WORKING WITH COLOURS

By Carita Saarinen, Futuregen, Pori, Finland.

Capital Alpacas, UK

I would like to pass my acknowledgements and thanks to Dr Kylie Munyard for patiently and generously guiding me to the basics of the science behind the alpaca colour genetics. And not forgetting the breeders/alpaca owners for kindly allowing me to have their animals in this article. I hope all of you readers will enjoy the article and that it gives you some more understanding and inspiration.

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Understanding how to breed for different colour/pattern has always fascinated alpaca and llama breeders – for an obvious reason. Alpacas and llamas come with the widest range of different base colours and patterns of all the other fibre production animals and professional alpaca breeders especially focus on a particular type of outcome. Many look for new breeding stock or stud services from preferred colour categories – with an expectation of what they will be getting. However, some essential stuff is easily missed, if we do not know the genotype of the colour. As experience has shown, surprises when we are only assuming are fairly common. Some interesting cases have been collected in this article.

Only in recent years has Neogen been providing a test based on the research by Dr Kylie Munyard at Curtin University, Australia, to find out the genotype of the two main genes in the DNA of an alpaca, MC1R & ASIP, that control the base colour and also the grey/non-grey status (classic grey pattern). Since 2022 the test has developed to also include the three non-functional MC1R and ASIP mutations to offer even more precise information. The intensity project (one of the three active alpaca colour research projects at Curtin University) is still working to find out the actual dynamics of these mutations, however, there is already a paper published in May 2023 (*Identification of six genomic regions associated with pigment intensity in alpacas – Naomi Gray[A], Ishani Shah[A], David Groth[A] and Kylie A.Munyard[A],**), if you wish to read further.

The DNA colour test is the first commercial genomic testing service to alpaca breeders to evaluate heritable genetic traits in their stock and might just potentially open the door on the world of genomics to the alpaca industry.

Being a complex topic, colour genetics, requires a bit more in depth study than reading one article or attending one seminar (which many alpaca breeders have got used when learning about the fibre histograms and traits alone). Once you are ready for it and get your own alpacas tested, it provides you with an excellent objective tool for more defined selection processes and to make your customers happy. It obviously works alongside other selection criteria.

The more breeders get involved, the more can be learned together as a European wide community!

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Alpaca Fleece Colour Test Results

REPORT DETAILS:

Customer Name: Futuregen
XXXXXX Alpacas
Order Number: XXXXXX
Date: 7/23/2023

Sample Barcode	Animal ID	Registration Number	MC1R + ASIP Genotype	Grey/Non-Grey Status^	MC1R_C901T	MC1R_A82G	MC1Rdel_224-227ACTT	ASIP_325-381del57_b	ASIP_C292T	ASIP_G353A
XXXXXXXXXX	XXXXXXXXX	Name	Ee Aa	Classic Grey	Ee1	Ee2	EE	AA	AA	Aa3
XXXXXXXXXX	XXXXXXXXX	Name	ee aa	Non-Grey	e1e1	e2e2	EE	AA	a2a2	AA
XXXXXXXXXX	XXXXXXXXX	Name	Aa	Non-Grey	Ee1	NR	EE	AA	AA	Aa3
XXXXXXXXXX	XXXXXXXXX	Name	ee aa	Non-Grey	e1e1	e2e2	EE	AA	Aa2	Aa3
XXXXXXXXXX	XXXXXXXXX	Name	Ee Aa	Non-Grey	Ee1	Ee2	EE	AA	Aa2	AA
XXXXXXXXXX	XXXXXXXXX	Name	Ee aa	Non-Grey	EE	Ee2	EE	Aa1	AA	a3
XXXXXXXXXX	XXXXXXXXX	Name	ee Aa	Non-Grey	Ee1	e2e2	EE	Aa1	AA	AA

NR designates inability to confirm genotype.
^Mating of 2 animals identified as Classic Grey has a 25% risk of leading to the death of the embryo early in the pregnancy (homozygous lethal).*
** Jones, M., Sergeant, C., Richardson, M., Groth, D., Brooks, S. and Munyard, K. (2019) A non-synonymous SNP in exon 3 of the KIT gene is responsible for the classic grey phenotype in alpacas (Vicugna pacos). Animal Genetics 50(5). 493-500.*

The MSIP & ASIP Genotype table is intended as a guide only. Base fleece phenotypes and progeny base fleece colour predictions are not guaranteed as current tests are unable to specify/identify specific alleles for white, fawn or bay (brown); nor can we predict dilution or intensity effects of the Classic Grey allele or other novel markers if present. Research continues to identify novel markers contributing to fleece colour and pattern.

An example of the current test report

Why test?

- Learn to work with your animals to get the most desirable outcome – combining the quality, health and colour.
- Improve colour uniformity within the herd to target a certain genotype and phenotype combination.
- Reduce the risks of BEW (blue eyed white) progeny.
- Improve your sales ads and make your customers happy by providing the colour genotype information so they know what they are getting when they are looking out for new breeding stock from your farm.
- Helps to prevent the sale of animals that may be useful in your breeding programme.
- Helps prevent you paying for something you will not get.
- Helps you price your animals correctly and prevent accidental discount for instance cryptic grey animals.

When to test?

- Carry out sampling at least two months before you need the results.
- There is no official recommendation to how old the animal should be for sampling but it might be a good idea to wait until the crias are about two months of age before samples are taken.

How the sample is taken?

- Blood sampling is known to be the most successful method of collecting material for genotyping, leading to the most success rate (99,15%) within different sampling techniques trialled *(Futuregen trial 2021)*.
- DNA blood sample can be collected from the edge of the ear or from the jugular vein in the neck of the alpaca.
- Does not involve an additional cost for the blood card. (Needles are not included.)
- Swab sampling is a simple, comfortable and an effective method of sampling and often only requires one person to do the job. Success rate with swabs is also high with 96,58% trialled *(Futuregen trial 2021)*.
- Samples taken from the nose have been more successful than the samples taken from the mouth *(Futuregen trial 2021)*.
- The cost of a swab is €3,50/pc (excluding VAT), however, this might save you additional vet costs and time.

Which animals to test?

- Any animal whose role you want to identify and target in your breeding programme.

>> Continued on next page

Case study: Teasing black to the daylight

Wuthering Heights Alpacas, Finland

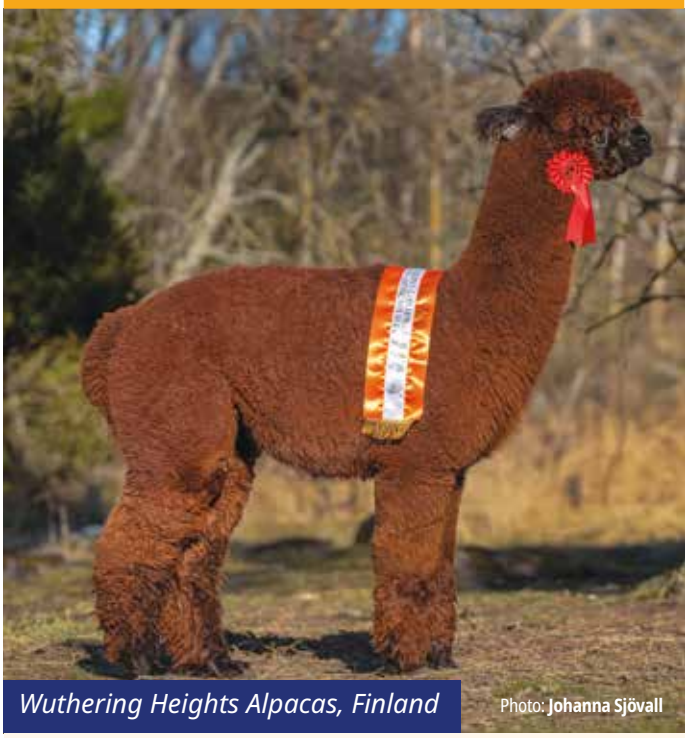
G134 JUANITA
Phenotypic colour: Solid light fawn
Genotypic colour: e^1e^1 a^1a^1 (a "hidden black")

As I personally have a strong affection for browns, I prefer animals that look and produce brown/darker pigments. With this female I have received both dark and lighter progeny. Before I could not tell what caused it or how to work with her.

When I received the results and gained more awareness, I was able to understand that all I had to do in order to get darker progeny off her, was to pair her with an EE (aa) male. The important part is to make sure, that the male most preferably has two large "E"s, so that the cria will definitely receive one functional MC1R copy. This is enough to allow at least a part of the eumelanin (black pigment) to pass through to the physical phenotype (two active MC1R copies is stronger than one).

Juanita is genetically black with her ASIP genotype, but just cannot express it due to the two non-functional MC1R copies. With a right pair, she will definitely always produce darker colours and never a fawn if this is what I wanted. On the contrary – if I wanted to go for fawn, I only need to pair her with a male with two small "e"s to make sure nothing could be produced other than fawn or potentially beige/white, depending on the ASIP copies of the partner. Simple. The sire of the black cria (who I named Touché Noire) is presented on the next page, Inca Othello.

Case study: A brown black



Wuthering Heights Alpacas, Finland Photo: **Johanna Sjövall**

INCA OTHELLO
Phenotypic colour: Solid medium brown with dark points
Genotypic colour: EE a^1a^2

Genetically this male is black – and he could just as well be! But he is brown – which is why he was originally selected. (More specifically, I selected him from my preferred colour category.)

I would like to note, that his genotype wasn't known as we purchased him with my friend Nana Hjelt (Finland), so it was a bonus for us to see how useful his genotype was for our coloured breeding programme. He has two active MC1R copies (EE) and two black ASIP copies, which matched perfectly with my female, Juanita, to provide a 100% chance of a cria with darker pigment. The chances for the phenotype of the cria were somewhere between medium/dark brown - bay black - black. And a black we got, Touchè!

Case study: Non-grey greys

Tinkan Alpakat, Finland

KARINIEMEN CENNI
Phenotypic colour: Dark silver grey fleece, white in the head
Genotypic colour: EE a^1a^2
Grey/Non-grey status: Non-grey[2]

Through her whole life this older female was always thought to be a classic grey. But she is a mixed tuxedo and possibly a roan or greying and this similar patterning has been inherited to most in her family line (some only with tuxedo and no roan/greying, some with both), and none of them are classic grey (three of them have been tested, that have this same pattern). Many thanks to Janika Ahti/Tinkan Alpakat from Finland for kindly allowing me to show Cenni´s case in this article.

Please see another similar case to this below, where breeders could get misled. Many thanks to Capital Alpaca/Kim for allowing me to show this case in the article. This animal is again a roan with a white spot and the fleece is medium rose grey according to the colour chart.

Modern grey – both body and fleece, Capital Alpacas, UK

Case study: What a grey surprise!

WINSAULA TOUCH OF FROST
Phenotypic colour: Solid white
Genotypic colour: ee Aa^2
Grey/Non-grey Status: Classic grey

Despite the pearl white appearance, this male is a classic grey with a hidden black gene (a^2). He has been doing really well at shows during the last two showing seasons, gaining a Suri Champion White Male at South of England Spring Show 2022 and came second at BAS National Halter Show 2023 in a Suri intermediate male white class. No one is able tell from his outer appearance, that this male is genetically a classic grey and I was able to witness that myself as I was allowed to have a closer inspection on him at the National Show this spring – and yes, he is indeed as white as snow! Thanks to the owner Paula Winsor for kindly allowing me to present him in this article. Knowing this does not take him to a grey class as far as shows, but from a breeding perspective, this obviously does change a thing or two and for his breeder it is very valuable information.

Winsaula Alpacas, UK

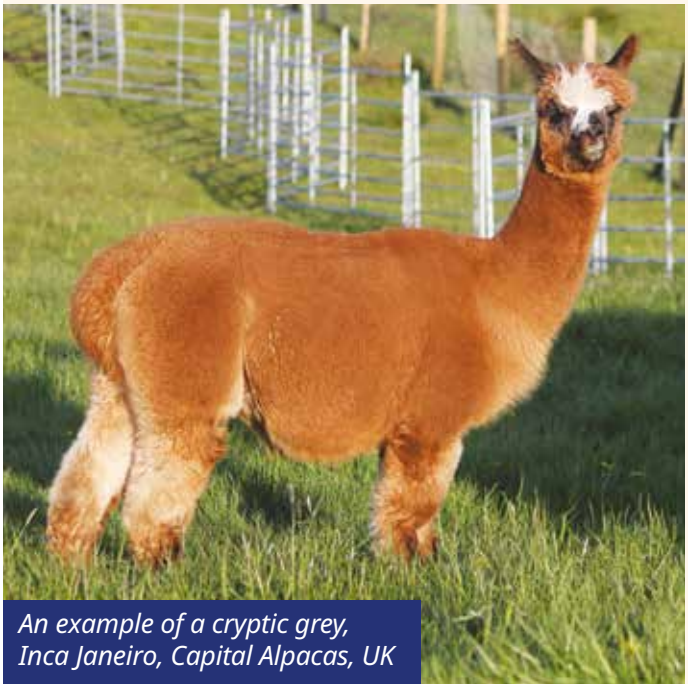
Worth noticing

Seeing his two non-functional MC1R:s (two small e:s) it is easy to understand why phenotypically he is so light. He is simply unable to express the level of eumelanin (black pigment) he has in his DNA. However, it seems as he has no yellow/red pigment (pheomelanin), as the MC1R does not have any effect on the pheomelanin being expressed, in other words, does not block it from showing even when the MC1R in non-active. His sample was not "calling" from the e^1 mutation, so we cannot say if he is either e^1 e^1, e^2 e^2, or e^1 e^2. To work the grey pattern to be visible on the offspring, the breeder would need to work with a right type female that will allow the cria to have a darker base colour. Therefore selecting a female with two large E:s to give a 100% chance for the cria to have one copy of a functioning MC1R, that will allow eumelanin to pass through to the phenotype. Of course the breeder also needs to have a look at ASIP genotype on the female. If the female had two non-functional ASIP copies (aa) this would give the best chances to a darker progeny, where the grey pattern would more likely become visible. Whether the cria will inherit the grey pattern is completely dependent on the parent(s) passing it or not.

Note to grey breeders: No homozygous greys have ever been found, as two copies of the grey allele is lethal and the embryo will die within the first weeks of pregnancy. This is the explanation to why breeding grey to grey generally has a lower stick rate. When breeding with any classic greys there is a 50% chance of getting the grey allele from the grey parent.

Out of 152 white alpacas tested for the grey research project at Curtin University, Australia, 9,2% were identified as classic greys and out of 89 fawns 13,4% were genetically classic greys. There was even one black out of 61 tested, that was identified carrying the grey allele! Greys can be both "hidden", like in the case above, but also "cryptic", when some outer expressions are visibly seen but they might get unnoticed, or misinterpreted, as they are not quite like the general classic greys. An example of a cryptic grey is pictured below.

This female, Inca Janeiro, belongs to Kim Murray from Capital Alpacas, UK, who kindly allowed me to share this case in my article. There are no white fibres anywhere in her fleece, just the white marking on her face. She could easily be identified as a multicolour and be discounted for that. However, she is a classic grey and with a genetically non-grey male she went on to produce the gorgeous rose grey progeny in the picture. Congratulations.



An example of a cryptic grey, Inca Janeiro, Capital Alpacas, UK

Capital Texugo

Case study: Black roulette

INCA NO STRINGS
Phenotypic colour: Solid Bay Black

Inca Alpaca, UK

BECK BROW JIMMY CHOOS
Phenotypic colour: Solid True Black

Now, black breeders, let's do a fun test: Which male do you think would most likely give you black progeny? Just by looking at the pictures and knowing the phenotypic colour.

Even though both males would work in a black breeding programme, one would give you black crias with a 50% larger likelihood than the other one.

Many would think that as Inca No Strings is a bay black and Jimmy Choos is true black, Jimmy Choos would have the higher likelihood of producing black progeny. However, Inca No Strings is testing out as EE aa (more specifically EE a^1a^3) and Beck Brow Jimmy Choos is Ee aa (more specifically Ee^2 a^2a^2).

Please take a look at the Neogen Colour Chance Table opposite for possible outcomes with different partners and the difference in the likelihood of getting the desirable black result:

Inca No Strings with EE aa has 50% more likelihood of producing a black cria, than Beck Brow Jimmy Choos with a Ee aa female. This is how we can simplify it to make it easier to work with. Overtime, breeders can work their herd towards any preferred genotype + phenotype combination, which can become really rewarding and for black breeders especially it solves the issue with the odd fawn crias being born in a black breeding program. Many thanks to Tim Hey from Inca Alpaca, UK, for generously allowing me to present these two stud males in this article!

Males like Jimmy Choos that would carry the genetics and traits that are desirable for the breeding program are still just as useful, but in order to also retain the desired colour outcome, the breeder would want to pair him with only EE aa females. Having the stud males tested is a very good start, especially if you are offering stud services, but because of the mathematics it is just as important to know the genotypes of the females to make this puzzle work. In other words the data will only really offer you tools to work with, when you have it complete from both parents. As you are able to see from the Colour Chance table, some pairs only have one single possible outcome, whilst some may have six different possibilities, depending on the genotype of both parties.

Neogen® Chance Tables for Alpaca Tests 2023

Base Colours

	EE AA	Ee AA	EE Aa	Ee Aa	ee AA	ee Aa	EE aa	Ee aa	ee aa
EE AA	EE AA 100%	EE AA 50% Ee AA 50%	EE AA 50% EE Aa 50%	EE AA 25% Ee AA 25% EE Aa 25% Ee Aa 25%	Ee AA 100%	Ee AA 50% Ee Aa 50%	EE Aa 100%	EE Aa 50% Ee Aa 50%	Ee Aa 100%
Ee AA	EE AA 50% Ee AA 50%	EE AA 25% Ee AA 50% ee AA 25%	EE AA 25% Ee AA 25% EE Aa 25% Ee Aa 25%	EE AA 12.5% Ee AA 25% ee AA 12.5% EE Aa 12.5% Ee Aa 25% ee Aa 12.5%	Ee AA 50% ee AA 50%	Ee AA 25% ee AA 25% Ee Aa 25% ee Aa 25%	Ee Aa 50% EE Aa 50%	EE Aa 25% Ee Aa 50% ee Aa 25%	Ee Aa 50% ee Aa 50%
EE Aa	EE AA 50% EE Aa 50%	EE AA 50% Ee Aa 50%	EE AA 25% EE aa 50% EE Aa 50%	EE AA 12.5% EE Aa 25% EE aa 12.5% Ee AA 12.5% Ee Aa 25% Ee aa 12.5%	Ee AA 50% Ee Aa 50%	Ee AA 25% Ee Aa 50% Ee aa 25%	EE Aa 50% EE aa 50%	Ee Aa 25% Ee aa 25% EE Aa 25% EE aa 25%	Ee Aa 50% Ee aa 50%
Ee Aa	EE AA 25% Ee AA 25% EE Aa 25% Ee Aa 25%	EE AA 12.5% Ee AA 25% ee AA 12.5% EE Aa 12.5% Ee Aa 25% ee Aa 12.5%	EE AA 12.5% EE Aa 25% EE aa 12.5% Ee AA 12.5% Ee Aa 25% Ee aa 12.5%	EE AA 6.25% Ee AA 12.5% ee AA 6.25% EE Aa 12.5% Ee Aa 25% ee Aa 12.5% EE aa 6.25% Ee aa 12.5% ee aa 6.25%	Ee AA 25% ee AA 25% Ee Aa 25% ee Aa 25%	Ee AA 12.5% Ee Aa 25% Ee aa 12.5% ee AA 12.5% ee Aa 25% ee aa 12.5%	EE Aa 25% EE aa 25% Ee Aa 25% Ee aa 25%	EE Aa 12.5% EE aa 12.5% Ee Aa 25% Ee aa 25% ee Aa 12.5% ee aa 12.5%	Ee Aa 25% Ee aa 25% ee Aa 25% ee aa 25%
ee AA	Ee AA 100%	Ee AA 50% ee AA 50%	Ee AA 50% Ee Aa 50%	Ee AA 25% ee AA 25% Ee Aa 25% ee Aa 25%	ee AA 100%	ee AA 50% ee Aa 50%	Ee Aa 100%	Ee Aa 50% ee Aa 50%	ee Aa 100%
ee Aa	Ee AA 50% Ee Aa 50%	Ee AA 25% ee AA 25% ee Aa 25% Ee Aa 25%	Ee AA 25% Ee Aa 50% Ee aa 25%	Ee AA 12.5% Ee Aa 25% Ee aa 12.5% ee AA 12.5% ee Aa 25% ee aa 12.5%	ee AA 50% ee Aa 50%	ee AA 25% ee Aa 50% ee aa 25%	Ee Aa 50% Ee aa 50%	Ee Aa 25% Ee aa 25% ee Aa 25% ee aa 25%	ee Aa 50% ee aa 50%
EE aa	EE Aa 100%	Ee Aa 50% EE Aa 50%	EE aa 50% EE Aa 50%	EE Aa 25% EE aa 25% Ee Aa 25% Ee aa 25%	Ee Aa 100%	Ee Aa 50% Ee aa 50%	EE aa 100%	EE aa 50% Ee aa 50%	Ee aa 100%
Ee aa	EE Aa 50% Ee Aa 50%	EE Aa 25% Ee Aa 50% ee Aa 25%	Ee Aa 25% Ee aa 25% EE Aa 25% EE aa 25%	EE Aa 12.5% EE aa 12.5% Ee Aa 25% Ee aa 25% ee Aa 12.5% ee aa 12.5%	Ee Aa 50% ee Aa 50%	Ee Aa 25% Ee aa 25% ee Aa 25% ee aa 25%	EE aa 50% Ee aa 50%	EE aa 25% Ee aa 50% ee aa 25%	Ee aa 50% ee aa 50%
ee aa	Ee Aa 100%	Ee Aa 50% ee Aa 50%	Ee Aa 50% Ee aa 50%	Ee Aa 25% Ee aa 25% ee Aa 25% ee aa 25%	ee Aa 100%	ee Aa 50% ee aa 50%	Ee aa 100%	Ee aa 50% ee aa 50%	ee aa 100%

		Parent 1	
		Classic grey	Non-grey
Parent 2	Classic grey	Classic grey 50% Non-grey 25% Lethal 25%	Classic grey 50% Non-grey 50%
	Non-grey	Classic grey 50% Non-grey 50%	Non-grey 100%

Note:

- The colours of the alleles illustrate the possible real life colours in each MC1R & ASIP genotype
- Where A = All non-black ASIP / Agouti phenotypes, i.e. white, fawn, bay or B&T
- The Chance Table is not capable of identifying / taking into account the different MC1R & ASIP mutations

Please contact **Carita Saarinen** at **Futuregen** for further assistance & individual planning:

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Understanding the terminology - Short descriptions

What are the MC1R and ASIP codes?*

The MC1R & ASIP codes you find on the report are the two main genes, that control the base colour.

The animal inherits one ASIP and one MC1R allele from each parent.

The MC1R & ASIP genotype plus the possible pattern defines how the fleece colour will look and how the colours are distributed over the body. Any base colour can co-exist with any pattern, more than one pattern, or none of the patterns.

The patterns are controlled by several genes. The patterns are for instance: roan (modern grey), tuxedo (white spotting), classic grey, vicuña, appaloosa.

ASIP / Agouti (agouti signaling protein)*

A = Wild type. White to fawn. Only pheomelanin is being produced.
a = Non-functional ASIP. Allows α-MSH to pass eumelanin.

ASIP is responsible for the kind and amount pheomelanin (yellow/red pigment) being produced and the allowance of eumelanin (black pigment) to be expressed on the animal. When ASIP is being active/functional, the result will be a capital "A" and when it is not, the result will show a small "a".

α-MSH (α-Melanocyte-stimulating hormone)*

Responsible for the kind and amount of eumelanin potentially being produced. When ASIP is not being functional and you get a small "a" in the results, α-MSH is able to affect the pigment and eumelanin will be passed in the melanocyte. This means that eumelanin is recessive to pheomelanin.

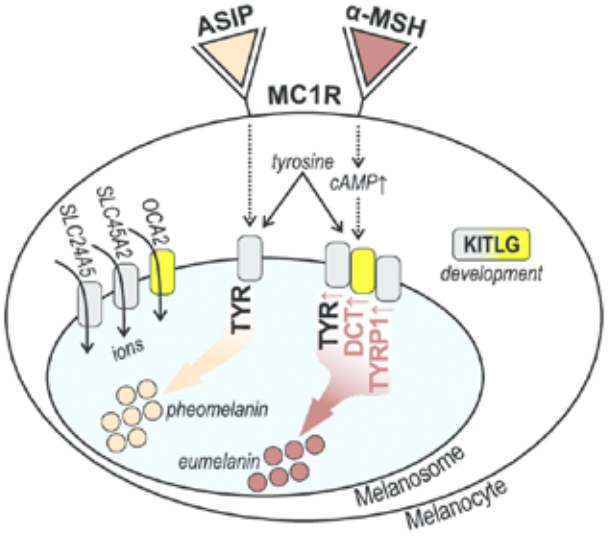
MC1R (melanocortin-1 receptor)*

E = Wild type/active. Allows eumelanin to be expressed. Two copies is generally more intense than one copy.
e = Non-functional MC1R. Blocks the production of eumelanin (black/brown) partially or completely.

MC1R translates the information from ASIP/α-MSH to the melanocyte and whichever level or depth of pigment the animal carries in it's DNA, will then be expressed in the phenotype you see.

The MC1R only affects eumelanin, not pheomelanin, so the animal may still look fawn even if both copies of the MC1R were non-functional.

- Both ASIP and α-MSH communicate through MC1R.
- Depending on which one has found the MC1R, either pheomelanin or eumelanin is being produced.
- As there are multiple MC1Rs in the melanocyte (cell that produces colour), both eumelanin and pheomelanin can be produced in the same melanocyte.
- It depends on the genes that the animal carries, whether it can produce either or both pigments.



Phenotypic alleles*

A^b = Bay allele / a phenotypic allele. Often brown with dark points, like bay horses.
a^t = Black and tan allele / a phenotypic allele or potentially a pattern (vicuña) on a black animal, that is yet to be separated in the DNA. Black and tan animals have a similar colouring/pattern to a doberman dog, so it is basically a reverse version from bay.

What is a "Phenotypic allele"?*

This means that it is known, that there is a certain genetic pattern or a gene that is being passed on/inherited and that produces a certain outcome, but it is yet to be identified/separated from the DNA. In other words it is known it exists, but not where exactly so that we could look it out with a test. In this case we can call this a "phenotypic allele".

What is a "wild type" in genetics?*

Wild type" refers to something that is original. All the mutations are then compared to the original "wild type". In horses the most common and the most original colour is bay and in alpacas the most original colouring is fawn with a vicuña pattern.

What does a "non-functional" gene mean?*

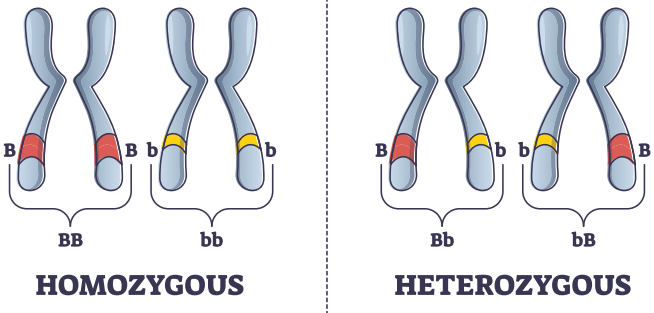
If a mutation in a gene leads to that gene not producing the protein (or RNA) that it should for normal function, then it's called "non-functional" or "not active". If it produces something that does more than its normal function, It's called "gain of function".

In reality "lost of function", "non-functional", or "not active" doesn't always mean no function at all.

When the non-functional versions don't do anything (or not much), the other copy of the gene remains functional, and sometimes one copy is enough for normal function, sometimes it's not.

What does "Homozygous" and "Heterozygous" mean?*

Homozygous = Similar
Heterozygous = Dissimilar/different



For example, EE is homozygous, Ee is heterozygous.

Some genotypes are homozygous lethal, like for instance the two grey alleles. There are no live homozygote greys, as they die early in the gestation.

This article was first published in the German magazine *AlpakaPost* in the summer 2023 (issue no 34).

References: Dr. Kylie Munyard, Curtin University

Curtin University