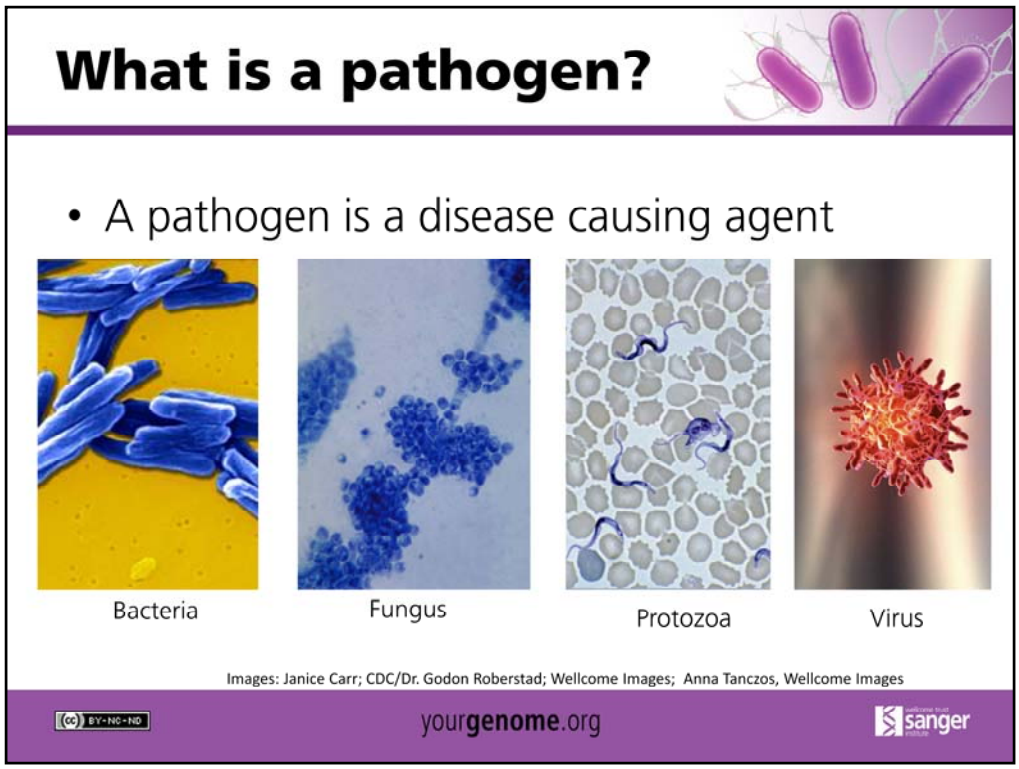
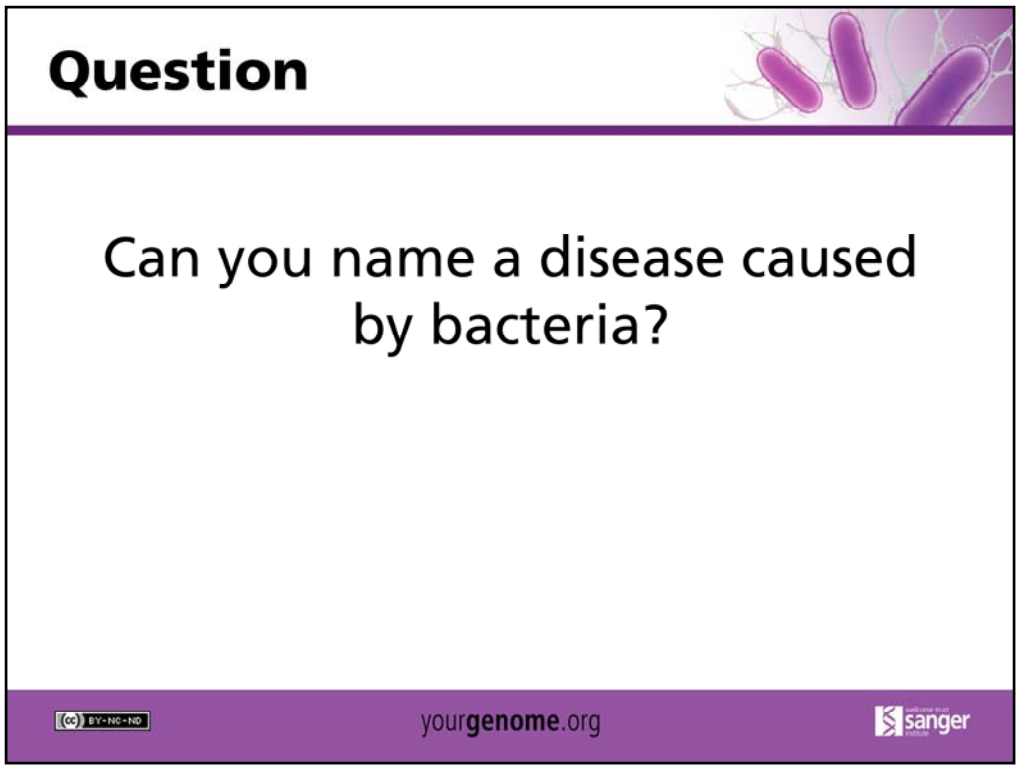
Build a bug activity

Introduction to *Salmonella*



A pathogen is an agent or microrganism that causes a disease in its host. Pathogens can be viruses, bacteria, fungi or protozoa.

Protozoa are single celled eukaryotic organisms. Some protozoa are pathogens. For example the protozoa *Plasmodium falciparum* causes malaria and species of the parasitic protist *Trypanosoma* (shown in slide) cause African sleeping sickness.



Encourage a discussion on bacterial species that can cause disease. Examples can include:

Food poisoning: caused by bacterial species including *Eschericia coli* (*E.coli*) and *Salmonella* species.

Typhoid fever: caused by *Salmonella* Typhi

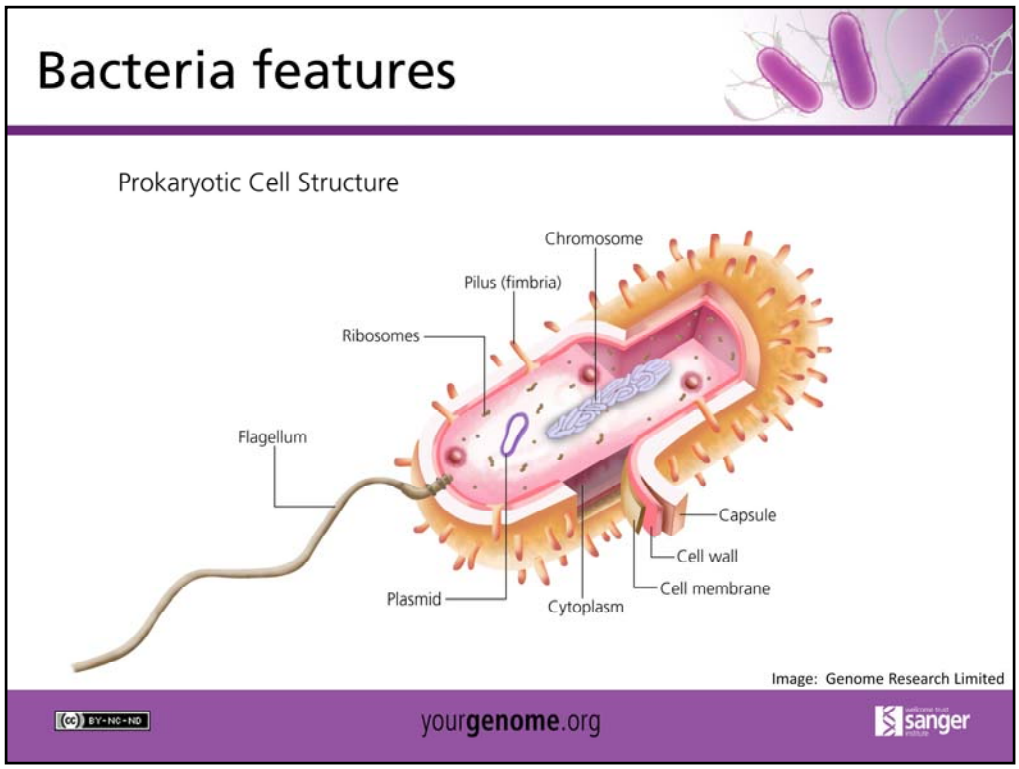
Bacterial meningitis: caused by *Neisseria menigitidis*

Pneumonia: caused by *Streptococcus pneumoniae*

Gastric ulcers: caused by *Helicobacter pylori*

Tuberculosis (TB): caused by *Mycobacterium tuberculosis*

Various infections can be caused by MRSA *(*Methicillin Resistant *Staphylococcus aureus)*



Point out the key structural features of the bacteria cell:

Cell wall: Composed of peptidoglycan (polysaccharides + protein), the cell wall maintains the overall shape of a bacterial cell. The three primary shapes in bacteria are coccus (spherical), bacillus (rod-shaped) and spirillum (spiral).

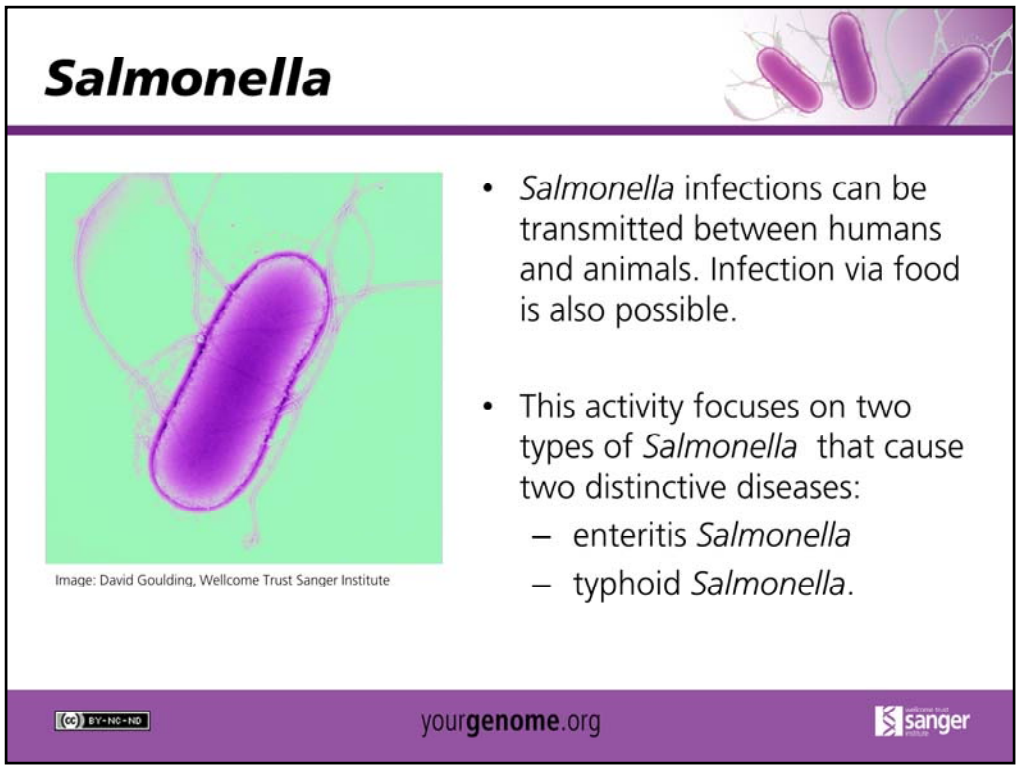
Capsule: Some species of bacteria have a third protective covering, a capsule made up of polysaccharides (complex carbohydrates). Capsules play a number of roles, but the most important are to keep the bacterium from drying out and to protect it from phagocytosis (engulfing) by larger microorganisms and cells of the immune system.

Flagella: Filamentous protein structures attached to the cell surface that allow the bacterial cell to swim in fluid environments.

Fimbria(e): Protein structures that allow the bacteria cells to stick to cell surfaces. They are major determinants of bacterial virulence because they allow pathogens to attach to (colonise) tissues and, sometimes, to resist attack by phagocytic white blood cells.

Chromosome: Unlike animal and plant cells, the bacterial DNA is not packaged inside a nucleus. Instead it is coiled into one “chromosome” which is found in the cytoplasm of the cell.

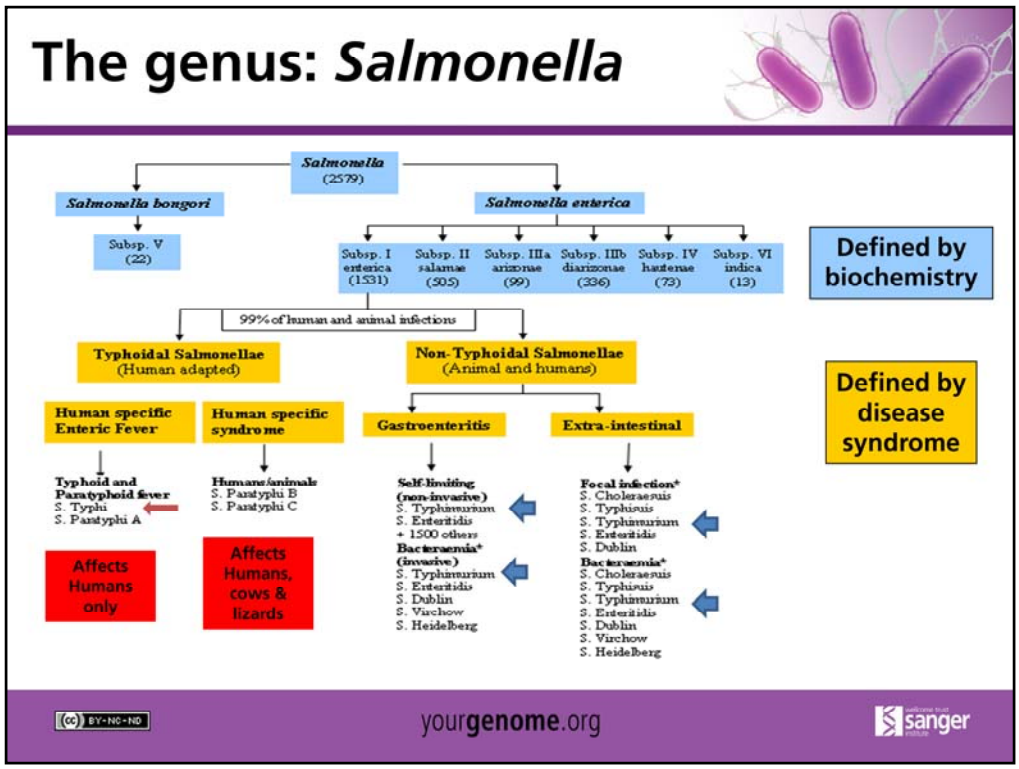
Plasmid: A circular ring of DNA that can carry genes that are advantageous but not essential to their bacterial host. Plasmids can be easily gained or lost by a bacterium and can be transferred between bacteria as a form of horizontal gene transfer. This process will be discussed in later slides.



Salmonella bacteria are most commonly associated with food poisoning (also known as *Salmonella* enteritidis). They can be naturally present in the intestines of a range of organisms including birds and mammals. As a result they can also be present in animal faeces. *Salmonella* can be spread by contact with contaminated food.

A common source of *Salmonella* food poisoning is poultry such as chickens. Eggs laid by infected chickens may contain the bacteria. It is therefore important to ensure that eggs are always well cooked to destroy any *Salmonella* in them.

Salmonella bacteria do not just cause food poisoning, but can also cause typhoid fever. This activity will focus on two species of *Salmonella*, one which causes food poisoning and one that causes typhoid fever.



The slide represents the classification of the genus *Salmonella*.

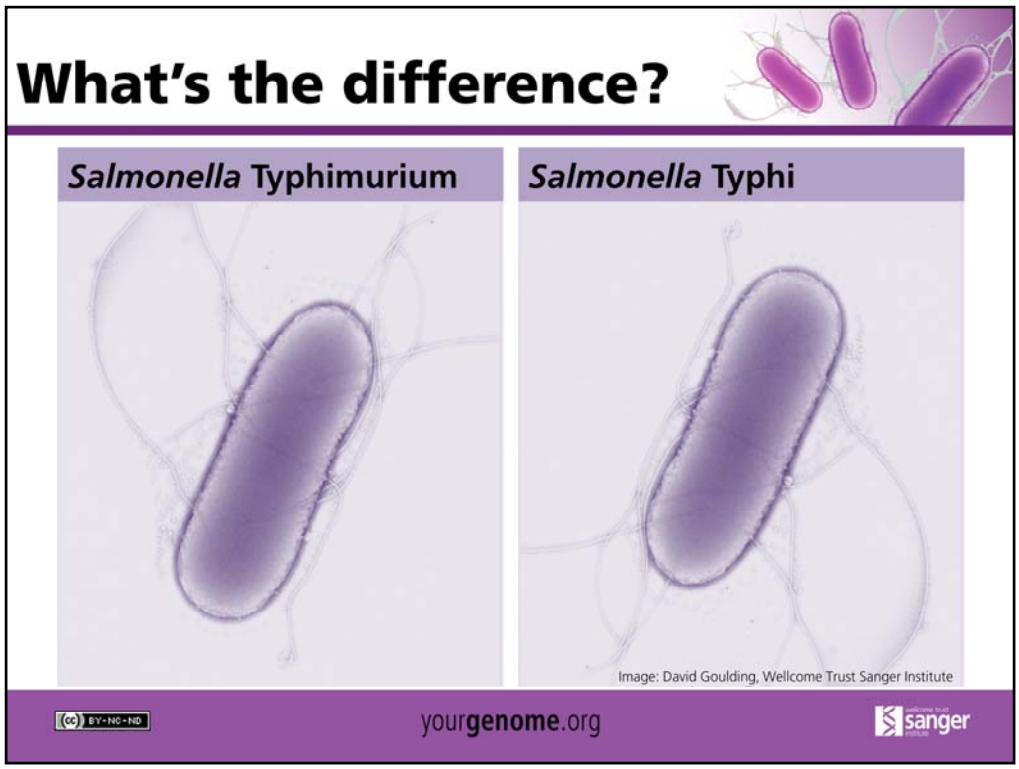
There are two main species of Salmonella and lots of subspecies or serovars. All serovars are very closely related but still distinguishable from each other by biochemical tests. For example, media growth tests look at whether the bacteria grow on media containing glucose or sucrose. This is a means of determining the phenotype, or characteristics, of the bacteria.

At the serovar level, further distinction is made by looking at the disease syndrome, i.e. what symptoms does the bacteria cause?

Most serovars infect a range of hosts. This means they are generalists. However, some serovars are highly specific and only infect humans.

This activity is going to look at one generalist serovar: *Salmonella enterica* serovar Typhimurium (*Salmonella* Typhimurium). This is known to cause food poisoning and diarrhoea. *Click for blue arrows.*

This activity will also look at one specialist serovar: *Salmonella enterica* serovar Typhi (*Salmonella* Typhi). This only infects humans and is known to cause typhoid fever. *Click for red arrow.*



Under the microscope it is impossible to distinguish between the two serovars. The symptoms and disease syndrome allow clinicians to identify which serovar has infected a patient. (*Click to fade out pictures*)

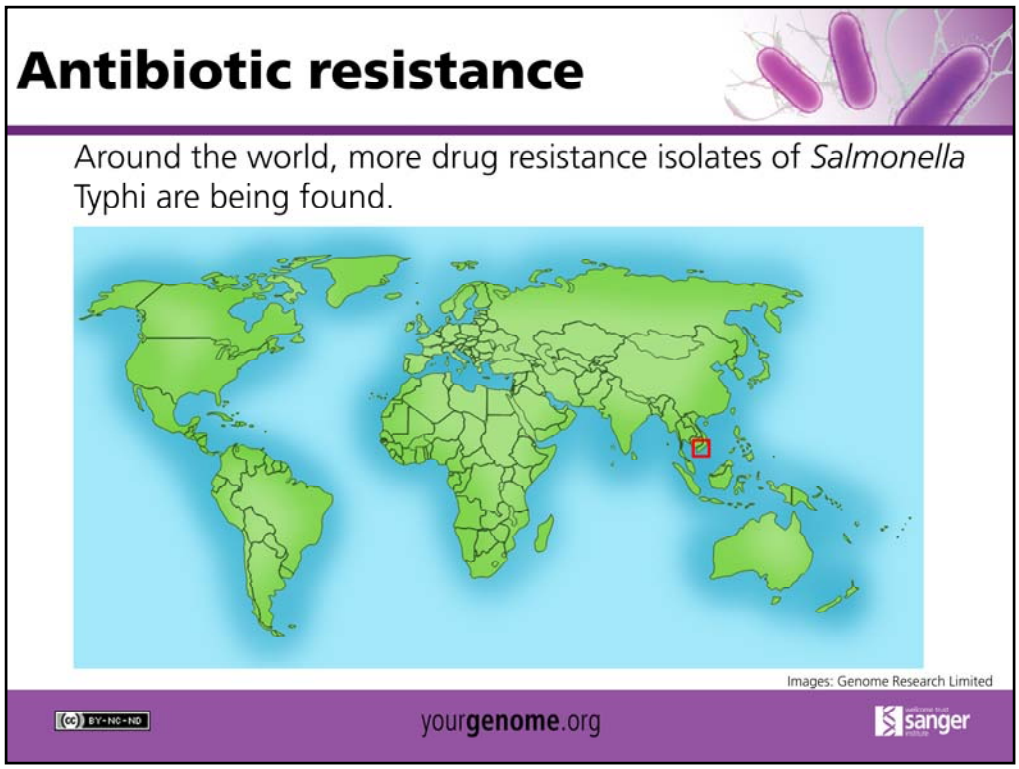
Salmonella Typhimurium causes *Salmonella* enteritis in humans. This is also known as **food poisoning.** Salmonella is a generalist meaning it can infect a range of different species and is not specific to one type of host. Symptoms include diarrhoea, vomiting and abdominal cramps, these occur after an incubation period of 6 – 24 hours.

Salmonella Typhimurium is a **zoonosis.** It can be transmitted by animals such as reptiles, birds and mammals.

Salmonella Typhi causes typhoid fever, a severe systemic infection that if left untreated can effect multiple organs in the body. Symptoms include a slow progressive fever as high as 40 °C (104 °F), profuse sweating and abdominal pain. If left untreated it can lead to intestinal hemorrhage or perforation, which can be fatal.

Salmonella Typhi is only carried by humans, and is usually contracted through direct contact with the faecal matter of an infected person.

Genome sequencing allows comparisons at the genetic level and provides a better means of distinguishing between the two serovars. *(Click to reveal gene figures).* For example, it allows us to identify unique genes found in the individual species. Through sequencing we know now that Typhimurium has 479 unique genes and Typhi 609.

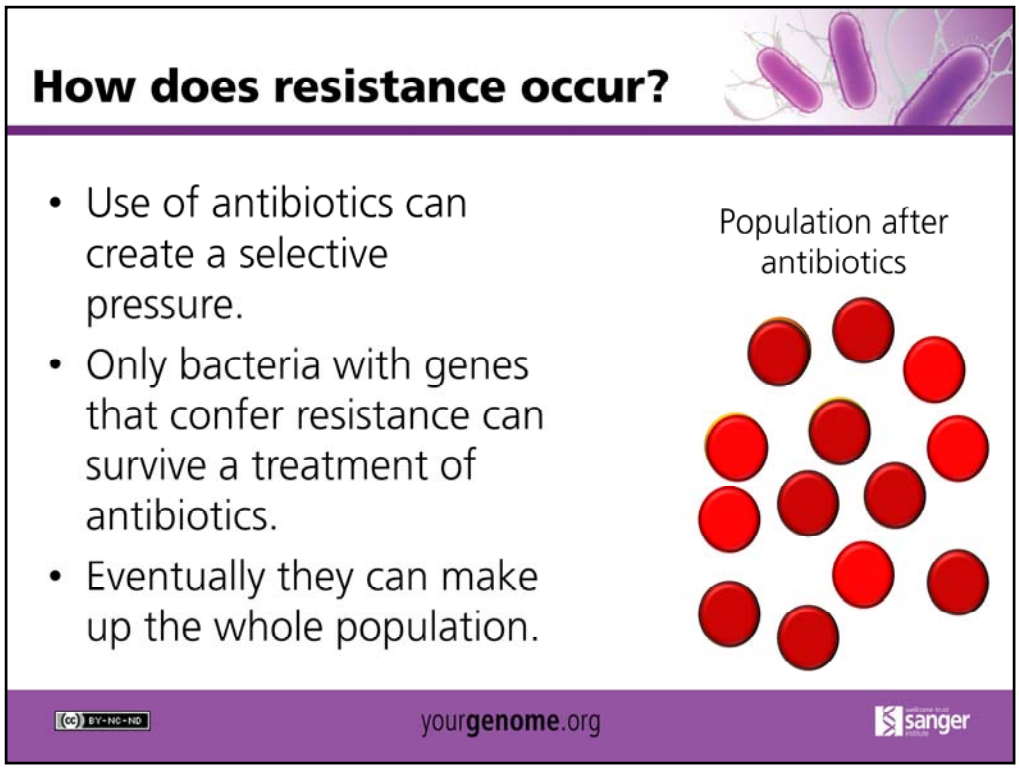


In many developing countries where safe water supply, environmental sanitation and food hygiene is not optimal outbreaks of typhoid are still a major problem. The common treatment for this disease are antibiotics such as ampicillin, chloroamphenical and co-trimoxazole. However the common use of a limited range of antimicrobials has led to the emergence of *Salmonella* Typhi isolates resistant to all three of these drugs.

The emergence of multi-drug resistance has great implications for therapy. For example, children infected with a drug resistant strain of *Salmonella* Typhi are seriously ill, have a longer duration of the illness and a higher mortality rate. The main problem is that there are no pathological features that distinguish a multidrug resistant strain from a drug sensitive strain at the initial presentation of symptoms.

The map shows the spread of a drug resistant strain of *Salmonella* Typhi in Vietnam over a three year period. *Click to reveal the zoomed in image*.

The local clinicians didn't recognise the infection as *Salmonella* Typhi as it did not respond to the usual antibiotic treatment.



Note: The slide is animated.

The use of antibiotics, starting with penicillin, has created a new selective pressure on bacteria. Researchers believe that bacteria have evolved increasing resistance to the various types of antibiotic as a result. The first antibiotic-resistant bacteria were isolated in 1947 – just four years after penicillin went into mass-production.

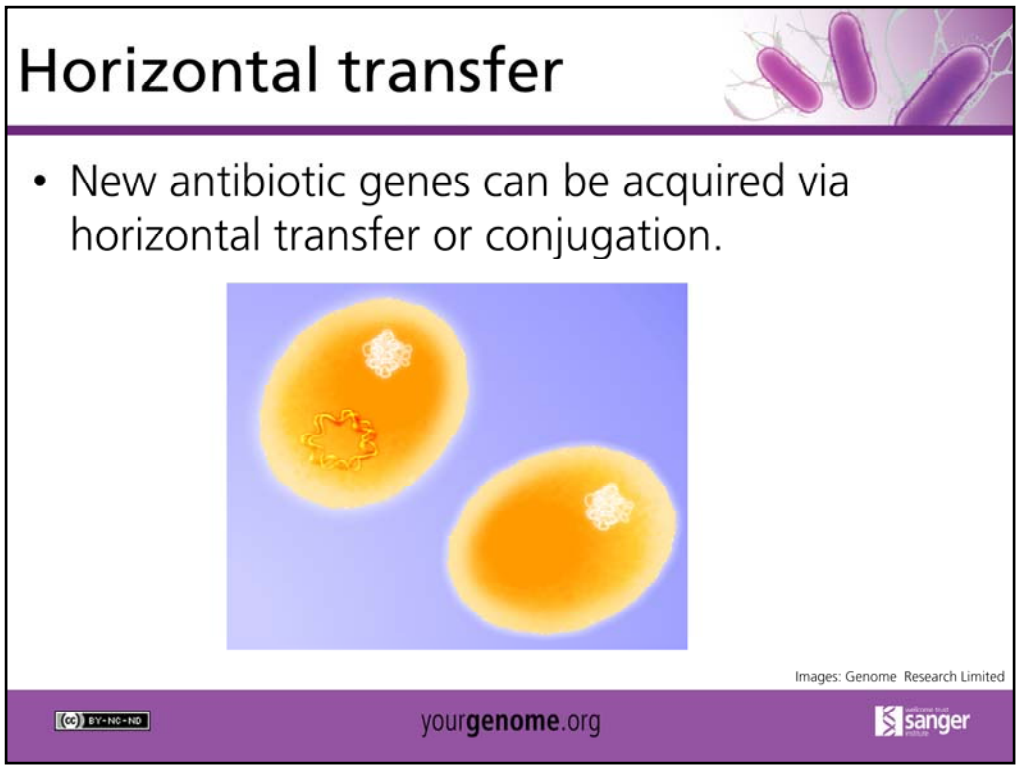
So how does this happen?

Click to show the effect of using an antibiotic on a bacterial population.

Antibiotic kills most bacteria in a population. However within that population there may be some that are resistant to that particular drug. This can be either through having naturally acquired mutations to key genes or having acquired new genes from other bacteria. These bacteria will survive the antibiotic treatment.

Click to show increase in resistant bacteria

This remnant population of resistant bacteria is not destroyed so continue to grow and reproduce and eventually come to make up the whole population.



Note: this slide is animated. A downloadable version of the full animation is available on the Build a bug web page in the *For Teachers* section at www.yourgenome.org.

Bacteria often exchange genes through the transfer of plasmids (circular pieces of DNA which sit outside of the main chromosome). This is known as conjugation and is a type of direct cell to cell horizontal gene transfer. This allows resistance to spread even faster than it would through random mutation and selection.

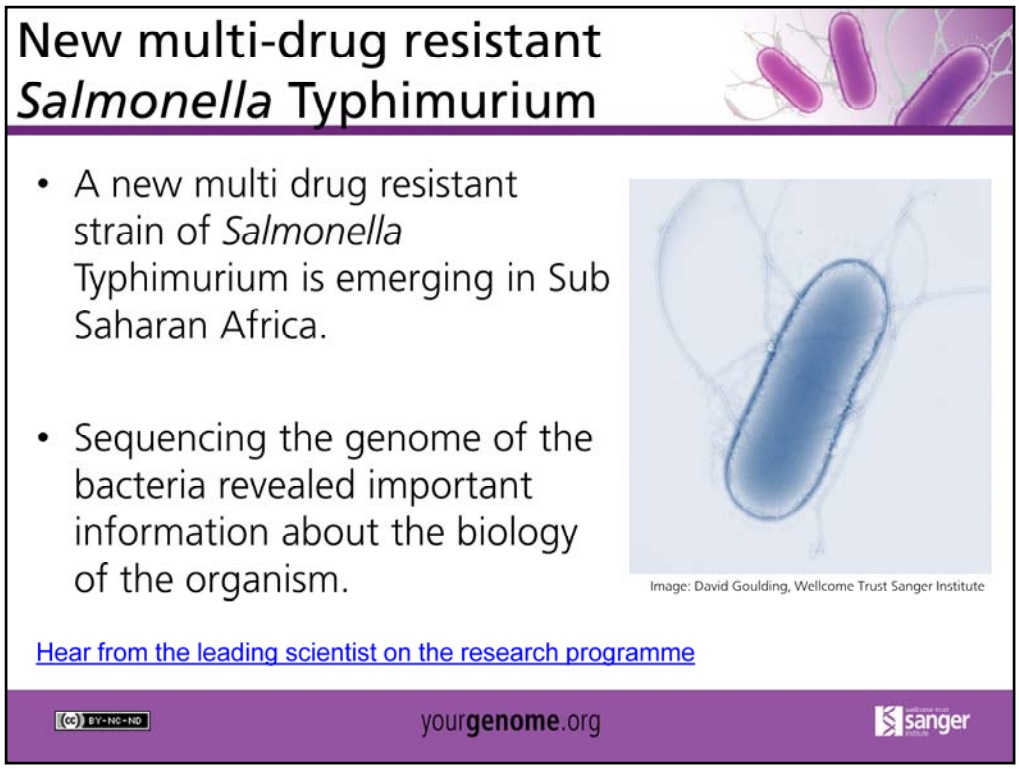
The bacteria can exchange genetic material using a specialised tube called a sex pilus. It acts like a bridge between the two cells along which genetic material can be transferred or exchanged.

Click to show sex pilus

The first stage of the process is when the "donor" bacterial cell produces a pilus that attaches to the "recipient" cell and brings the two cells together. The mobile plasmid is "nicked" and a single strand of DNA is then transferred to the recipient cell along the pilus.

Click to show next stage

Both cells synthesize a complementary strand to produce a double stranded circular plasmid. Both cells are now viable donors and can exchange the newly acquired material with other bacteria in the population.

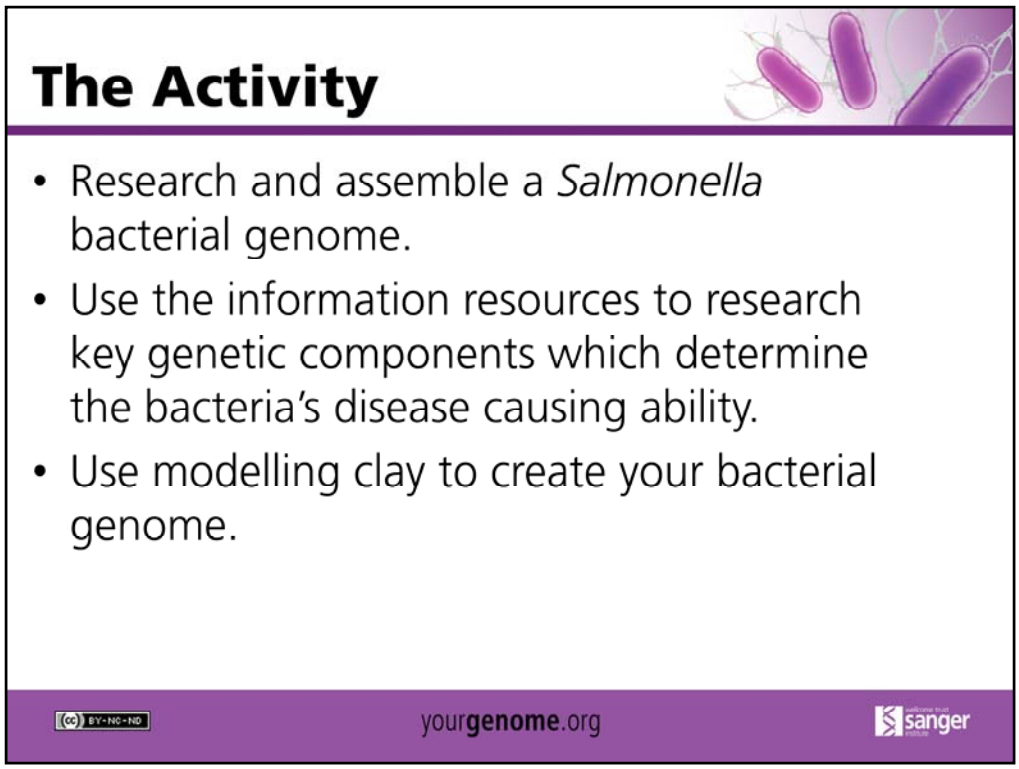


This slide links to an online video which discusses a newly emergent drug resistant strain of *Salmonella* Typhimurium that has been sequenced at the Wellcome Trust Sanger Institute.

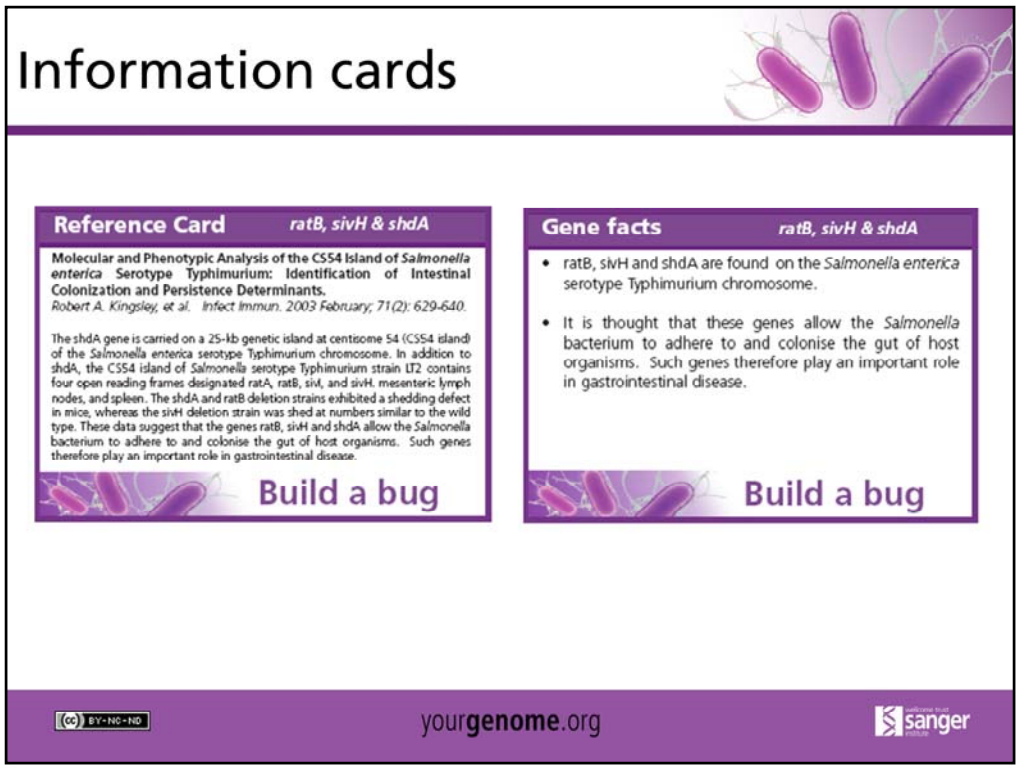
A new multi drug resistant strain of *Salmonella* Typhimurium (ST313) is being isolated in sub-Saharan Africa. The new strain is causing life threatening disease. This type of *Salmonella* bug normally causes diarrhoea and is rarely fatal, however, the new strain infects vulnerable children and adults with compromised immune systems in many regions of sub-Saharan Africa leading to death in up to one in four cases. It appears to be better adapted to affect humans, is resistant to several commonly used antibiotics and may spread from person to person.

Previously, it had been widely assumed that this wasn't a new deadly form of the organism but, rather, that the existing diarrhoea-causing strain of the pathogen was taking advantage of the weakened immune system of patients with underlying health problems to cause a more severe disease. Genome sequencing at the Sanger Institute* revealed that the bacteria had acquired drug resistance genes but also lost some gene function, similar to *Salmonella* Typhi – suggesting this was adapting specifically to a human host, unlike the more generalist strains of *Salmonella* Typhimurium.

*Kingsley RA *et al*. (2009) **Epidemic multiple drug resistant *Salmonella* Typhimurium causing invasive disease in sub-Saharan Africa have a distinct genotype.** *Genome Research* 2009; 19(12): 2279-2287.
Available online at doi: http://dx.doi.org/10.1101/gr.091017.109



In this activity you will be using reference sources, similar to those used by researchers at the Wellcome Trust Sanger Institute and other genome research institutions.

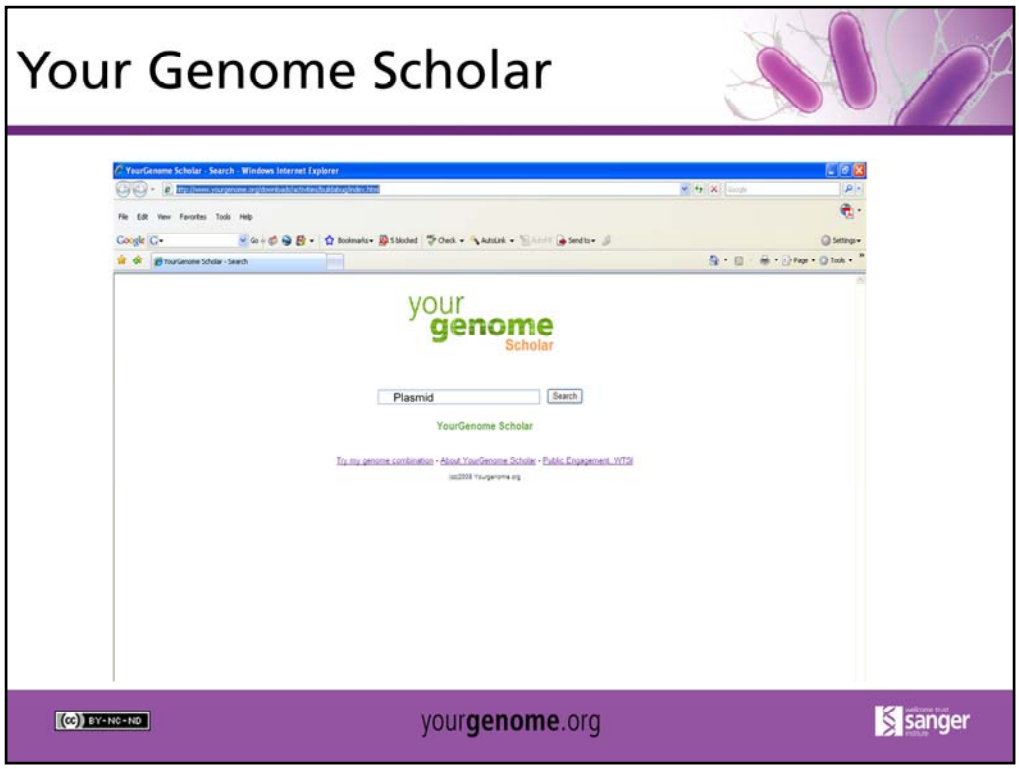


The information cards present abstracts and information taken from published scientific research papers.

There are two levels of information card available. Reference Cards feature an abstract style exert from a research paper. The Gene Facts card provide more general and concise facts on the gene and its function for the students.

You can provide both to the students or alternatively just choose one which is more appropriate for your students level of understanding.

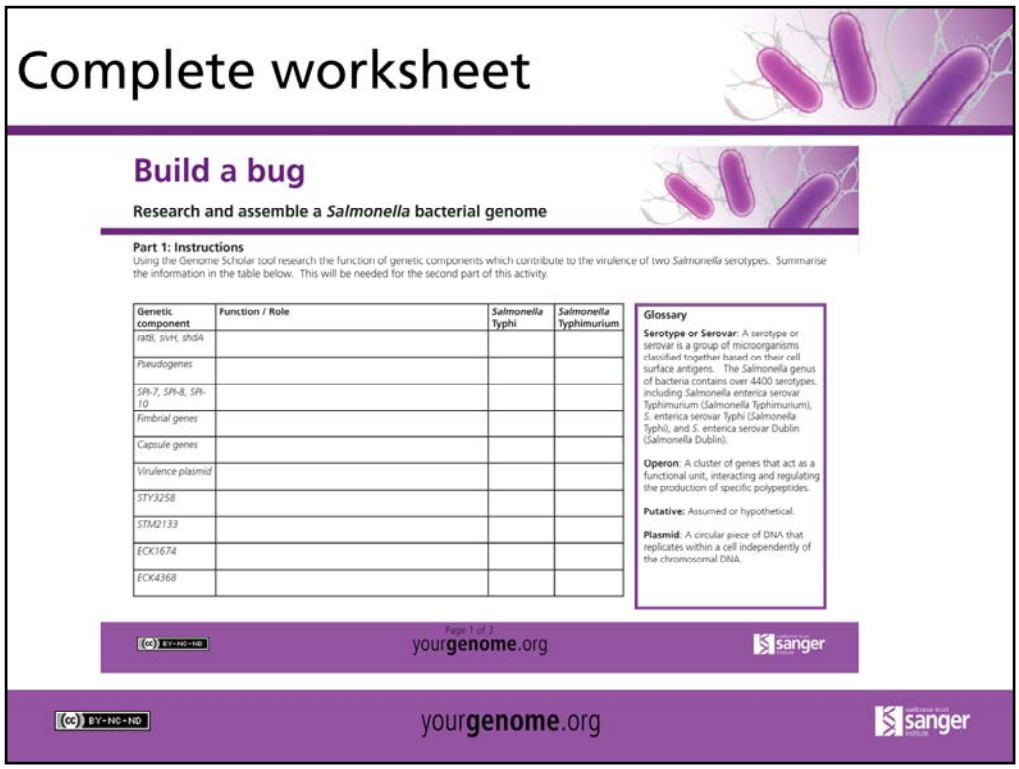
The cards will provide the students with all the information they need to complete the worksheet (shown in the next slide).



Genome scholar is a specially designed web tool to help students research the genetic components featured in this activity. It functions just like a normal search engine.

Enter the name of the genetic component of interest e.g. Plasmid in the search box and press search. *Click to reveal next image*

This will bring up a short abstract from a referenced research paper that features the genetic component of interest. This should provide enough information for students to complete the worksheet. However if interested students can view the whole research paper by clicking on the title of the paper.



This slide shows the worksheet – Part 1 should be completed before attempting to assemble the genome.

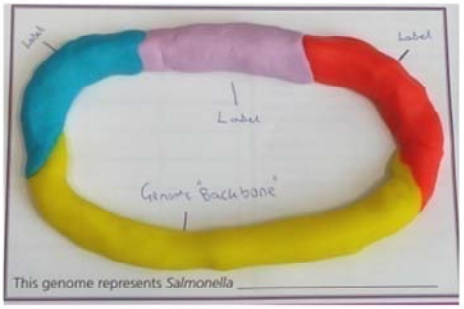
The genes are shown in the first column, they should write the function or role of the gene in the second column and tick whether it is found in *Salmonella* Typhi (column 3) or *Salmonella* Typhiumurium (column 4).

Hints for the students:

1. For the purposes of this exercise none of the genes are found in both, i.e. it should either be one or the other.
2. There are also a few red herrings thrown in that are not found in either bacteria.

Assemble a genome

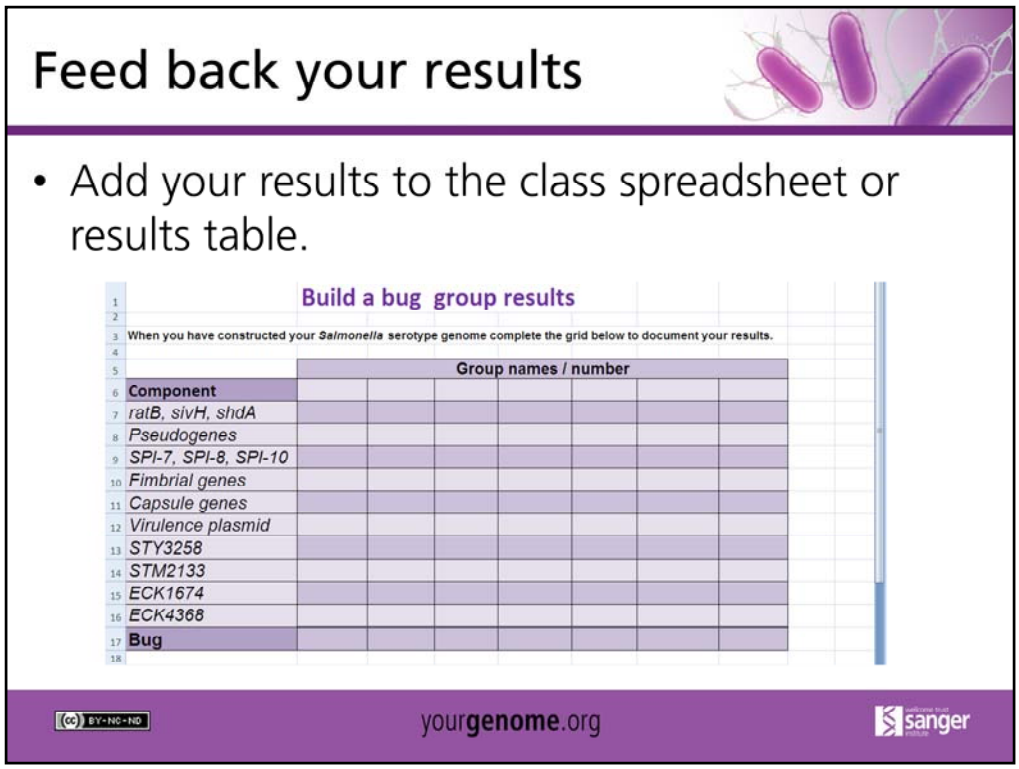
- Using modelling clay or the paper genome provided assemble three key genetic components on a chromosome backbone for either:
- *Salmonella* Typhi or
- *Salmonella* Typhimurium.



yourgenome.org

sanger

If making a model, it is important that the students construct a chromosome "backbone" to which the three accessory genetic components can be added. An example is shown in the slide. The "backbone" represents the rest of the essential genetic material for the bacterium to survive and function.



Once the students have identified three critical components, they can add their findings to the class spreadsheet or results table.

Answer summary

Component	Description / Function	*Salmonella* Typhi	*Salmonella* Typhimurium
ratB, sivH, shdA	The proteins encoded by these genes allow the bacterium to adhere to and colonise the gut of host organisms		✓
Pseudogenes	Genes which no longer function or have been inactivated; implicated in the ability of *Salmonella* to cause Typhoid fever.	✓	
SPI-7, SPI-8, SPI-10	Clusters of genes unique to the *Salmonella* Typhi bacterial chromosome.	✓	
Fimbrial genes	Clusters of genes linked with causing diarrhoea in humans.		✓
Capsule genes	The proteins encoded by these genes form a protective layer around the bacteria which prevents it being detected by the host's immune system.	✓	
Virulence plasmid	A ring of DNA found in the bacterial cell which increases the rate at which the bacteria replicates itself in the host's tissues beyond the intestines.		✓
STY3258	This gene is found on the *Salmonella* Typhi genome but there is no information on its specific function.	Red herring (×)	
STM2133	This gene is found on the *Salmonella* Typhimurium genome but there is no information on its specific function.		Red herring (×)
ECK1674	This gene is found on the *Escherichia coli* genome but there is no information on its specific function.	×	×
ECK4368	This gene is found on the *Escherichia coli* genome but there is no information on its specific function.	×	×

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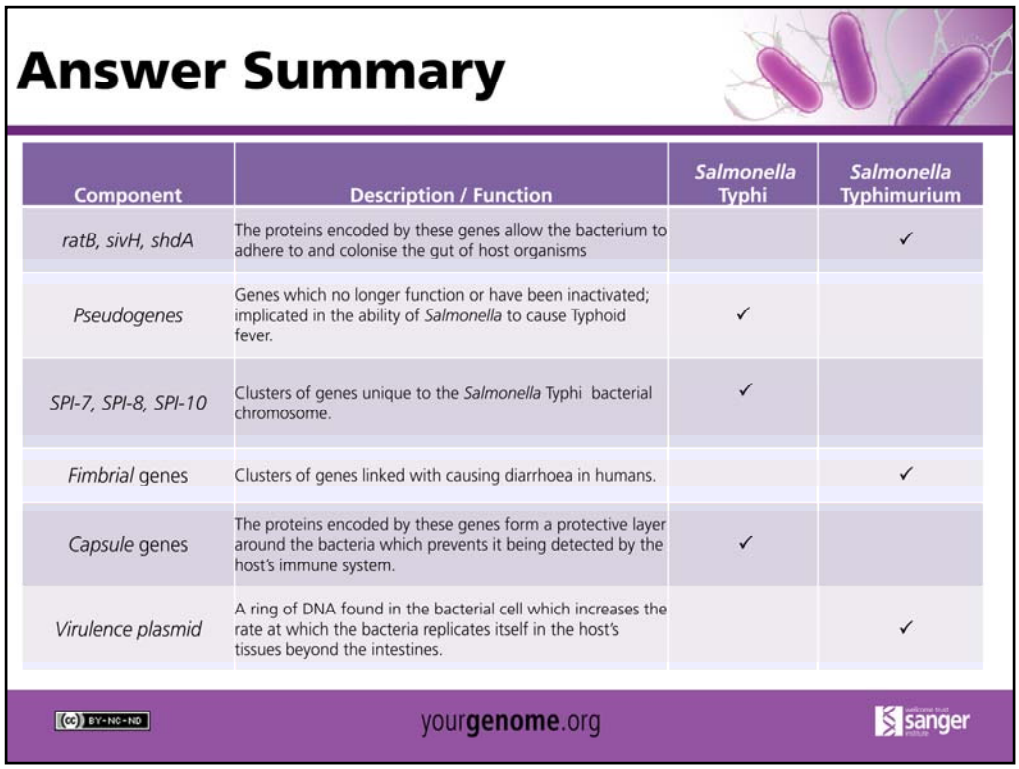
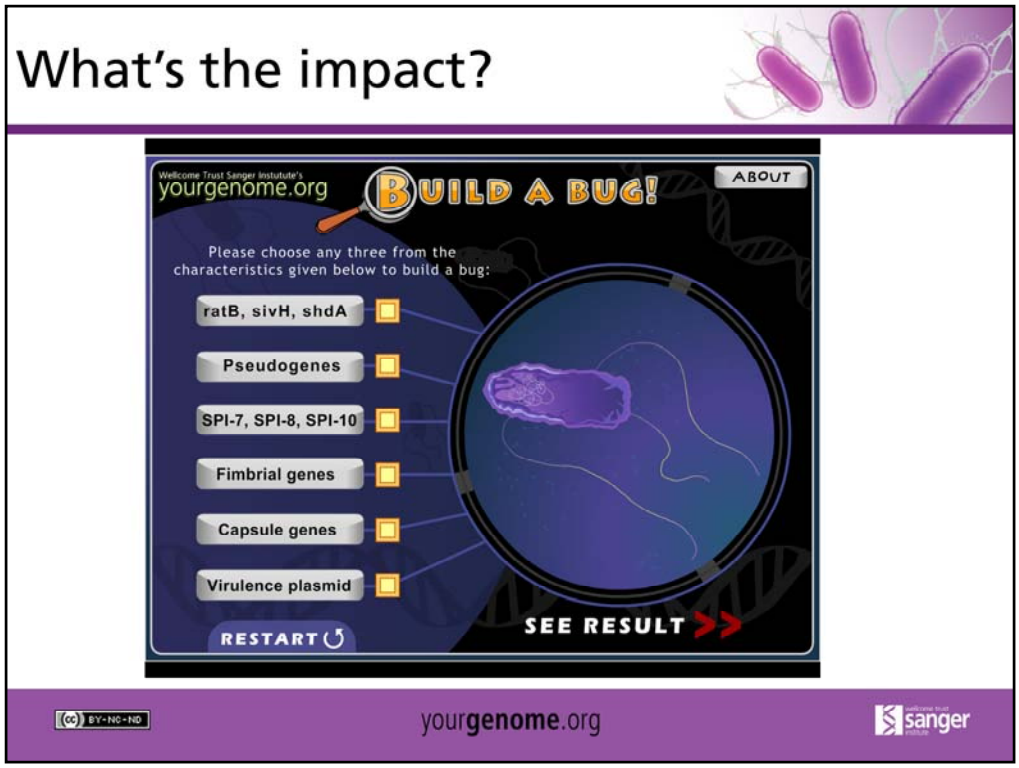
The table shows the full results which can be discussed.



This shows the six key genetic components. If you wish to discuss their roles and functions more details are found in the teacher's notes.

The final stage of the activity is to reveal the impact of these genes using the animation provided. This will have to be open and minimised when running.



Have the animation file running and minimised so you can quickly switch from this slide to the animation. Alternatively click on the image to launch an online version.