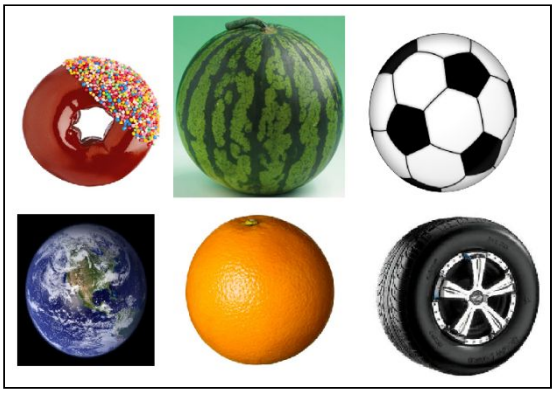
A221: Microbiology
Problem 7: Identity Crisis
WORKSHEET

Question 1: Are you here or there?

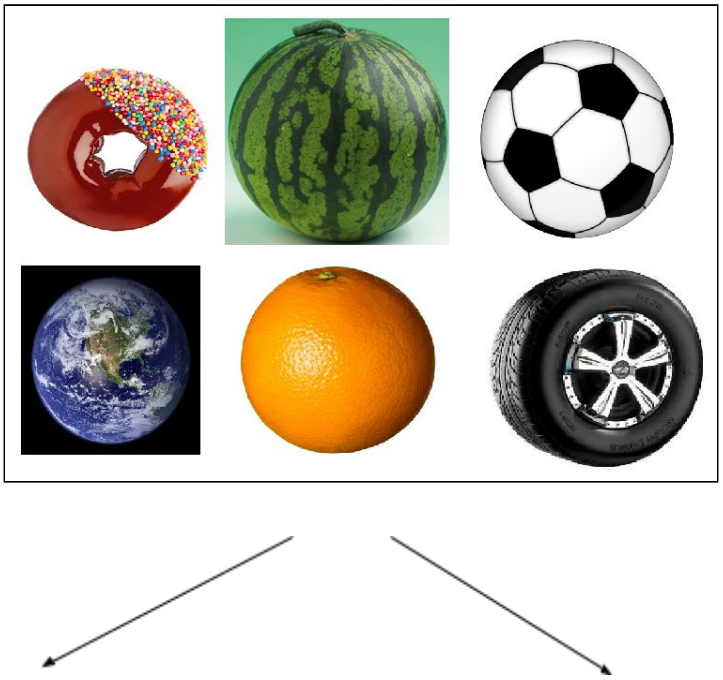
Group A

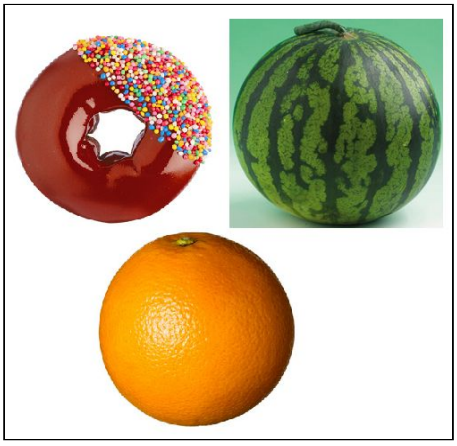


Group B

a) What is the basis of placing these items in the specific groupings above?
These items are organised based on their shapes, circle and square.

The items from Group A are now sub-divided as shown in the diagram below.





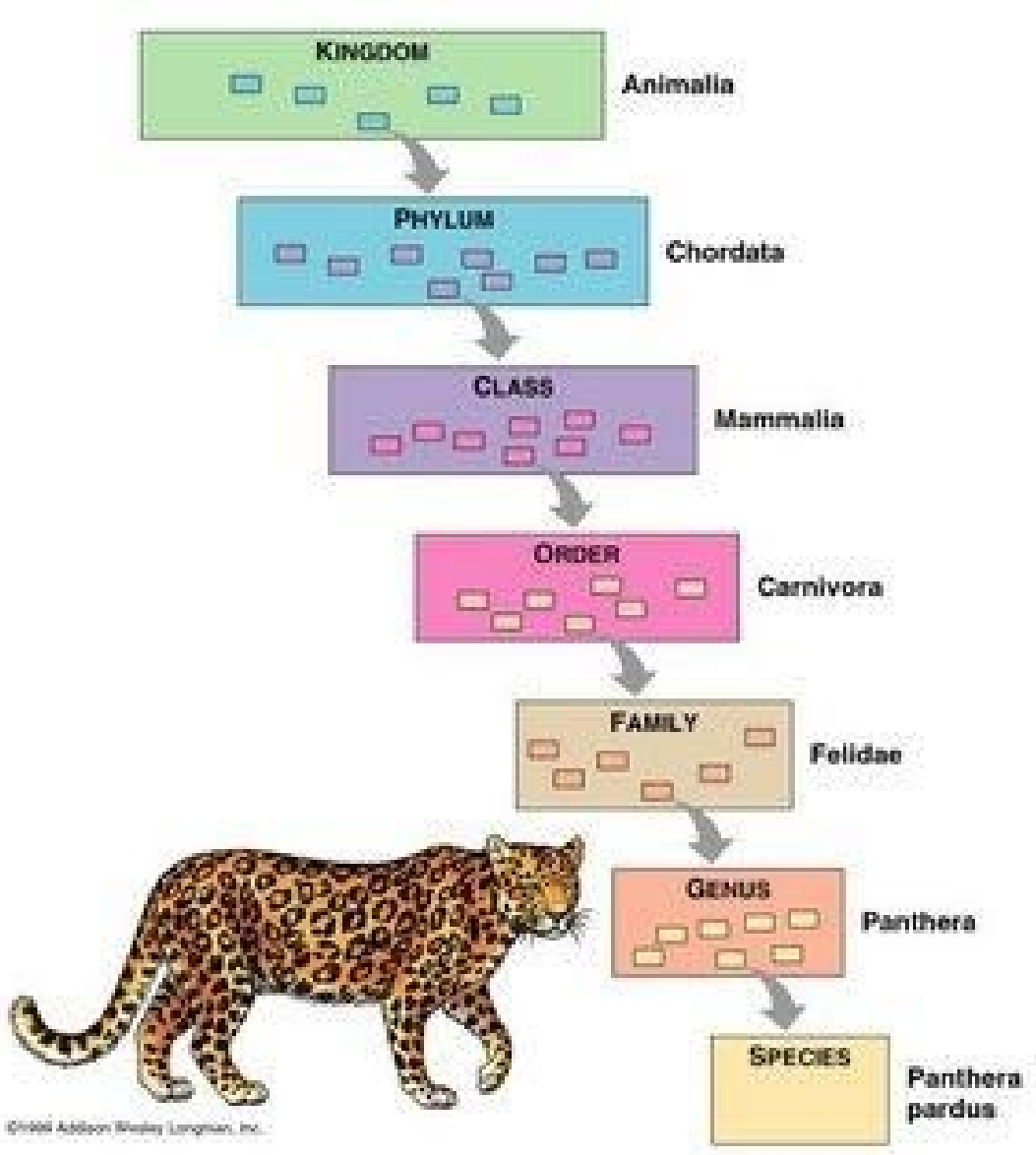
b) What are the different categories that Group A items are now categorised into?
Group A items are categorised into edible and non-edible.

c) What is the rationale that items must be classified?
They have similar points/characteristic for easy identification and to sort things into useful categories.

Question 2: Let's rank…

The figure below shows a leopard.

a) Refer to the schematic diagram below and discuss in your teams the relevance of ranking and naming the leopard shown below.



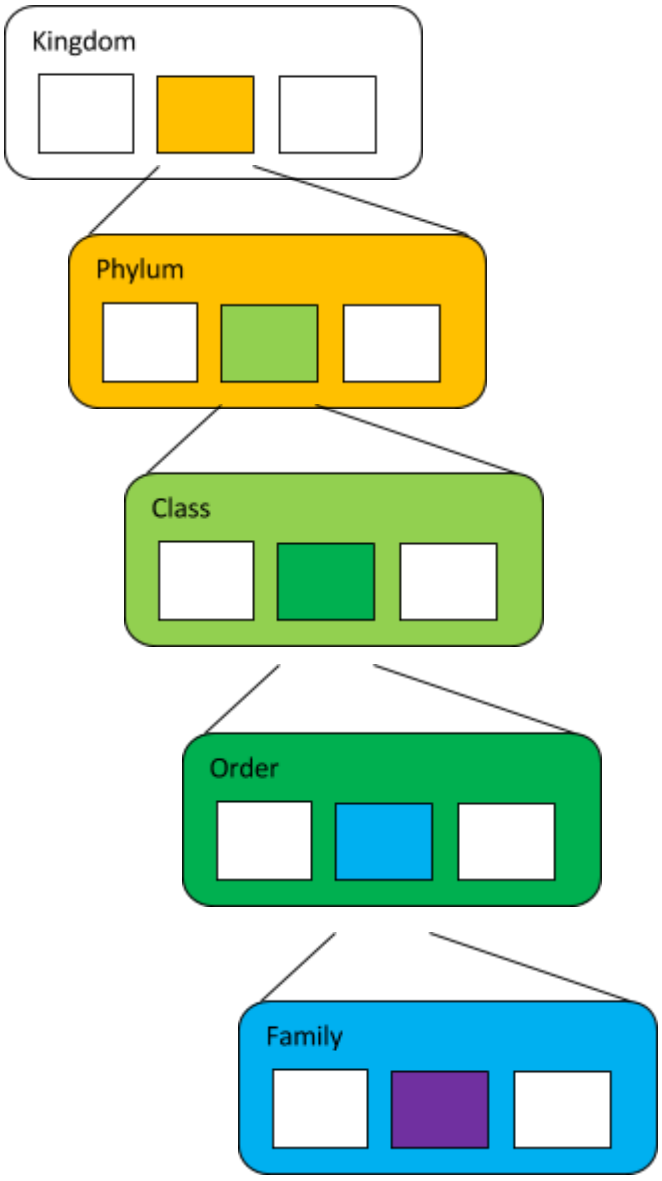
b) Name the different kingdoms for classifying living organisms into.
Animals, Plants, Fungi, Protista, Eubacteria, Archaebacteria

Monera/bacteria (bacteria and archaea), protista (protozoa and algae), fungi, plantae (plants), animalia (animal)

c) In your opinion, which kingdom would bacteria belong to?
Eubacteria

Monera kingdom

d) With reference to the chart below, place the naming (of human and *E. coli*) into their respective boxes in the table below.

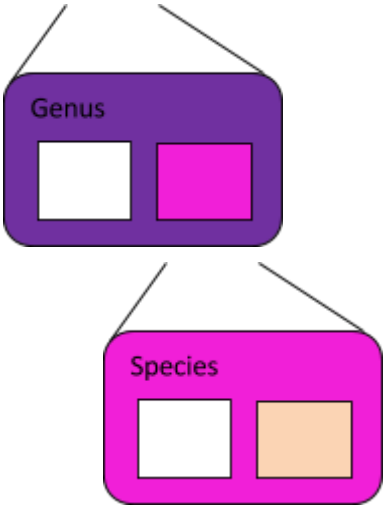


Human beings

- Hominidae
- Chordata
- Mammalia
- Primate
- Homo
- *Homo sapiens*
- Animalia

E.coli

- Gamma proteobacteria
- *Escherichia coli (E. coli)*
- Escherichia
- Bacteria
- Enterobacteriales
- Enterobacteriaceae
- Proteobacteria



Kingdom	**Animalia**	**Bacteria**
Phylum	**Chordata**	**Proteobacteria**
Class	**Mammalia**	**Gamma proteobacteria**
Order	**Primates**	**Enterobacteriales**
Family	**Hominidae**	**Enterobacteriaceae**
Genus	**Homo**	**Escherichia**
Species	***Homo sapiens***	***Escherichia coli (E. coli)***
Strain	**-**	***E. coli* O157:H7**

Question 3: Can you recall?

Both *Escherichia coli* and *Klebsiella pneumoniae* belong to the Family Enterobacteriaceae. Some basic characteristic of the two bacteria are listed in the table below.

	Characteristics		
Escherichia coli	Gram-negative	Rod	Utilize lactose as food source
Klebsiella pneumoniae	Gram-negative	Rod	Utilize lactose as food source

a) With reference to the above table, discuss how bacteria in the same family are classified.

It is classified by gram status, the shape of bacteria and the nutrient requirement

The bacteria are classified based on their closeness/ similarity of one bacteria to another bacteria

b) Why is there a need to classify bacteria into different categories?

There is a need to classify bacteria into different categories so that we can differentiate between the two types of bacteria.

It is useful for microbiologists working in clinical labs to help with the differentiation of microorganisms based on characteristics that are easy to identify. Researchers all over the world will be able to share their findings/ novel isolation into these categories

c) What were some of the experiments that were conducted in the previous weeks (Problem 4-6) for determining the identity of *E. coli*?

Gram staining, simple staining, using the microscope to identify shape of the bacteria

Observation of the cell colony morphology (including the shape, size, edge, elevation, etc.) to do cross-comparison with known samples, staining (simple and Gram staining, with observation of their shape as well as arrangements. e.g. in chains or clusters etc), growth and nutritional characterics (appearance in liquid and solid culture)

d) Recalling what you have learnt in Problem 6, what is the basic identification system that is usually used for preliminary identification of bacteria?

The cell shape and cell organisation of bacteria

The bacteria are determined primarily by their Gram stain status; Gram-positive and Gram-negative. They are then sub-classified according to their shape (rod, cocci, spiral, etc.)

e) Are you confident that the culture that you had isolated during the practical session into pure culture and observed under the microscope to be Gram-negative rod is truly *E. coli*?

No. there are other bacteria that are rod-shaped and gram-negative (e.g. Salmonella enterica subsp. enterica)

No. There can be other bacteria that are Gram-negative rod as well. (To show the limitation of identification using Gram staining alone)

Question 4

The physiological reactions to nutrients and other substrates provide excellent indirect evidence of the types of enzyme systems present in a particular species of bacteria. The Bergey's Manual of Determinative Bacteriology is the main resource for determining the identity of bacteria species based on the specific characteristics of the bacteria.

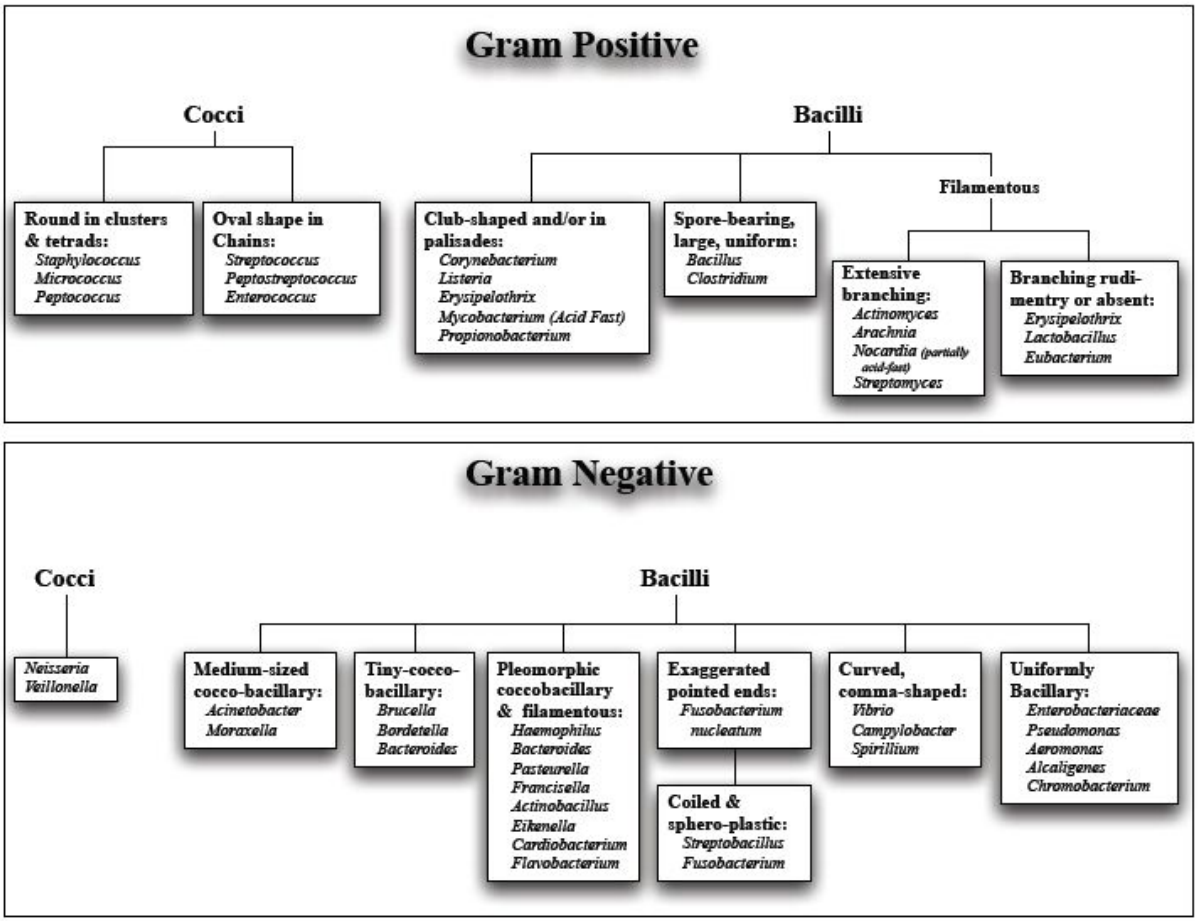
Bergey's Manual.pdf

It is used to classify bacteria based on their structural and functional attributes by arranging them into specific familial orders.

a) Using the chart below that was extracted from the Bergey's Manual, will it be possible for Bacteria Z to be of *Lactobacillus* spp., given that Bacteria Z is a Gram-negative bacillus?

No, it is not possible for Bacteria Z to be of *Lactobacillus* spp.

No, it cannot be of *Lactobacillus* spp. because from the information provided in Bergey's Manual, *Lactobacillus* is a Gram-positive bacillus



b) What is the likelihood of Bacteria Z to be classified under *Enterobacteriaceae*?
There is a chance that bacteria Z is under *Enterobacteriaceae*, but with only this information, it cannot be confirmed that bacteria Z is under enterobacteriaceae as not all Gram-negative bacteria are *Enterobacteriaceae*.

c) Given that Bacteria Z has the following characteristic, determine the identity of Bacteria Z using information available in Bergey's Manual.

Oxidase Test -

Lactose fermentation +

Indole Test +

Citrate Test -

Bacteria Z is *E.coli*.

Question 5

Just like the ability to carry out differential staining of bacteria, once a preliminary classification of a bacterium is done, special types of media can be used in conjunction with biochemical tests for identification of the bacteria. Selective medium and differential medium are good examples of such media.

a) Briefly describe what selective and differential media are.

Selective media allows the growth of only certain types of microbes while inhibiting the growth of others. When specific microbes are present in differential media, the medium or bacterial colonies will exhibit a colour change that provides information about their identity.

A selective medium is a culture medium containing one or more agents that allows the growth of certain types of microorganisms, while inhibiting the growth of other microorganisms.

Differential media distinguish one microorganism type from another growing on the same media via the biochemical characteristics of a bacteria growing in the presence of specific nutrients or indicators (eg. Dye in the media) added to the medium to visibly indicate the defining characteristics of the bacteria

b) Knowledge of microorganism's normal habitat is often useful in selecting a suitable culture medium because its natural environment reflect its nutritional requirements. Selecting the correct medium will enable specific microorganisms to grow which will help in the identification of a particular species. Fill in the following table to illustrate the type of media for isolation of different microorganisms.

Media	**Classification**	**Media components responsible for their selective, differential or enriching ability**	**Type of organism isolated and identified**
Mannitol salt agar	Selective and differential	7.5% NaCl: inhibit most bacteria	For the isolation of *Staphylococcus*

		other than Staphylococci **Mannitol**: bacteria that is able to ferment this sugar will produced acid. **Phenol red:** pH indicator turns yellow when acidic. Colonies that is able to ferment mannitol, resulting in the production of acid, will turn yellow	species and differentiation between pathogenic and non-pathogenic *Staphylococcus* spp
MacConkey's agar	Selective and differential	**Crystal violet and bile salts**: inhibit most gram positive bacteria while permitting gram negative rods to grow. Good for isolating intestinal bacteria **Lactose:** bacteria able to ferment this sugar, will produce acid **Neutral red:** pH indicator that turns pink when acidic. Colonies that is able to ferment lactose, resulting in the production	Selective for gram negative enteric bacilli Differentiation between lactose and non-lactose fermenter

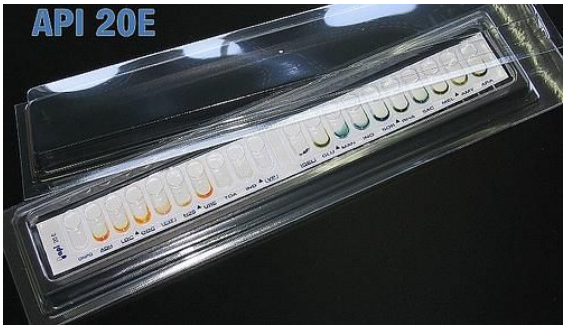
		of acid, will turn pink	
Blood Agar	Differential and Enriched	**5% defibrinated sheep blood:** supports the growth of most bacteria and differentiate bacteria based on their hemolytic properties	Isolation of fastidious bacteria differential for hemolytic organism

Going Further (Optional):

Question 6

a) What might be a disadvantage of performing the various biochemical tests one by one?
Time consuming to perform all the test one by one

Technological advances have enabled the development of techniques to not only speed up the process of identification, but also ensure standardization and reproducibility. Many of the tests listed in the Bergey's manual can be performed with rapid, miniaturized systems that can simultaneously determine up to 23 characteristics in small individual cups or space. One such example is the API-20E test strip as shown in the picture below.



i. What is API-20E test strip used for?
The API-20E test strip system consist of a plastic strip of 20 individual, miniaturized tests tubes (cupules) each containing a different reagent used to

determine the metabolic capabilities, and, ultimately, the genus and species of enteric bacteria in the family Enterobacteriaceae.

ii. How is the test carried out?

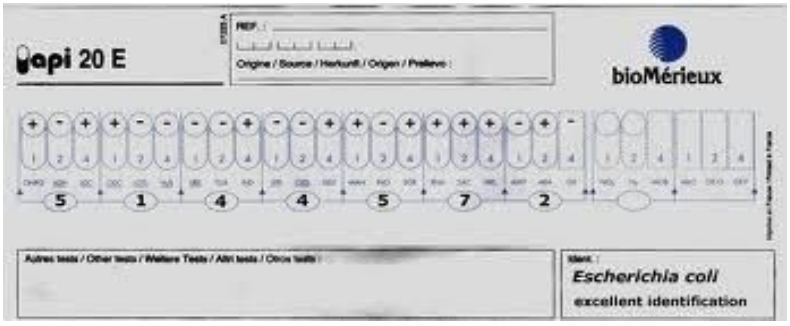
Loading the Cupules :

Each cupule is inoculated with a saline suspension of a pure bacterial culture, rehydrating the dried reagent in each tube. Some of the tubes are to be completely filled (tests CIT, VP and GEL), whereas others are topped off with mineral oil so that that anaerobic reactions (reactions that occur in the absence of oxygen) can be carried out (tests ADH, LDC, ODC, H2S, URE)

Incubating the API20E:

The strip is then incubated in a small, plastic humidity chamber for 18-24 hours at 37 degree celsius. Living bacteria produce metabolites and wastes as part of the business of being a functioning cell. The reagents in the cupules are specifically designed to test for the presence of products of bacterial metabolism specific to certain kinds of bacteria

iii. The figure below shows the result slip for reading and recording of results from the API-20E test strip. Briefly describe how the results should be read and how the results are interpreted.



After incubation, each tube (an individual test) is assessed for a specific colour change indicating the presence of a metabolic reaction that sheds light on the microbes identity. Some of the cupule contents change colour, due to pH differences, others contain end products that have to be identified using additional reagents.

Interpretation of the 20 reactions, in addition to the oxidase reaction (which is done separately), is converted to a seven digit code. The code can then be fed into the manufacturer's database via the computer where a search will be carried out in the ……

This system provides the identification, usually down to the genus and species. Traditionally, the code can has to be looked up in a reference book

iv. What is the advantage of such a test system?

API-20E offers a fast and convenient way for identification of enteric bacteria in the family Enterobacteriaceae

Question 7

What you have done so far in the worksheet is the phenotypic identification of bacteria. Bacteria can also be identified via genotypic methods, where the DNA sequence encoding the 16S rRNA (a component of the 30S ribosomal subunit) of prokaryotic bacteria can be identified.

a) What is the function of ribosomes?
The function of ribosomes is to produce proteins (protein synthesis in cells).

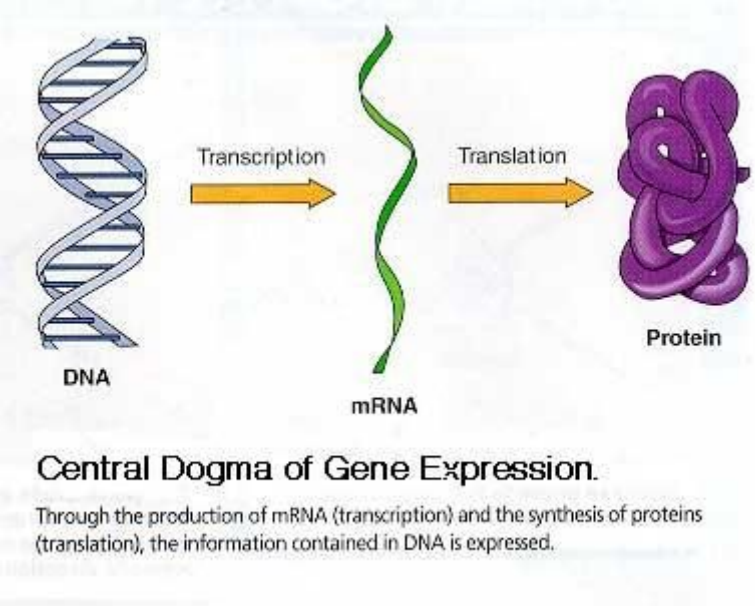
b) Due to the same function that ribosomes have in all cells, would you expect ribosomes to remain rather stable or unstable in their nucleic acid content over long periods?
Stable

c) With reference to your answer in 7b above, explain why 16S rRNA is suitable as a marker to be used for genotypic identification of bacteria?
Ribosomes tend to remain more or less stable in the nucleic acid content over long periods (due to same function-protein synthesis in all cell), hence can be used to identify bacteria. Any major difference in sequence, or "signature", of the rRNA is likely to indicate the distance in ancestry

d) The piece of DNA used for identifying bacteria is the region that codes for a small subunit of the ribosomal RNA (16S rRNA). With reference to the central dogma shown below, explain how determination of 16SrRNA DNA sequence would allow identification of bacteria.

since ribosome are translated from mRna which is transcribed from DNA, determination of DNA codes can allow identification of unique species. THe identification can be performed by matching the DNA sequence from the bacterial sample against

(Extra info: The 16SrRNA gene is used for phylogenetic studies as it is highly conserved between different species



Central Dogma of Gene Expression.

Through the production of mRNA (transcription) and the synthesis of proteins (translation), the information contained in DNA is expressed.

Sequence analysis of the 16S rRNA sequences is done with the help of several primers, called " universal primers". These primers target the conserved region of 16S rRNA gene and amplify the target in parts. FInally he several amplified parts could be assembled together to have the entire sequence of amino acids

In addition to highly conserved primer binding sites, 16S rRNA gene sequences contain hypervariable regions that can provide species-species signature sequences useful for bacterial identification. The identification relies on matching the sequences from the bacterial sample against a database of all known 16S rDNA sequences

This technique is powerful at 2 levels: It is effective for differentiating general group differences, and can be fine-tunes to identify at the species level.

References:

Principles of bacteria identification:

1. http://amrita.vlab.co.in/?sub=3&brch=76&sim=1109&cnt=1

Selective and Differential media:

2. http://www.highlands.edu/academics/divisions/scipe/biology/labs/rome/selectivedifferential.htm
3. http://www.austincc.edu/microbugz/macconkey_agar.php
4. http://iws2.collin.edu/dcain/CCCCD%20Micro/msa.htm
5. https://catalog.hardydiagnostics.com/cp_prod/Content/hugo/ColumbiaBldAgar.htm
6. http://www.austincc.edu/microbugz/blood_agar_test.php

References for Going Further:

Ribotyping:

7. http://amrita.vlab.co.in/?sub=3&brch=76&sim=1421&cnt=1

API:

8. http://www.youtube.com/watch?v=RpFnwXMyC-w
9. http://www.youtube.com/watch?v=PXIis18qN9k&feature=related
10. http://www.youtube.com/watch?v=ew3amo02_b0&feature=related
11. http://www.youtube.com/watch?v=mGa5zjhtGD0&feature=related

~End of worksheet~