Build a bug
Teachers' notes

Background to the activity
This is a classroom-based activity that allows students to explore the features of two bacterial pathogen genomes. The aim of this activity is to highlight the role of different genetic components in two closely related subspecies of *Salmonella enterica* and to identify how these contribute to the bacteria's ability to infect its host, causing two very distinct illnesses. The activity can be carried out using either paper-based resources or a web based tool.

Note on nomenclature
The activity focuses on two subspecies of the bacteria *Salmonella enterica* called *S. enterica* serovar Typhimurium and *S. enterica* serovar Typhi. To avoid confusion between serovars and species, serovar names are printed in Roman type (not italics) starting with a capital letter. In order to simplify the naming of serovars a shortened nomenclature is used: *Salmonella enterica* serovar Typhimurium becomes ***Salmonella*** **Typhimurium** and *Salmonella enterica* serovar Typhi becomes ***Salmonella*** **Typhi**. This convention will be used throughout this activity.

Activity overview
Three of the genetic components featured in the activity are critical to the genome of *Salmonella* Typhimurium which causes food poisoning / gastroenteritis (stomach pain and diarrhoea). Another three are critical to the genome of *Salmonella* Typhi which causes typhoid fever.

The students decide which *Salmonella* serovar (subspecies) they want to assemble. Using either the information cards (Reference Cards or Gene Fact cards) or the Genome Scholar web tool, they choose the correct genetic components critical to that bacterium. The students can illustrate their choices by either creating a basic model of the bacterial genome or using the cut out components provided.

Students collate their findings in a central spreadsheet. Once all results are in, the teacher or session leader can present the correct genetic combinations and their outcomes using an animation.

To run the activity you will require
- Introductory presentation to pathogens, *Salmonella* and the activity
- Student worksheet (paper version or modelling version)
- Information cards (Reference Cards or Gene Fact cards)
- Computer with *Build a bug* animation (teachers' use only)
- Flip chart / whiteboard
- Results spreadsheet (optional)
- Genome Scholar web tool (an online alternative to using the information cards)
- Computer with internet access (only needed if using Genome Scholar web tool)

Modelling alternatives to the cut out paper genome (separate worksheet is provided):
- Playdough / modelling clay (four different colours per group)
- Tangles (four different colours per group) and printer labels
- Pipe cleaners (four different colours)

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Activity preparation

The following components need to be prepared before the activity commences:

1. Information cards
Print off all of the Reference Cards or Gene Fact cards and cut them out. One set of cards is required per pair or group. It is recommended that the cards are laminated to prevent damage, however this is not essential. Two levels of information cards are available (see Page 3). Reference Cards provide an abstract-like summary from a published research paper. These are suitable for Post-16 students. Gene Fact cards provide a more concise description of the gene and its function. These are more suitable for GCSE students. Decide which would be the most appropriate set of cards to use for your group.

2. Modelling materials (optional)
If you have chosen the modelling option (modelling clay, playdough, pipe cleaners etc.), make sure that you have four different colours of modelling materials to construct a bacterial genome backbone and the three other critical components. Also provide sticky tape to attach the model to the worksheet.

3. Spreadsheet or results table
An Excel spreadsheet is provided to compile the students' results. If viewed at 110% this will fill the screen when projected.

Alternatively, you can use the following summary chart to create your own flipchart or whiteboard versions.

Group / Name											
Component											
ratB, sivH, shdA											
Pseudogenes											
SPI-7, SPI-8, SPI-10											
Fimbrial genes											
Capsule genes											
Virulence plasmid											
STY3258											
STM2133											
ECK1674											
ECK4368											
Bacteria											

4. Genome Scholar web tool (optional)
To save time, bookmark and save the following link to the desktop of the students' computers:
http://www.yourgenome.org/downloads/activities/buildabug/index.html.

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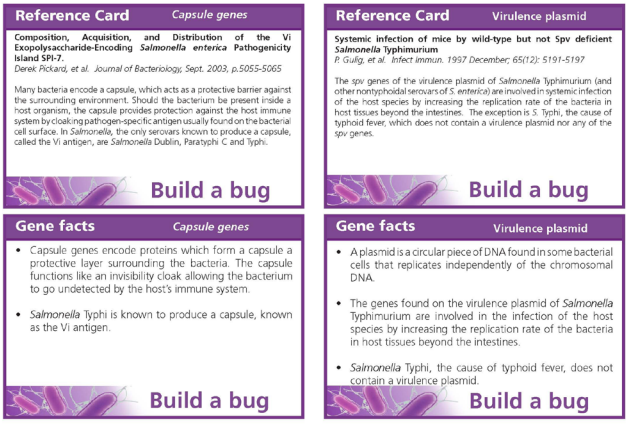
Instructions for running the activity

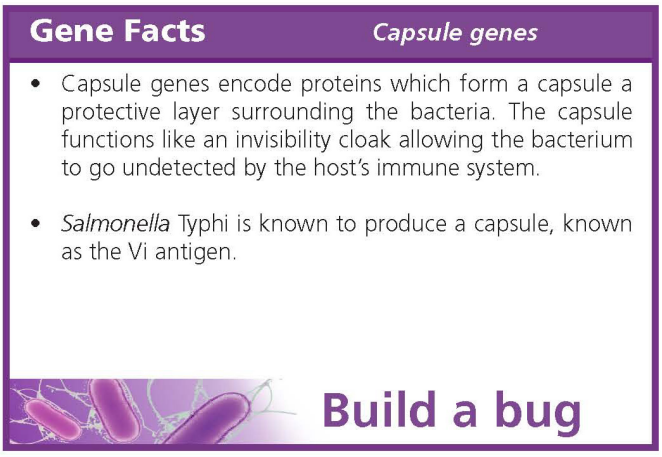
Stage 1: Introductory presentation (15 minutes)
The *Build a bug* presentation introduces the concept of pathogens as agents that cause disease. It focuses specifically on two serovars of *Salmonella*.

The presentation provides an introduction (or refresher) on pathogens and bacterial cell structure (Slides 3 – 4). Slide 3 encourages class participation in naming bacteria that cause disease. Slides 5 – 8 focus specifically on the bacteria of interest: *Salmonella* Typhimurium and *Salmonella* Typhi, providing details on their classification and biology. Slides 9 – 11 discuss the issue of antibiotic resistance in *Salmonella*. The final section (Slides 12 – 17) introduces the activity and gives instructions on how to complete the task. If using the information cards for the activity, hide Slide 14. If using the Genome Scholar web tool, hide Slide 13.

Stage 2: Complete the worksheet (10 – 15 minutes)
The worksheets have two parts:
- Part 1: research
- Part 2: genome assembly

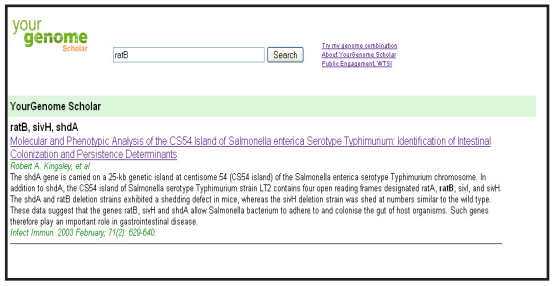
Part 1 should always be completed first. By reading the information cards students can identify and summarise the key functions of the genes listed on Page 1 of the worksheet.





Alternatively the Genome Scholar web tool can be used by students to source information on the genes listed on Page 1 of the worksheet. The content of the Genome Scholar web tool is the same as the Reference Cards.

To use the Genome Scholar web tool:
1. Go to http://www.yourgenome.org/downloads/activities/buildabug/index.html
2. Type in a search term or name of the genetic component of interest, e.g. *ratB*
3. Press 'search'
4. Repeat the process for each listed genetic component



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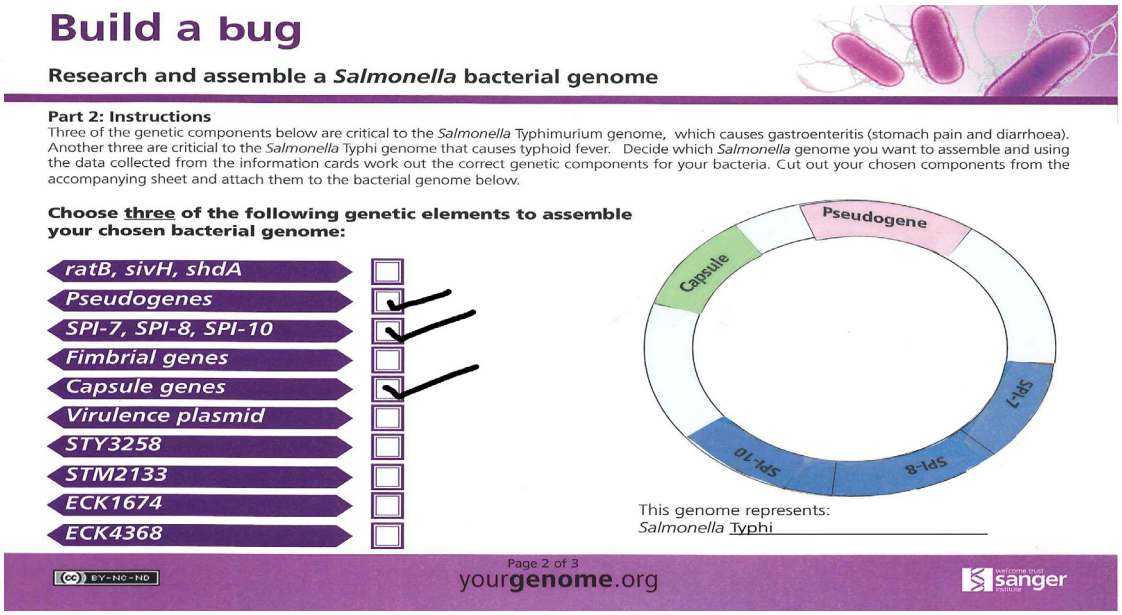
When Page 1 has been completed, students should decide which species' genome they will assemble. On Page 2 tick the three genetic components for the bacterial genome.

If using the paper version, cut out the genetic elements (Page 3) and stick to the genome backbone on the worksheet.



Build a bug

Research and assemble a *Salmonella* bacterial genome

Part 2: Instructions
Three of the genetic components below are critical to the *Salmonella* Typhimurium genome, which causes gastroenteritis (stomach pain and diarrhoea). Another three are criticial to the *Salmonella* Typhi genome that causes typhoid fever. Decide which *Salmonella* genome you want to assemble and using the data collected from the information cards work out the correct genetic components for your bacteria. Cut out your chosen components from the accompanying sheet and attach them to the bacterial genome below.

Choose three of the following genetic elements to assemble your chosen bacterial genome:

- ratB, sivH, shdA ☐
- Pseudogenes ☑
- SPI-7, SPI-8, SPI-10 ☑
- Fimbrial genes ☐
- Capsule genes ☑
- Virulence plasmid ☐
- STY3258 ☐
- STM2133 ☐
- ECK1674 ☐
- ECK4368 ☐

This genome represents:
Salmonella Typhi

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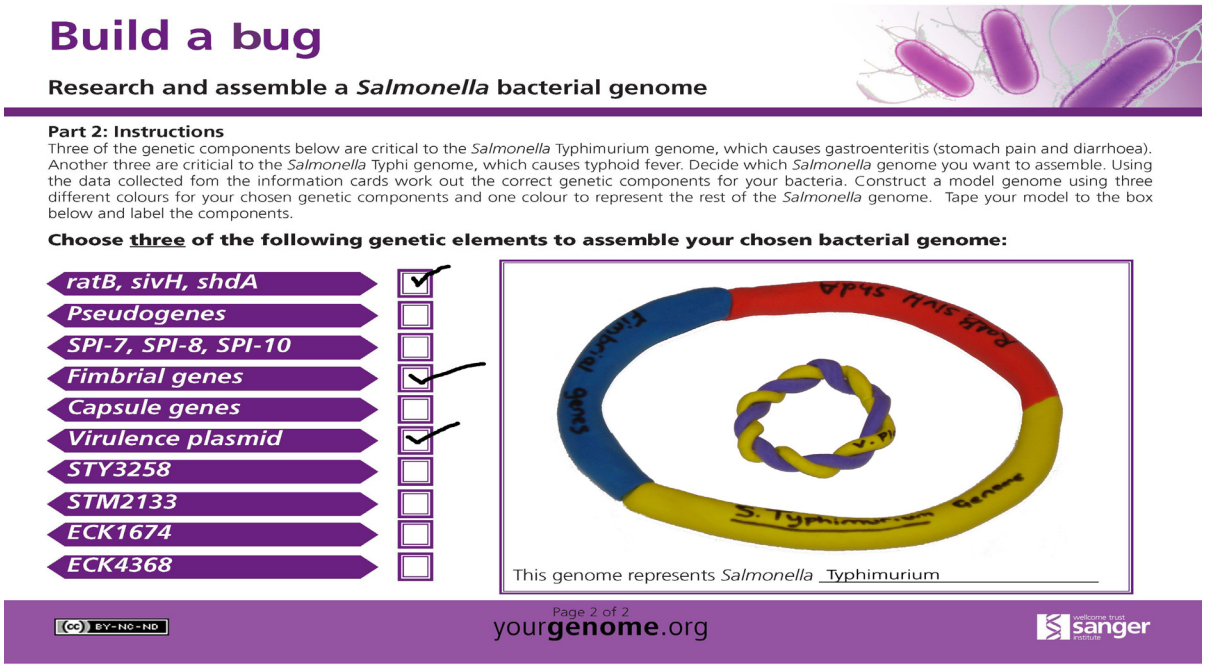
yourgenome.org

If using the modelling option, create the model using the materials provided and stick it to the worksheet. A playdough example is shown below.



Build a bug

Research and assemble a *Salmonella* bacterial genome

Part 2: Instructions
Three of the genetic components below are critical to the *Salmonella* Typhimurium genome, which causes gastroenteritis (stomach pain and diarrhoea). Another three are criticial to the *Salmonella* Typhi genome, which causes typhoid fever. Decide which *Salmonella* genome you want to assemble. Using the data collected fom the information cards work out the correct genetic components for your bacteria. Construct a model genome using three different colours for your chosen genetic components and one colour to represent the rest of the *Salmonella* genome. Tape your model to the box below and label the components.

Choose three of the following genetic elements to assemble your chosen bacterial genome:

- ratB, sivH, shdA ☑
- Pseudogenes ☐
- SPI-7, SPI-8, SPI-10 ☐
- Fimbrial genes ☑
- Capsule genes ☐
- Virulence plasmid ☑
- STY3258 ☐
- STM2133 ☐
- ECK1674 ☐
- ECK4368 ☐

This genome represents *Salmonella* Typhimurium

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yourgenome.org

Stage 3: Feedback and results (15 minutes)
Once students have completed Part 2 they can collate their results using the spreadsheet provided or a results table on a whiteboard or flip chart. Slides 18-19 of the presentation provide a summary of all the results, which can be presented to the class. Slide 20 of the presentation shows an image of the *Build a bug* animation. This can be used to introduce the final stage of the activity before switching to the animation.

Discussion point

Red herrings

Four of the genes (*STY3258, ECK1674, ECK4368, STM2133*) are classed as 'red herrings'. They are present in bacterial genomes but there is currently no information on their specific function. They are therefore not considered to have a role in the disease causing capabilities of the bacteria at this stage. Because these genes are not critical to the *Salmonella* genomes they have not been included in the animation.

Stage 4: Run the animation

The animation illustrates three scenarios: a *Salmonella* Typhimurium infection, a *Salmonella* Typhi infection and a non infection scenario (incorrect combination of genetic elements). The correct combinations are below:

Component	*Salmonella* Typhimurium	*Salmonella* Typhi
ratB, sivH, shdA	✓	
Pseudogenes		✓
SPI-7, SPI-8, SPI-10		✓
Fimbrial genes	✓	
Capsule genes		✓
Virulence plasmid	✓	

Any other combinations will result in the third scenario, i.e. no effect.

Discussion points

Why sequence bacterial genomes?

The activity highlights that there are key genetic difference between the serovars and these determine the virulence and pathogenicity of the bacteria. Whole genome sequencing reveals these key differences and allows researchers to better understand the biology of the organism which can lead to the development of new drugs and vaccines.

Genome information can also reveal how the bacteria have evolved. Comparing the two *Salmonella* serovars has highlighted that their evolution has been driven by acquiring or losing gene function. This information can help build up a clearer picture of the molecular basis of epidemics and how new virulent strains emerge (Sabbagh et al, 2010).

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The key differences between the two bacteria servovars are explained below. Use this information to discuss their role in the disease causing abilities of the two bacteria.

The key genetic elements of *Salmonella* Typhimurium

ratB, sivH, shdA
The ability of the bacterium to invade and colonise the gut of its host causes symptoms such as diarrhoea and gastroenteritis.

The genes *ratB, sivH* and *shdA* encode proteins that allow the bacteria to colonise the gut of their hosts. Experiments where mutations have been induced in these specific genes reduced the bacteria's ability to colonise areas of the host's gut such as the caecum (first part of the large intestine).

The genes are all implicated in intestinal colonisation by *Salmonella* Typhimurium but are all pseudogenes (inactivated) in *Salmonella* Typhi.

Fimbrial genes
Fimbriae are small hair-like projections from the surface of the bacterium. These protein projections (encoded by fimbrial genes) are not used to help the cell move about, but instead make it possible for bacteria to adhere to each other, surfaces and host cells. Basically fimbriae help bacteria stick to things.

Fimbriae are also a major virulence factor (the ability of a bacterium to cause disease), because these structures enable *Salmonella* to colonise the epithelial cells of the hosts digestive system, in particular the intestines. This causes inflamation and leads to symptoms of the disease such as diarrhoea.

All fimbrial operons are intact in *Salmonella* Typhimurium whereas pseudogenes are found in six fimbrial operons in *Salmonella* Typhi. These differences in "active" fimbrial operons may explain in part the different colonisation abilities of the bacteria.

Virulence plasmid
The virulence plasmid is found in several *Salmonella* serovars. It is a circular piece of DNA separate from the chromosome that has a number of genes that can aid the bacteria's survival within a host. For example, *spv* genes (*Salmonella* plasmid virulence) have been shown to increase the infection and growth of *Salmonella* in its host's intestinal tissues causing severe diarrhoea.

All *Salmonella* virulence plasmids contain the *spv* genes (Chu & Chui, 2006), but they often carry different drug resistance genes. In addition, some plasmids can be transferred between serovars via horizontal transfer or conjugation (see presentation Slide 11). Such events allow the bacterium to acquire new genes which can give it a survival advantage in an unfavourable drug environment. This can lead to the evolution of a more virulent and drug- resistant strain.

No plasmid has been identified to date in *Salmonella* Typhi that has been associated with virulence.

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The key genetic elements of *Salmonella* Typhi

Pseudogenes
A pseudogene is a sequence of DNA that is very similar to the sequence of a known gene but is no longer functional: it cannot be transcribed into a working protein.

More than 200 pseudogenes have been identified in the genome sequence of *Salmonella* Typhi. Several of these are related to genes that are known to contribute to virulence in *Salmonella* Typhimurium. It is thought that this genetic degradation or loss of gene function may contribute to the human-restricted host range for *Salmonella* Typhi. In other words, it has lost functions because it's specialised to just one specific host.

SPI-7, SPI-8, SPI-10 (*Salmonella* Pathogenicity Islands 7, 8 and 10)
Pathogenicity islands (PI) are clusters of genes or genetic elements found on the chromosomes of a large number of pathogens and are considered to be 'quantum leaps' in bacterial evolution (Groisman and Ochman). The acquisition of PI by horizontal gene transfer enables bacteria to rapidly gain complex virulence functions from other species. Virulence genes located on PI play a crucial role in *Salmonella* infections.

It is thought that *Salmonella* pathogenicity islands (SPI) allow the bacteria to invade the host cell and cause the break down of tissues in the intestines. There are thought to be 12 SPI across the *Salmonella* species, however *Salmonella* Typhi is the only species to encode SPI-7, SPI-8 and SPI-10.

Capsule genes
Capsule genes encode proteins that form a protective layer around the bacteria. It provides protection from its host's immune system by cloaking or hiding the surface antigens specific to that bacterium. Without the capsule the host's immune system would identify the surface antigens of the bacterium, recognise them as foreign and destroy them.

The production of a capsule is restricted to *Salmonella* Typhi, *Salmonella* Paratyphi C and *Salmonella* Dublin. It is absent in *Salmonella* Typhimurium.

References
Chu C and Chiu C-H. Evolution of the virulence plasmids of non-typhoid *Salmonella* and its association with antimicrobial resistance. *Microbes and Infection* 2006; Volume 8, (7): 1931 – 1936.

Groisman EA and Ochman H. Pathogenicity islands: bacterial evolution in quantum leaps. *Cell* 1996; 87: 791 – 794.

Sabbagh SC, Forest CG, Lepage C, Leclerc J-M and Daigle F. So similar, yet so different: uncovering distinctive features in the genomes of *Salmonella enterica* serovars Typhimurium and Typhi. *FEMS Microbiology Letters* 2010; 305: 1-13.

Further reading

Sabbagh SC, Forest CG, Lepage C, Leclerc J-M and Daigle F. So similar, yet so different: uncovering distinctive features in the genomes of *Salmonella enterica* serovars Typhimurium and Typhi. *FEMS Microbiology Letters* 2010; 305: 1-13.

Kathari A, Pruthii A and Chugh TD. The burden of enteric fever. *Journal of infection in Developing Countries* 2008; 2: 253-259

Bhutta Z A and Threlfall J. *Addressing the global disease burden of typhoid fever.* Journal of the American Medical Association 2009; 302: 808-809

Recent news

Defining DNA Differences to Track and Tackle Typhoid. Wellcome Trust Sanger Institute press release. 27th July 2008. http://www.sanger.ac.uk/about/press/2008/080727.html

First high-throughput functional analysis of every Salmonella Typhi gene. Wellcome Trust Sanger Institute press release. 16th October 2009. http://www.sanger.ac.uk/about/press/2009/091016.html