</i> = insertion at 0-1 min	<i>zag</i> = insertion at 6-7 min
<i>zab</i> = insertion at 1-2 min	<i>zah</i> = insertion at 7-8 min
<i>zac</i> = insertion at 2-3 min	<i>zai</i> = insertion at 8-9 min
<i>zad</i> = insertion at 3-4 min	<i>zaj</i> = insertion at 9-10 min
<i>zae</i> = insertion at 4-5 min	
<i>zaf</i> = insertion at 5-6 min	<i>zaa</i> = insertion at 0 min

<i>zba</i>	=	insertion at 10 min	<i>zga</i>	=	insertion at 60 min
<i>zca</i>	=	insertion at 20 min	<i>zha</i>	=	insertion at 70 min
<i>zda</i>	=	insertion at 30 min	<i>zia</i>	=	insertion at 80 min
<i>zea</i>	=	insertion at 40 min	<i>zja</i>	=	insertion at 90 min
<i>zfa</i>	=	insertion at 50 min			
<i>zxx</i>	=	insertion with unknown location			
<i>zzf</i>	=	insertion on F-plasmid			

Mini-transposon derivatives we commonly use can be designated as follows:

Tn10dTet	=	Tet resistance, deleted for Tn10 transposase
Tn10dCam	=	Derived from Tn10dTet, Cam resistance substituted for Tet resistance
Tn10dKan	=	Derived from Tn10dTet, Kan resistance substituted for Tet resistance
Tn10dGen	=	Derived from Tn10dTet, Gen resistance substituted for Tet resistance
MudJ	=	Kan resistance, forms <i>lac</i> operon fusions, deleted for Mu transposase
MudJ-Cam	=	Derived from MudJ, Cam resistance marker disrupts Kan resistance
MudCam	=	Cam resistance substitution between ends of Mu

- Plasmids.** Plasmids should be indicated by a / slash after the genotype. Indicate the name of the plasmid, the plasmid origin, and the relevant genotype or phenotype carried by the plasmid. Insertions of suicide plasmids into the chromosome can be indicated with double colons as for transposons. If a duplication is generated it can be described as indicated under chromosomal rearrangements.
- Phage.** Prophages or plasmids integrated into an attachment site can be indicated by the name of the attachment site followed by a double colon and the phage genotype indicated in brackets. For example, *att::[P22 mnt::Kan]*.
- Chromosome rearrangements.** Chromosome rearrangements including deletions, duplications, and inversions should be indicated by a three letter symbol indicating the type of rearrangement, followed by the genes involved indicated in parenthesis, followed by the allele number.

Deletions	=	DEL(genes)allele number
Inversions	=	INV(join point gene #1 – join point gene #2)allele number
Duplications	=	DUP(gene #1*join point*gene #2)allele number

The strain collection is an extremely important resource.

Please follow these rules carefully.

If you are unsure, ask!