Stocks, Breeding Records, and Tank Tags

Good record keeping is essential. We can trace most of our stocks all the way back to founding lines at the University of Oregon (c. 1980). We aim to preserve this continuity and in so doing avoid errors that could cost the lab months or years of lost effort.

Stock basics

- All stocks of fish have an assigned stock name. Sometimes this name is provisional; names can change as projects are published, as crosses are performed, etc. Please ask Dave if you are not sure what a particular stock should be called, or whether two or more stocks are equivalent. Never, ever combine stocks without checking with Dave.
- Some stocks look alike but are very different genetically. For example, the lab maintains several lines of "wild-type" zebrafish that are nearly indistinguishable from one another. Some of these are inbred stocks used for genetic mapping (e.g., AB, wik, Tubingen); others are out-bred stocks used for embryo and larva production (e.g., WT). Phenotypes of several different mutants also resemble one another, as do some of our other danio species. Precautions must be taken to avoid accidental mixing of these phenotypically similar but genetically distinct stocks, including: placing tanks far apart on the racks, avoiding simultaneous breeding or tank cleaning, keeping tanks covered at all times, etc.
- Fish jump. Be aware of this as the fish can themselves contribute to stock mixing through such ballistic activity. Tank covers are a deterrent but do not eliminate jumping: fish occasionally launch themselves through feeding holes, sometimes landing on other tanks and diving in. Really. If you find a live fish outside of a tank, do *not* try to guess where it came from; instead, put it in a beaker with fish water and ask Dave what to do with it. Likewise, if you see a tank with an obvious contaminant phenotype, check with Dave to see what steps should be taken.
- Despite one's best intentions, mistakes happen. Please consult with Dave If you know or suspect you have made a mistake that affects a stock (e.g., accidentally mixing two stocks or mislabeling something). Most such errors are easily corrected. It is much better to fix problems as they arise than to discover them only weeks, months, or years later.[1]

Allele Designations

New mutants or transgenic lines are given allele designations that must always remain associated with the stock. Careful naming—and tracking—of alleles is critical for all of our analyses. Use the following guidelines in naming alleles:

- all formal allele designations carry the *wp* lab identifier (i.e., UW-Parichy lab); some older alleles are *ut*

[1] Ask Dave for some specific and frightening examples.

- our lab allele designations keep track of species, locus, mutation source, and allele number; this differs from many labs that use only sequential numbers
- the correct format for naming is:
    - *wp.(species)(locus number)(mutation source)(unique allele ID)*
    - species: *r*=rerio; *a*=albolineatus; *b*=burma; *c*=choprae *n*=nigrofasciatus; *s*=shanensis
    - locus number: each mutant locus in the lab has a unique identifier; currently used numbers are: *1=tuba8l3a/puma*; *2=erbb3/picasso*; *3=trpm7/nutria*; *4=fms/csf1r/panther*; *5=ednrb1*; *6=TS38*; *8=pissarro*; *10=cx41.8/leopard*; *11=kir7.1/jaguar*; *12=oberon*; *13= sox10/colourless*; *14=kita/sparse*; *15=seurat*; *16=bnc2l/bonaparte*; *17=cezanne*; *18=degas*; *19=duchamp*; *20=chagall*; 21=*dali*
    - mutation source: *e*=ENU-induced; *g*=gamma-ray induced; *s*=spontaneous or from natural populations
    - allele ID: typically a series starting at 1 for each locus, though sometimes referring to the stock number of origin
    - using these conventions, for example, the ENU-induced *D. albolineatus kit* mutant is: $kit^{wp.a14e1}$
- temporary allele designations can be used for on-going screens; typically these will have just the locus name and the stock number in which they were identified (with a, b, etc. for multiple alleles from the same stock)
- transgenic lines should be named as:
  wp.(species)(transgene type)(clone ID)-(unique line ID)
    - transgene type: *t*=tol2, *b*=bac
    - clone ID: as appears in the Parichy lab clones database (typically the LR clone); for example, wild-type from the second founding line for hsp70:XlDIO3-DrIRES-nlsCFP would receive the “allele” designation: *wp.t532-2* (and might have on their tank tag $hsp:DIO3\text{-}CFP^{532\text{-}2}$; see below)

Breeding records

All stocks of fish have a stock number and a stock name. Both are assigned shortly after embryos are fertilized and this information travels with the fish throughout their lives. For filling out breeding records (kept in the stock book), please see the example below follow these guidelines:

- Breeding records (stock number, stock name, etc.) are completed the same day that embryos for the stock are produced. Usually this is done when the embryos are sorted (see **Embryo Care** SOP). It is imperative that breeding records be written the day of breeding so that mix-ups can be avoided.

S# 1234-1...3 investigator DP	(squeeze) natural	birthdate 24 MAR 05
Description kit gfp x kit		Comments stock propagation 3 pair
Mother #, Desc. 101 kit gfp		
Father #, Desc. 120 kit		

- For assigning a stock number, use the following system:
    - New stocks are always assigned the next stock number in sequence. Check the current stock book to see what the last stock number (S#) was. (Fish arriving from other facilities also are assigned the next number in sequence, though such fish may remain in quarantine.)
    - If fish are bred pairwise so that every family has *known parents*, and the families are to remain separate, assign a numerical suffix to the stock number to identify families (e.g., 1234-1, 1234-2). This information can greatly assist subsequent stock propagation and interpretation of phenotypes.
    - If fish are allowed to spawn naturally and *in groups* (i.e., >2 fish per tank), then it will not be certain how many parents contributed to the next generation. This information also is very important for stock propagation decisions and interpreting phenotypes. If batches are to be kept separate, then label the progeny resulting from each tank or batch with an alphabetical suffix (e.g., 1234A, 1234B); if the batches are to be combined subsequently, add an “N” to the stock name to indicate natural group spawning (1234N).
- For assigning a stock name, you should provide as much detail as possible, given the constraints of the tank tags (see below). In general:
    - This information should include the strain and allele (possibly in abbreviated form); for example, homozygous *fms*$^{ut.r4e174a}$ fish are labeled simply “fms-174”; homozygous fish doubly mutant for *fms*^{j4e1} and *kit*^{b5} are labeled simply “fms kit”.
    - Include in the name information about genetic background; this is especially true for outcrosses. For instance, fish outcrossed to wik should be named “stock name x wik” whereas fish outcrossed to AB should be named “stock name x AB” (e.g., “fms-174 x AB”).
    - If a homozygous stock is intercrossed (i.e., brother-sister matings), no special designation is required.
    - If a heterozygous stock is intercrossed and the embryos can be sorted before they are placed on the rack, it can be labeled with the homozygous designation; for example progeny of kit/+ fish can be named “kit” if they are sorted for the embryonic *kit* mutant phenotype and fish placed on the rack are all mutants. If the heterozygous stock cannot be sorted, or there

is some other reason for keeping mixed phenotypes, the intercrossed stock name should include the designation “i.c.” (e.g., “kit/+ i.c.”).

  - Naming of transgenic lines should include the allele designation (which refers to the clone identification number), as well as any pertinent information about promoter, fluorophore, and protein expressed. Additionally, fish should be labeled according to whether they are F0 (newly injected and mosaic) or F1 (ifrom a germ-line carrier and presumably non-mosaic).
  - As always, if you are not sure how to name a stock, ask Dave!

- Record your initials and the date the fish were bred (or when they arrived) in the breeding record. Note that fish room dates are always in the format “numerical day / alphabetical month / numerical year”; e.g., “24 JUN 05”.
- Record the stock numbers and complete stock names for the maternal and paternal stocks. Be careful that information read off of tank tags is transcribed accurately!
- Record whether the stock resulted from natural spawning or in vitro fertilization by circling “natural” or “squeezed”, respectively. This is important to record because fish generally need longer to recover from in vitro fertilization than natural spawning.
- In the “Comments” box indicate why the fish were bred (e.g., stock maintenance, complementation test, non-complementation screening, double mutant production) and how many were successfully bred (e.g., 2 pair; 4 male, 2 female; etc.). This is critical information that allows correct handling of the stock later on, and also can provide important information about the extent of inbreeding, etc.

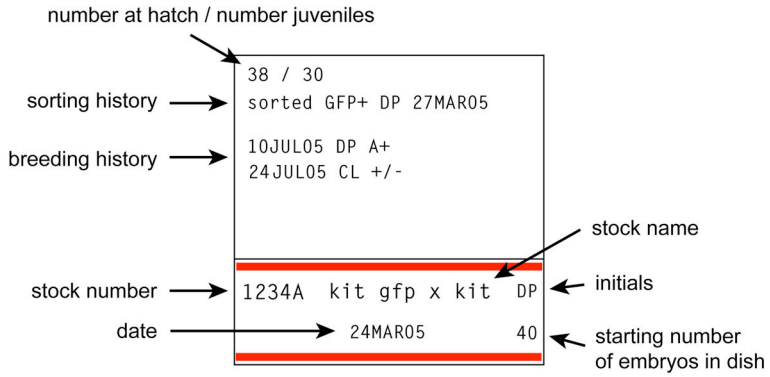
Tank tags

All tanks, dishes, or other containers with embryos or fish are labeled with a tank tag. This tag travels with the fish throughout their lives and contains several important pieces of information. It is very important that all tags use a consistent format so that mistakes can be avoided. Please use the following guidelines:

- Tank tags must be written the day a stock is generated (or arrives).
- Every dish, tank, etc. must have its own tank tag.
- Labels must be written legibly in permanent ink (i.e., fine point sharpie).
- The bottom of the tag (illustrated below) includes the stock number, stock name, date of breeding (or arrival) in standard fish room format, initials of the researcher who generated or received the stock, and starting number of individuals in the container.
- The top of the tag is reserved for: survival data (how many remaining at hatching, etc.), whether fish have been sorted (must include initials and date of sorting), and breeding history (date, initials, and an indication of how successful the

breeding was). Collectively, these data are extremely important for stock maintenance and correct use.

- Tags are placed on the upper right corner of tanks so as not to obscure fish from view. Should a tag become too full of information or too faded, a new tag with just the bottom information is written and the old tag retained on the side of the tank.
- When fish are discarded, the tank tags are retained in a notebook kept in the fish room for this purpose.



I have read and understood the **Stocks, Breeding Records, and Tank Tags** SOP and I agree to abide by it.

Name	Signature	Date