Chapter 2 Basic Genetics

2.0 Genetics

This file introduces some very basic Mendelian genetic concepts. Adult zebra fish are diploid they have two copies of each chromosome, one from the mother and one from the father. In contrast haploid cells such as egg and sperm also known as gametes have only one copy. Each chromosome is comprised of thousands of genes so adult fish have two copies of each gene, again one maternal one paternal. If we represent a gene as **abc** we could represent the genotype as:

Fish genotype = **abc** (maternal) / **abc** (paternal)

Genes are units of information about specific traits derived from parents and passed on to offspring. The gene is a section of DNA that encodes a series of amino acids to make a protein. If the gene encodes a fully functional copy of the protein it is called a wild type allele (allele means "version") of the gene. If there is a mutation in the DNA that leads to a non-functional or partially functional protein we call this a mutant allele of the gene. If the protein is required in the embryo, then in its absence the embryo will not develop normally and will exhibit an abnormal or mutant phenotype. The phenotype of an embryo is a description of how it looks, its observable traits.

Alleles are typically represented in superscript. The wild type allele can be described as **abc^{wt}** and a mutant allele as **abc^{mutX}** where mutX defines a specific mutation in the gene **abc**. There are a many different mutant alleles possible arising from different mutations in the DNA. The wild type allele is sometimes represented as **WT** or often just **+**. So a fish which has one mutant copy of a gene **abc** and one wild type copy of the same gene could be represented as:

abc^{mutX}/abc^{wt} or **abc^{mutX}/wt** or **abc^{mutX}/+**

To use a particular line of wild type fish found in our fish facility **AB*** the wild type allele is **AB*.** If this fish was bred to a fish carrying a mutant allele (outcross) it could then be described as **abc^{AB*}/abc^{mutX}** or more commonly **AB*/abc^{mutX}** this means that the offspring of these fish has a wild type allele of the gene from a **AB*** line of fish and its other copy of the gene is a mutant allele designated **mutX** from the other line of fish. This could then be described as a heterozygous fish, one that has a pair of nonidentical alleles at a gene locus, again one wild type allele and one mutated allele. In contrast, fish that have two copies of the same allele either wt or mutant are called homozygous.

When labeling these fish it is important that you do not use the gene name to refer to the mutant allele. For example do not write **+/abc** to describe a fish heterozygous for a mutation in gene **abc** instead of writing **abc+/abc^{mut}**. For example, a fish line carrying a mutation in the gene sparse should be referred to as **spa^{wt}/spa^{b5}** or **+/spa^{b5}** or **AB*/spa^{b5}** but **not as AB*/spa**. Sparse (**spa**) is just the name of the gene not the name of the mutant allele. The fish facility is communal and has many researchers from different laboratories if we have or if we get a second allele it would be important for us always to record the

allele name appropriately to prevent mix up between the two separate mutations in the same gene.

2.1 Genetic Inheritance of Gametes and Embryos

Adult fish are diploid and possess two copies of each gene. Eggs and sperm are haploid, possessing only one copy of each gene. It is entirely random which copy of the gene is inherited by each gamete (sperm and egg) during the meiotic cell divisions that generate these gametes. Half the sperm and eggs will have one copy of a gene and the other half will have the other copy. An adult fish heterozygous for a wild type and a mutant allele of the gene **abc** will give 50% germ cells carrying the wild type allele and 50% carrying the mutant allele.

Table 2.0 Heterozygous fish carrying a wild type allele and a mutant allele of the gene **abc**.	Genotype	DNA Content
Parent	**abc+/abc-**	Diploid
Possible Germ cells	**abc+** and **abc-**	Haploid
Frequency	50% | 50%	-

When gametes combine in to a fertilized egg, it gets one copy of a gene from its mother and one from its father to make the embryo diploid once again. Based upon this inheritance of alleles, it can be predicted how often an embryo of any given genotype will arise.

2.2 Intercross

If we cross two heterozygous carriers of a mutation in the gene **abc**, what percentage of the embryos will show the mutant phenotype resulting from being homozygous for the mutant allele?

Table 2.1 A cross of two heterozygous carriers of a mutation in gene **abc.**	Female	Male
Parental genotype	**abc+/abc-**	**abc+/abc-**
Germ cell genotypes	**abc+** or **abc-**	**abc+** or **abc-**

Table 2.2 A punnett-square predicting the probable outcome of a genetic cross between two heterozygous carriers of a mutation.		Male Gametes (sperm)	
		abc+	**abc-**
Female Gametes (eggs)	**abc+**	**abc+/ abc+**	**abc+/ abc-**
	abc-	**abc-/ abc+**	**abc-/ abc-**

Fifty percent of the female's eggs will get the allele **abc-**. For each of the eggs that gets the **abc-** allele, there will be a 50% chance that is also gets an **abc-** allele from its farther so the chance that any single embryo gets **abc-** alleles from both it mother and its father is 50% X 50% =. 25%. There are four possibilities for allele combinations from this cross: **abc+/ abc+, abc+/ abc-, abc-/ abc+, and abc-/ abc-**. Each of these combinations has a 25% chance of occurring.

Table 2.3 Outcome of genetic cross between two heterozygous carriers of a mutation.				
Parental genotypes	**abc+ / abc- x abc+ / abc-**			
Progeny	**abc+/ abc+**	**abc+/ abc-**	**abc-/ abc+**	**abc-/ abc-**
Frequency	25%	25%	25%	25%
Phenotype	WT	WT	WT	Mutant

In this cross 25% of the embryos will exhibit the mutant phenotype. Assuming that the mutation is recessive (heterozygotes look the same as the homozygous wild type fish), then the other 75% will look normal. However, those that express the WT phenotypes are not genetically identical, 2/3 of them are heterozygous **abc+/ abc-** and 1/3 are wild type **abc+/ abc+**.

Special note, if mutations are caused by translocation they may not give the expected frequency of mutant embryos.

2.3 Identifying Carriers

Outcrossing an identified heterozygous carrier of a mutant allele to a wild type fish will result in 50% of the progeny being carriers of the mutant allele. Therefore, when screening pools of outcrossed fish, only one in four random crosses will generate offspring in which 25% of the embryos will show a mutant phenotype. Due to this, when crossing two non-genotyped fish, you must not discard the adults if no mutant progeny are discovered because one of fish may still be a carrier.

Table 2.4 Outcome and frequency of an outcross between a heterozygous carrier of a mutant allele to a wild type fish.				
Parental genotypes	**abc^{wt} /abc^{mutX}** x **abc^{wt}/ abc^{wt}**			
Embryo genotypes	**abc^{mutX}/ abc^{wt}**	**abc^{wt}/ abc^{mutX}**	**abc^{wt}/ abc^{wt}**	**abc^{wt}/ abc^{wt}**
Frequency	25%	25%	25%	25%

Note: Only when you cross two heterozygous carriers of a mutation in a gene will you see the homozygous mutant phenotype –described above in 4.2 Intercross and below.

When identified carriers are established, you can cross non-genotyped fish with the identified carriers. If m/+ represent heterozygous carrier of a mutant (m) allele and ?/+ is a potential heterozygous carrier (test fish) with the ? being either a mutant or a wild type allele the following outcomes are possible either m/+ x m/+ the unknown fish is a heterozygous carrier or m/+ x ++ wild type.

m/+ x ?/+ can give either

Table 2.5 If ? is the mutant allele m+/m+.		Male Gametes (sperm)	
		m	+
Female Gametes (eggs)	m	mm (mutant)	m+ (wt)
	+	+m (wt)	++ (wt)

In this case 25% of the embryos have a mutant phenotype. The test fish is a heterozygous carrier of the mutation.
or

Table 2.6 If ? is the wild type allele m+/++.		Male Gametes (sperm)	
		m	+
Female Gametes (eggs)	+	+m (wt)	++ (wt)
	+	+m (wt)	++ (wt)

In this case all the embryo phenotypes look normal. The test fish is not a carrier.
If the progeny do not show the mutant phenotype then the non-carrier fish can be discarded.

In the course of doing genetic crosses it is crucial that you do not discard the wrong fish. One must be able to distinguish the carrier from the test fish. This can be easy if you have a set of fish that exhibit adequate sexual dimorphism or different pigmentation patterns. This may not always be the case so you need to select other differences. Often even if it seems to be easy to tell the male and female fish apart when you first set them up it maybe harder to tell after the female fish has just laid her eggs. She may start to look more like a well-fed male. together. You can always tell a male from a female fish by carefully looking at the skin around the anal flap – learn how to make this distinction. You can also choose two fish of different sizes, again making careful not of which fish is which and keeping a detailed spawning log book including physical descriptions of the fish.

2.4 Propagating and Maintaining Fish Lines

Timely propagation of stocks is necessary to maintain the reproductive and physical fitness of the fish. Zebra fish are most productive between the age of 6-12 months beyond this reproductive capabilities drop. Commonly used stocks should be regenerated at least every 9 months from at least 10 successful pair-wise crosses. Heterozygous carriers should be outcrossed to healthy wild type fish to produce the next generation of fish. Generally the next generation should not be derived from the surviving siblings of a cross between heterozygotes that generated mutant embryos. The only time you might want to do this is if you are desperate for heterozygous fish as an incross will generate more carriers than an outcross 66% versus 50% respectively. Remember when your doing pair-wise test crosses you must be able to distinguish between the carrier and the wild type fish.

Maintain at least 12 males and 12 females from identified mutant carriers. Keep a generous supply of outcrossed fish for all lines that you maintain. Although space is always limited try to keep ~150 potential carriers at different ages of the lines you are responsible for. With commonly used stocks be sure to keep at least two different tanks on two different fish system. Ideally some tanks in the H221 and some in J083b facilities. Freeze sperm whenever possible from genotyped males in case of a disaster.

Wild type lines are maintained exclusively as inbred lines. To insure there productivity and overall well being the next generation should be based on at least a dozen pair-wise crosses. Since these fish lines are used for egg production, mapping, outcrossing, etc it is important to insure the purity of the wild type line. Fish used for outcrossing should be kept in a separate tank and not placed back in to the wild type pure line.

In vitro fertilization (see section 3.2) is a useful tool for propagating lines because it can produce a large number of fertilized eggs for your next generation. This is a particularly good idea if you have fish that do not give well or have only a few fish to work with. A new generation should never be based on a single fish or set of fish if it all possible.

After 3 to 4 months the generation will reach maturity. At this time check sex ratio and genotypes of the fish by pair wise crossing. If you have adequate sex ratios and enough identified carriers the old line of fish can be euthanized.

Keep track of your fish as they pass from the nursery, to juvenile rearing and finally to adult tanks. Consolidate fish whenever possible to conserve space.

2.5 Labeling Fish Tanks

Every fish container needs to be properly labeled. First because the fish facilities are communal you need to select the appropriate labeling tape color. For those doing work under the protocol of David Raible use blue tape, David Kimelman use orange tape, Susan Brockerhoff use yellow tape, James Hurley use magenta tape and those working with the mutation screen fish use green tape. White labeling tape is generally used by facility staff, and red labeling tape is used for marking fish that need to be euthanized. Labeling tape can be found at every workstation. In the upper left-hand corner of the label place the exact genetic information, and then alongside or just below it place the stock number. On the bottom section of the labeling tape place the date of birth and the primary researcher's initials. A stock number can be used in place of genetic information if you have completely filled out the zebra fish database. For more information on stock numbers and the zebra fish database see Chapter 1 Basic Methods. Place the label on the upper left-hand corner of the front tank. Other appropriate tank labels include those that identify the last time the fish were breed or squeezed, and any special feeding directions. Dates can be placed along the sides of the face (front) of the tank. Special feeding instructions can be recorded on white tape and placed on the front or top of the tank, but **do not cover the front of the tank with labels**. Fish facility staff needs to be able to readily see in to these tanks everyday.

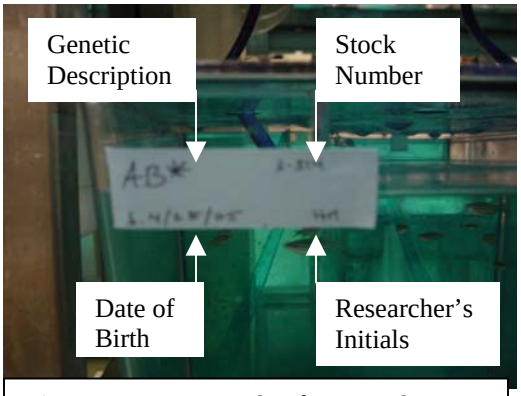

Figure 2.0 An example of a properly identified fish tank.

As previously mentioned when labeling these fish do not use the gene name to refer to the mutant allele. For example do not write **+/abc** to describe a fish heterozygous for a mutation in gene **abc** instead of writing **abc+/abc^{mut}**. For example, a fish line carrying a mutation in the gene should referred to as **spa^{wt}/spa^{b5}** or **+/spa^{b5}** or **AB*/spa^{b5}** but **not as AB*/spa**. Sparse (**spa**) is just the name not the name of the mutant allele.