## Activity Answers

### Activity 1

### Task

The sequence below is a stretch of mRNA encoding a human protein. “Translate” it computationally into a sequence of amino acids, using EMBOSS Transeq, and find out what this protein is using protein BLAST.

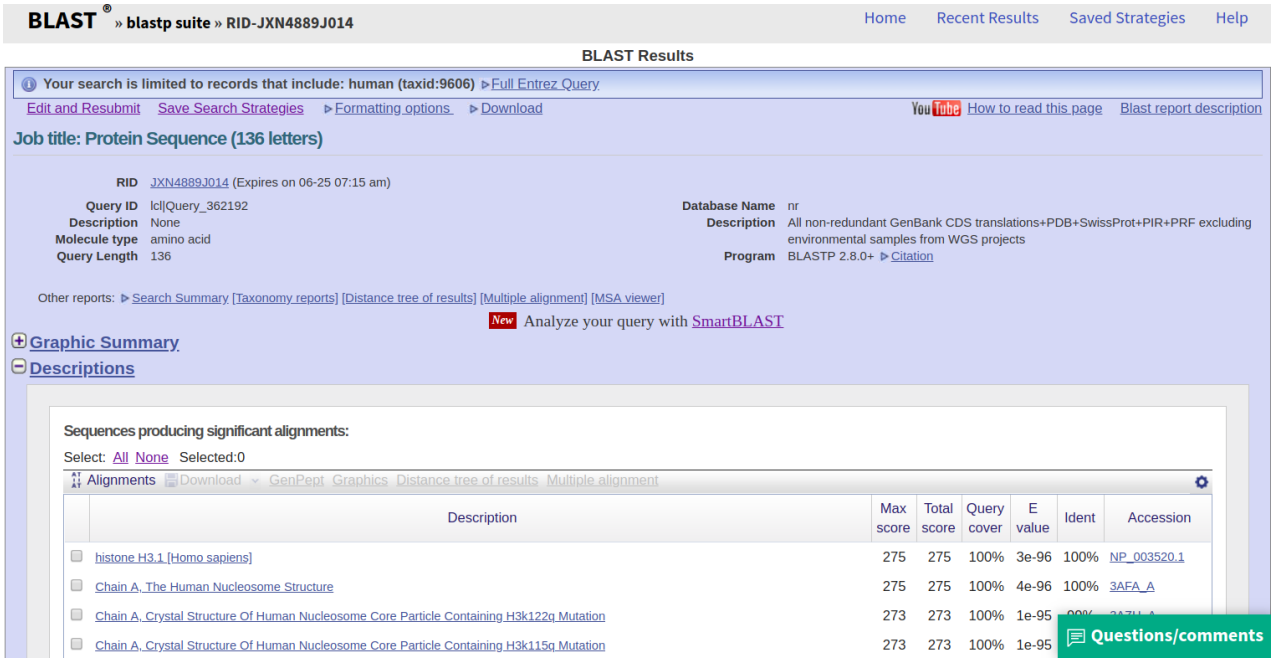
It is histone H3.1, one of the core constituents of a nucleosome. The amino acid sequence “translated *in silico* is:

MARTKQTARKSTGGKAPRKQLATKAARKSAPATGGVKKPHRYRPGTVALREIRRYQKSTE

LLIRKLPFQRLVREIAQDFKTDLRFQSSAVMALQEACEAYLVGLFEDTNLCAIHAKRVTI

MPKDIQLARRIRGERA

And the BLAST output is:




### Activity 2

### Task

Outside of CpG islands, the CG sequence is actually rare – much more rare than one would expect by chance. Given that the total percentage of C’s and G’s in the human genome is

about 40%, calculate the expected occurrence of the CG sequence. In reality, this sequence only appears at a frequency of ~0.8%. What could be the reason for this combination being under-represented?

*(Hint: think about the mechanisms by which the genome sequence evolves).*

If the total percentage of C's and G's in the genome is 40%, then the probability of appearance of each of them at a certain genomic position is 0.2. Therefore, theoretically the occurrence of the CG sequence should be 0.2*0.2 = 0.04, or 4%.

The reason this combination is underrepresented is that methylated cytosine tends to mutate to thymine (T) by spontaneous deamination. Hence, in the course of evolution, methylated CG sequences become TG. It is anticipated that CpG island appear due to lower methylation rates in these regions, which has a functional role of promoting gene expression.

**Additional resources:**

One of the original papers on CpG frequency of occurrence by Prof Adrian Bird

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC324012/pdf/nar00424-0061.pdf

**Task**

Thinking about the physical properties of the DNA and the acetyl group, can you guess what effect histone tail acetylation has on chromatin conformation?

At physiological pH (~7.5), the acetyl group loses its proton and is negatively charged. The same happens to the phosphate groups in the DNA backbone, and thus DNA also become negatively charged. Since like charges repel, acetylation of histones loosens the association between the DNA and the histone complex, thus making chromatin less condensed and more accessible.

**Task**

Suggest a mechanism by which HP1-associated chromatin regions can "spread".
*(Hint: think about its interaction partners).*

As you have learned, HP1 associates with H3K9me2/3 and is able to recruit other proteins. Some of them will contribute to silencing of this genomic region, while others will help this chromatin state to spread. You can imagine that if one of HP1's interaction partners was able to modify the neighbouring nucleosomes, adding methyl groups to their H3K9, these newly added marks would again recruit HP1. Indeed, this is what happens – through the action of histone-lysine-N methyltransferase Suv39h.

**Additional resources:**

EpiGenie on HP1

https://epigenie.com/key-epigenetic-players/chromatin-modifying-and-dna-binding-proteins/heterochromatin-protein-1-hp1/

**Task**

True or false?

1. DNA methylation is involved in bacterial protection from viruses.
2. There exist regions with both active and repressive chromatin marks.
3. Chromatin conformation plays a role in silencing endogenous viruses incorporated in our genome.
4. If you inject purple petunias with a gene encoding the purple pigment, they become white.

1. True. To protect themselves from viruses, bacteria cut any foreign genome that is injected into them. They use DNA methylation to distinguish an invader from their own genome.
2. True. Such regions are termed “bivalent chromatin”, and are mostly found in embryonic stem cells. They allow these regions to be rapidly activated or silenced, depending on the type that the cell decides to differentiate into.
3. True. Endogenous retroviruses comprise 5-8% of the human genome, and they need to be repressed to prevent production of viral RNA and proteins. Most of them exist in very densely packaged chromatin with a variety of repressive histone marks.
4. True. This is due to a phenomenon called RNA interference (RNAi), brought about by non-coding RNAs.

**Additional sources:**

Khan Academy on bacterial restriction-modification system

https://www.youtube.com/watch?v=U2cKywEn6KY

Wikipedia on bivalent chromatin

https://en.wikipedia.org/wiki/Bivalent_chromatin

The Atlantic on endogenous retroviruses

https://www.youtube.com/watch?v=Opdp5L1bVAY

NOVA scienceNOW and Nature Video on RNA interference (great videos!)

https://www.youtube.com/watch?v=Vh3-NHdjnyQ&t=443s

https://www.youtube.com/watch?v=cK-OGB1_ELE

**Activity 3**

**Task**

In Figure 3, you see a female tortoiseshell cat. Can you think of how its fur colour develops?

Will a cloned cat be identical to the parent?

The gene encoding the orange fur colour is located on the X chromosome. After the random inactivation of one of the X chromosomes at an early developmental stage, cells divide further, each giving rise to a group of cells. If the two X chromosomes contain different alleles of this gene (orange and non-orange), then the inactivation leads to sole expression of one of the protein versions. Since the identity of the inactivated X chromosome is inherited by all the descendants of a given cell, groups of skin cells will express the same allele of the fur colour gene, which might however be different from that expressed by a neighbouring group of cells. Overall, this gives rise to the 'patchy' pattern of the fur.

Although the cloned cat will have the same genetic content as the donor cat, the process of X chromosome inactivation will be random. Thus, the fur of the "copycat" will have a different pattern than that of the parent.

**Additional resources:**

ACSH on calico cats (same principle as tortoiseshell cats)

https://www.acsh.org/news/2016/07/27/calico-cats-are-a-walking-genetics-lesson

Guardian on the copycat (not very informative though)

https://www.theguardian.com/science/2002/feb/15/genetics.highereducation

Bozeman Science on X inactivation
https://www.youtube.com/watch?v=Y9vXhmI5FXM

**Task**

Why are people with an odd number of chromosomes (e.g. women with triple X syndrome) usually infertile?

There are two types of cell division: meiosis and mitosis. Mitosis is what allows a single cell to make up an entire organism. As a result of it, normally two daughter cells are produced which are genetically identical to each other and have a full diploid set of chromosomes (46 for humans). Meiosis, in turn, is a more complicated process, that results in progeny cells

only containing half of the genome (23 chromosomes for humans). However, the distribution of chromosomes between the daughter cell is non-random: each of them has to inherit one of the homologous chromosomes, so that they have at least one copy of each gene in the genome. Mitosis happens with somatic cells, while meiosis produces gametes – eggs and sperm, – which enables sexual reproduction to happen and the genes to "shuffle", giving rise to a unique new organism. During meiosis, there exist robust control mechanisms that make sure that each of the daughter cells has got a correct set of chromosomes. However, if the mother cell has 47 chromosomes instead of 46 (for humans), it is impossible to divide those equally. Thus, such cells do not get a "green light" to produce gametes, leading to infertility of the organism.

**Additional resources:**

HealthSketch on Turner syndrome
https://www.youtube.com/watch?v=YQG8o5b4lKg

A paper on meiotic checkpoints by Subramanian and Hochwagen (rather complicated)

http://cshperspectives.cshlp.org/content/6/10/a016675.full

**Task**

Although in placental mammals (including us) X inactivation is random, in earlier marsupials (e.g. kangaroos) the paternal X chromosome is silenced in all cells of the body. What could be the evolutionary advantage of random X inactivation?

Sex-linked diseases occur due to mutations on the X chromosome. For males, there is no escape: they only have one X chromosome, so they have to live (or die) with whatever mutations it has. However, for females, the situation is different. If they inherit one defective X chromosome, then due to X inactivation, each cell at the blastocyst stage of embryonic development has a 50% chance of inactivating the "disease" chromosome. If the same chromosome was inactivated in all of those cells, the organism would have a 50% chance of not inheriting the disease (or of survival in the extreme case). However, if different X chromosomes can be inactivated in different cells, the "correct" protein produced in cells with the defective X chromosome inactivated might be able to rescue the rest of the cells. This is indeed what happens in colour blindness: although female carriers do have defective photoreceptors, they also have many "healthy" ones, which can do the job almost perfectly. Even more peculiar is the fact that for some X-linked disorders (e.g. Rett syndrome), there is a "skew" towards inactivating the mutated X chromosome!

**Additional resources:**

Why aren't more women colourblind?

http://www.color-blindness.com/2011/11/07/why-arent-more-women-colorblind/

Wikipedia on skewed X chromosome inactivation

https://en.wikipedia.org/wiki/Skewed_X-inactivation#Primary_Nonrandom_Inactivation

**Activity 4**

**Task**

For a cell to be able to develop into an embryo, it has to have the diploid genome, which is normally obtained as a result of fusion of egg and sperm, which each are haploid. Can an embryo develop from a fusion of two eggs?

No, since in both haploid genomes the same genes are imprinted. Thus, some developmentally important genes will be completely repressed, leading to non-viability of the embryo. Embryos with two copies of the paternal DNA sometimes appear due to incorrect fertilisation, which results in molar pregnancies.

**Additional resources:**

Human Reproduction Update on molar pregnancy

https://academic.oup.com/humupd/article/11/2/137/763311

**Task**

Why does the above evidence suggest, but not prove that methylation of stress-related genes is indeed responsible for the variation in behaviour between the pups? Can you spot the limitations of the experiment?

The first two experiments (measuring levels of anxiety of progeny of “good” and “bad” mothers and subsequently swapping the pups) merely indicates that the observed phenotype is not inherited and that it correlates with methylation patterns. Although the degree of correlation deduced in the paper is impressive, correlation does not imply causation. For example, if the number of the supporters of a football club is correlated with the frequency of them winning, it does not mean that this team wins because they have more fans! The third experiment is more powerful in this sense: by changing what is anticipated to be the cause of the pups’ behaviour (i.e. methylation), scientists observe an effect on their anxiety levels. However, they have not changed methylation pattern specifically at the stress gene site – instead, they globally reduced methylation using a drug. Therefore, the observed change might be the result of changing methylation at some other gene, or even some other off-target effect. Taking both arguments into account, it is likely that the authors’ hypothesis about stress gene methylation being the means by which the environment (mother’s behaviour) affects pups’ anxiety is right, but unfortunately that is not

set in stone (yet). New more subtle experimental methods are needed to prove their point of view.