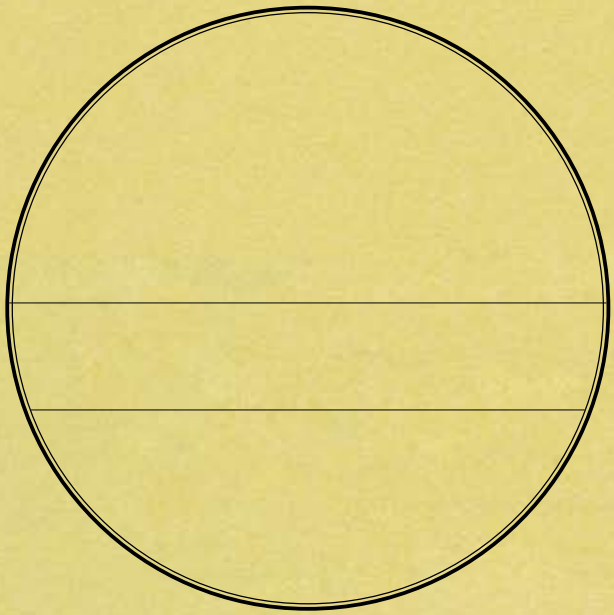
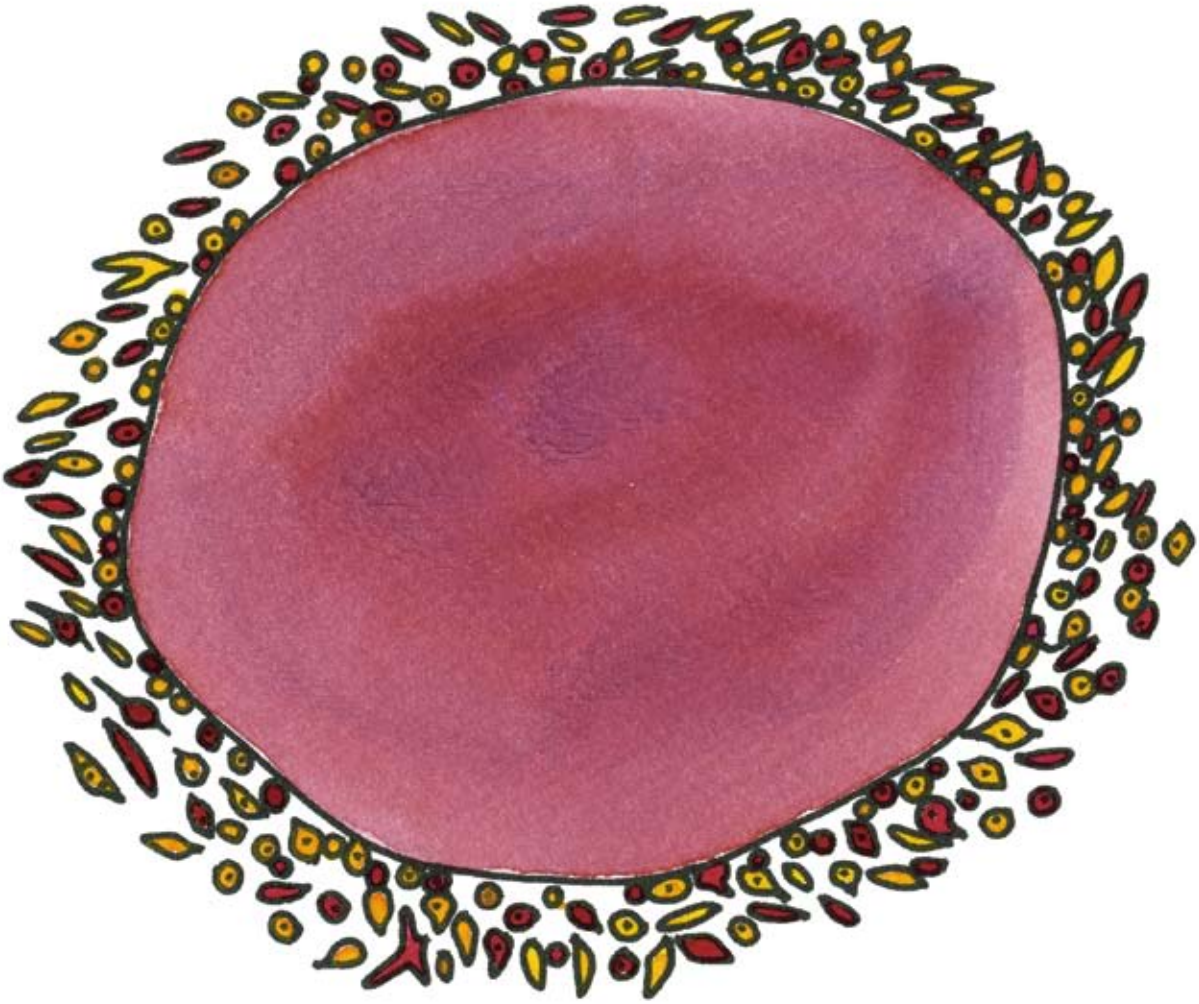
iCeMS - CiRA Classroom

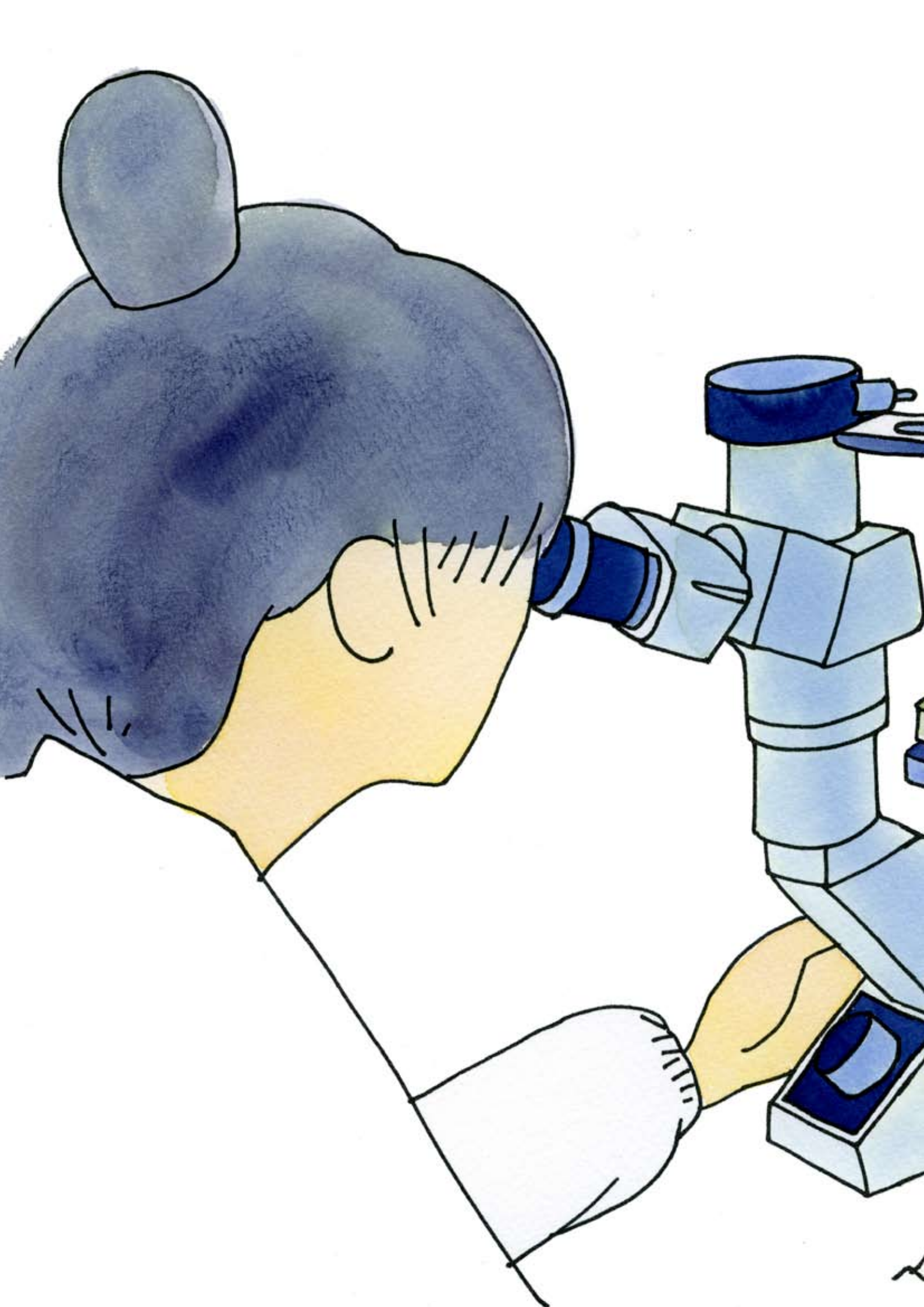
Hands-on with Stem Cells!

STUDY BOOK

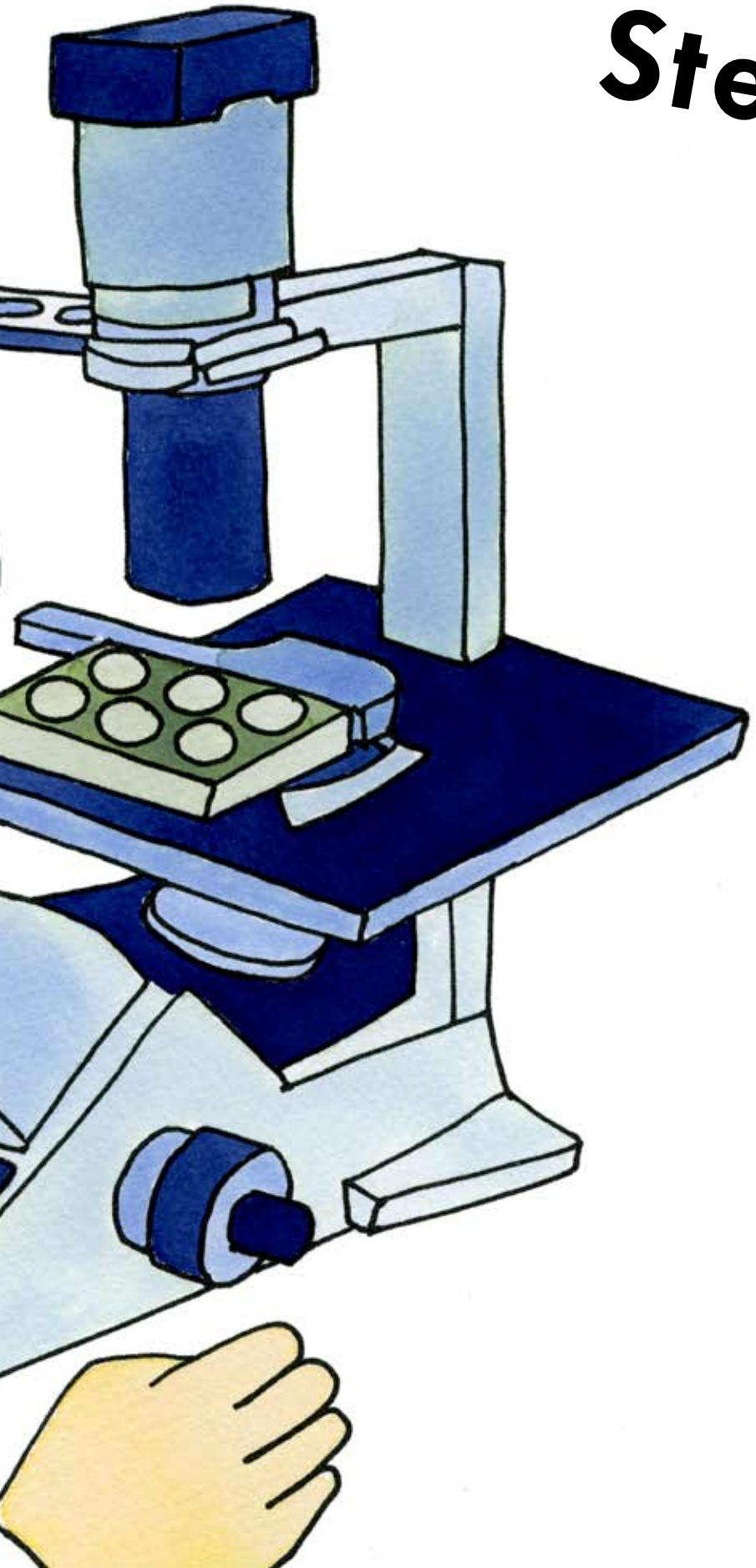
Institute for Integrated Cell-Material Sciences, Kyoto University (iCeMS)
Center for iPS Cell Research and Application (CiRA), Institute for Integrated Cell-Material Sciences, Kyoto University







Hands-on with Stem Cells!



Dear Participants,

How is cutting-edge research born?
How do “scientists” live their lives?
You will experience some “laboratory exercises” and
“discussions” on embryonic stem (ES) and
induced pluripotent stem (iPS) cells.
Let’s learn about some scientific activities
you will not find in school text books.

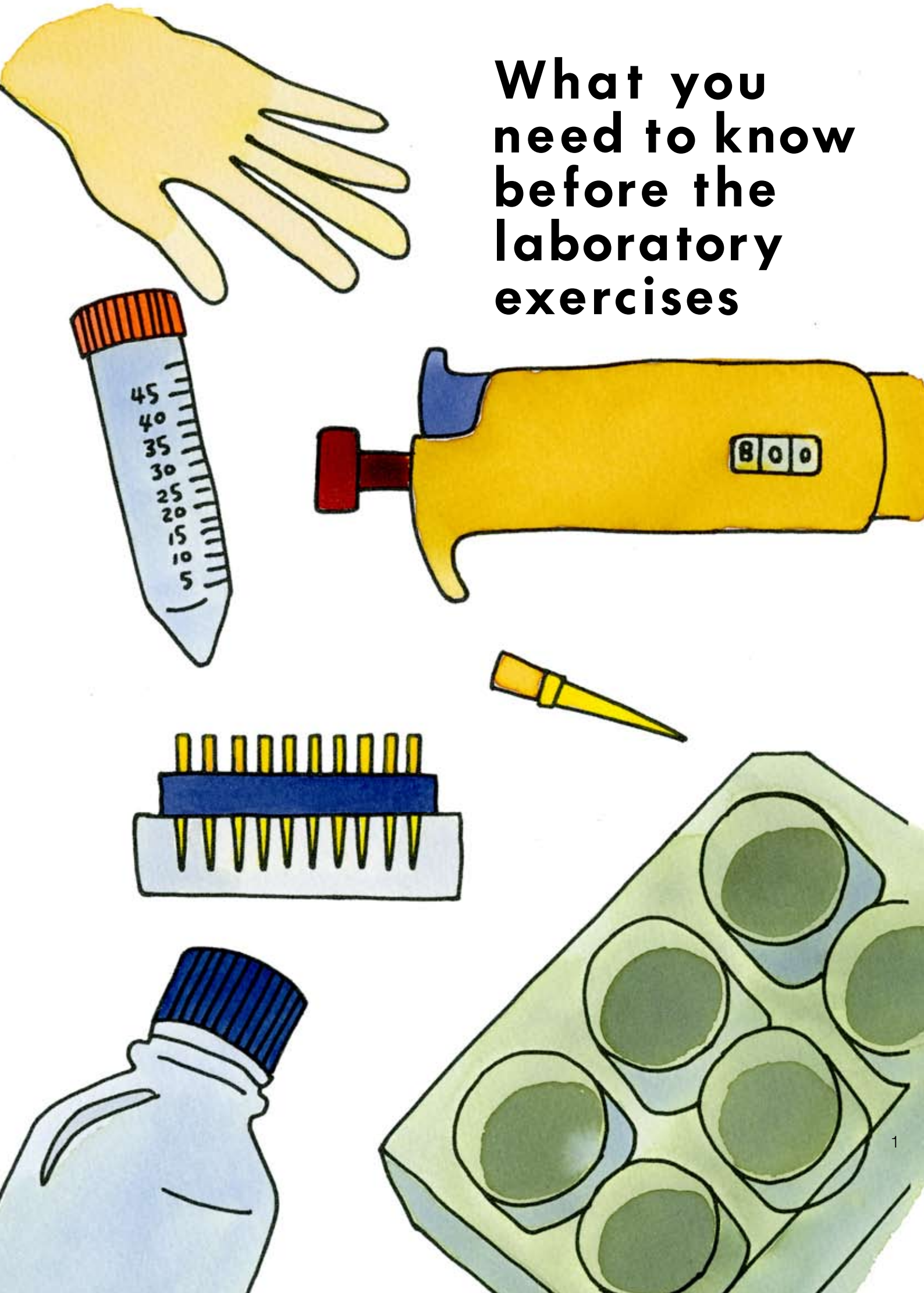
Contents

To ensure your safety and enjoyment during the laboratory exercises, we ask for your cooperation in carefully observing some basic regulations and guidelines:

1 Keep valuables in your possession at all times.
2 Wear your name tag at all times.
3 Do not bring any food or drink into the laboratory.
4 Wash your hands before entering and exiting the laboratory.
5 Do not touch any equipment without being instructed to do so.
6 If you are injured or sick, please notify the staff immediately and follow their instructions.

Please contact the laboratory staff if you have any questions.

What you need to know before the laboratory exercises



Part 1

How to use microscopes

Observation using a phase-contrast inverted microscope

1. Turn the light intensity control <a> counterclockwise until reaching MIN.

2. Set the main switch <b> on the side panel of the microscope frame to “I” (ON).

3. Turn the objective lens <c> to select the magnification. At first, select the lowest magnification (4×).
   CAUTION! Be careful not to crash the objective lens into the stage.

4. Slide the phase slider into position.
   ✳ Appropriate combination of the phase slider with the objective lens is as follows: 4× lens - Left; All other lenses - Middle.

5. Turn the light intensity control <a> to adjust the brightness.
   ✳ White light enters from above.

6. Turn the coarse adjustment knob <h> or fine adjustment knob <i> to adjust the focus.

7. The specimen can be moved to the desired position by turning the Y-axis knob <j> and the X-axis knob <k> .

Observation using a fluorescent microscope

1. Turn the light intensity control <a> counterclockwise until reaching MIN.

2. Set the main switch <b> on the

side panel of the microscope frame to “I” (ON).

3. Turn the objective lens <c> to select the magnification.

4. Turn the phase contrast ring <d> into position as follows: 4× – PhL; 10× – Ph1; 20× – Ph1; 40× – Ph2. Following this, close the fluorescence shutter <e> and set the filter selector ring <f> to empty.

5. Turn the light intensity control <g> to adjust the brightness.

6. Set the main switch <b> to “OFF”.

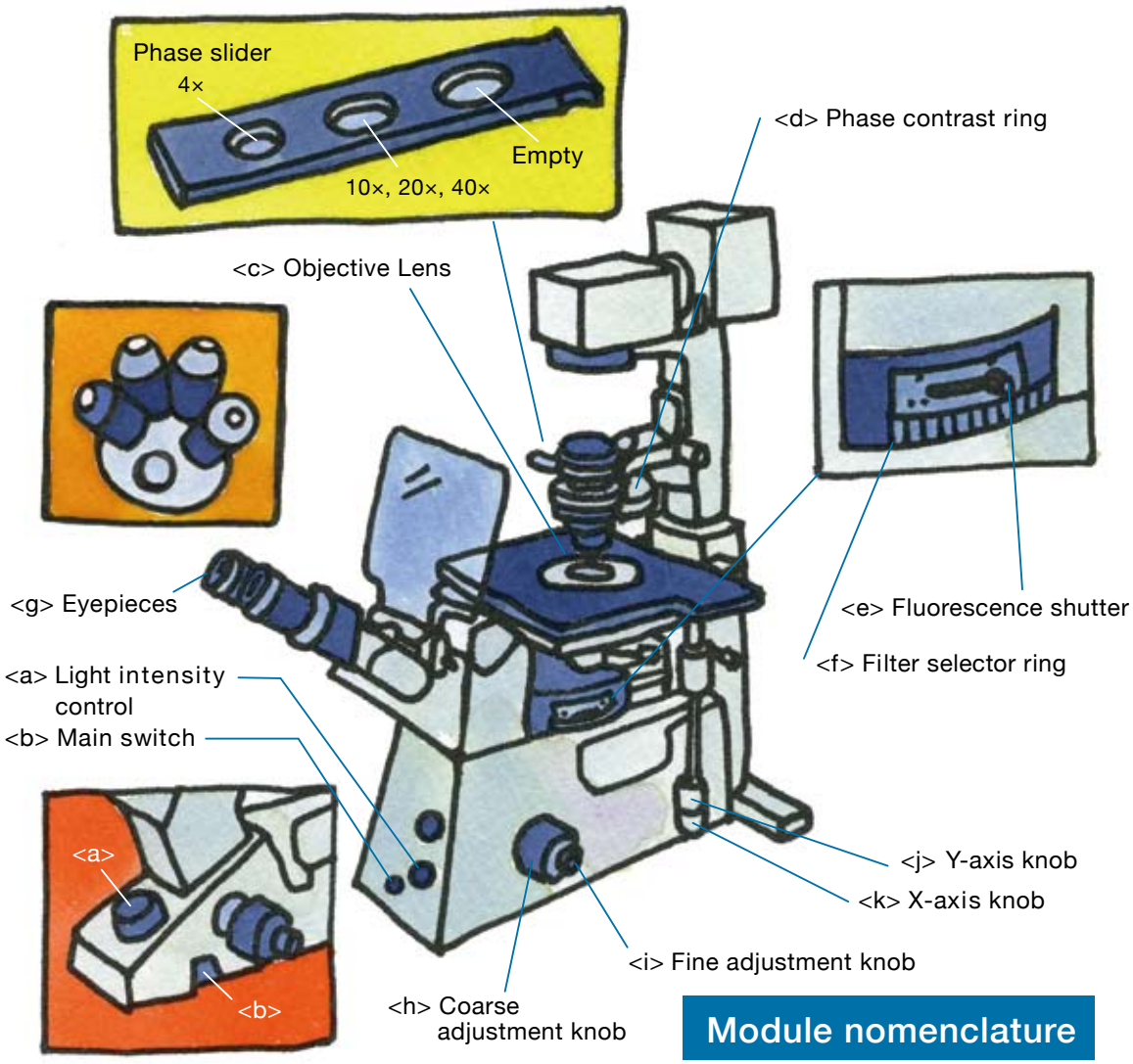
7. Set the objective lens <c> .

8. Set the filter selector ring <f> to GREEN.
CAUTION! When you proceed to the next step 9., strong light is emitted from the objective lens. Never look into the lens.

9. Open the fluorescence shutter <e>. ✳Blue light is emitted from the objective lens.

10. Turn the coarse adjustment knob <h> or fine adjustment knob <i> to adjust the focus.

11. The specimen can be moved to the desired position by turning the Y-axis knob <j> and X-axis knob <k>.



Module nomenclature

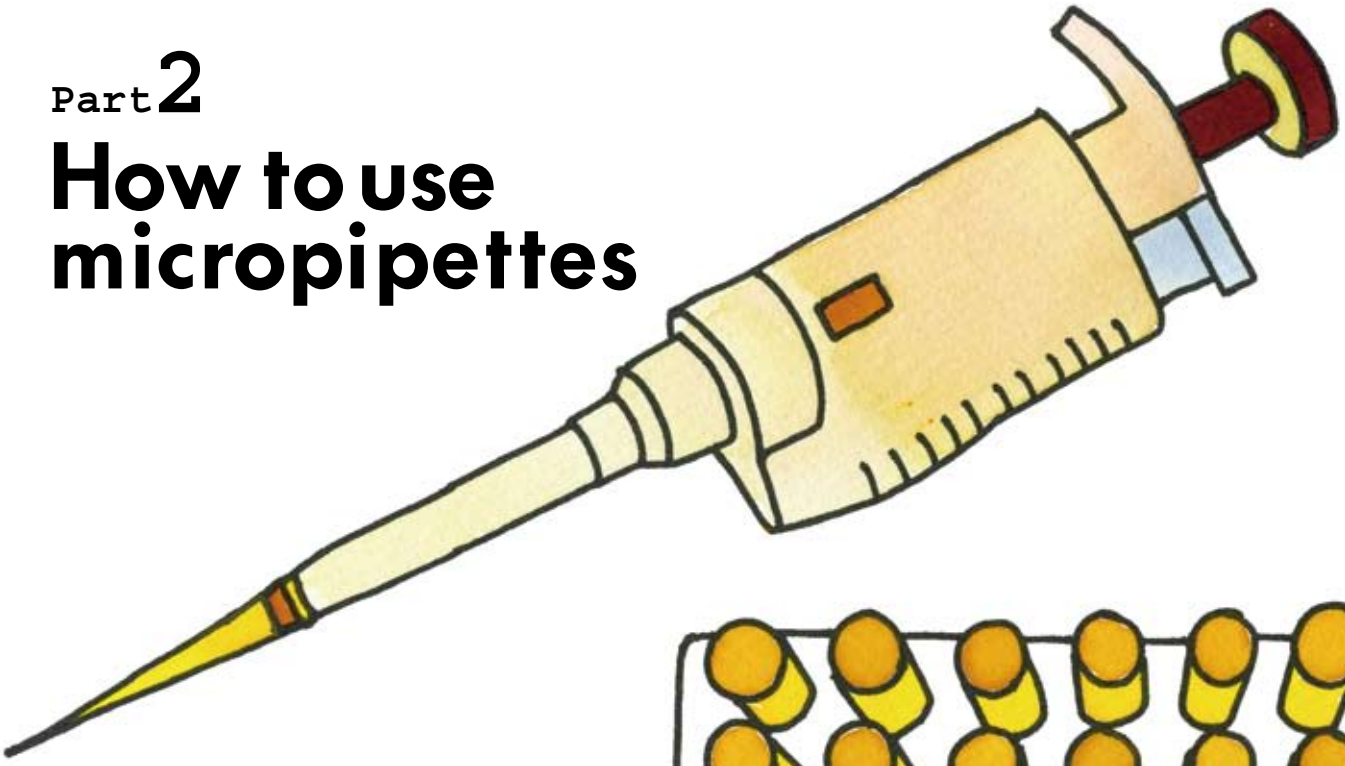
Practice

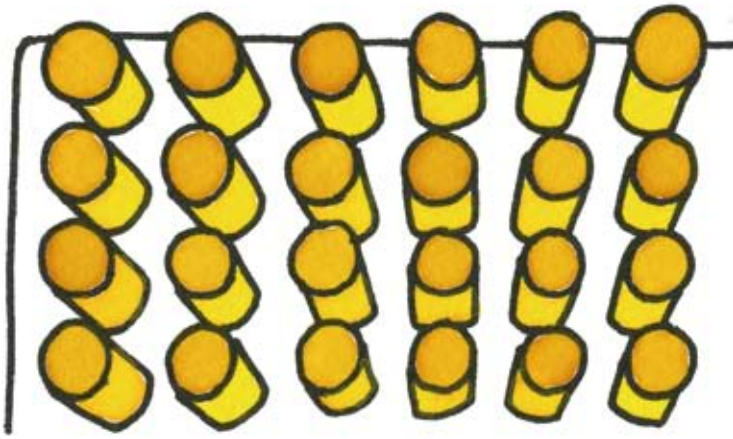
Let’s observe mouse cells using a microscope.

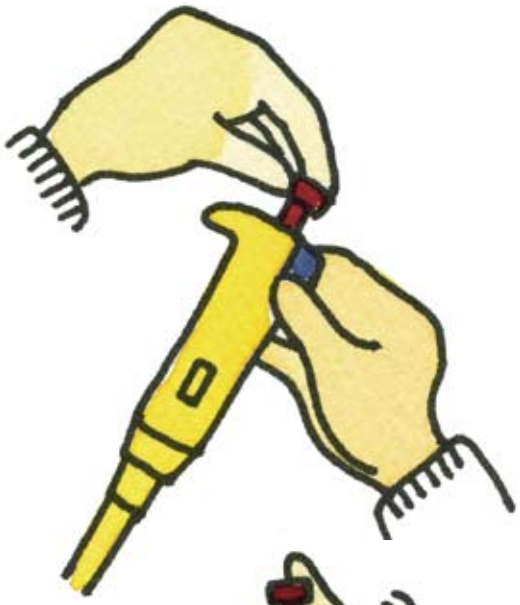
An example of drawings

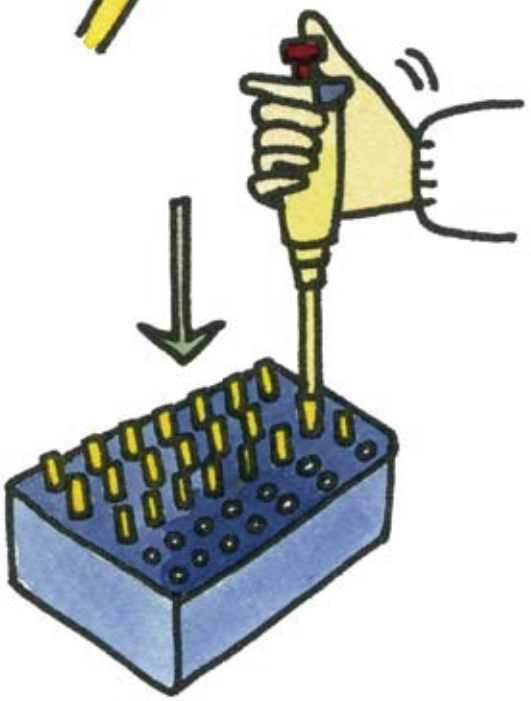
Part 2

How to use micropipettes









1. Volume setting using the volume adjustment knob

* Micropipettes covering volumes ranging from 100µL to 1000µL.
  You can measure from 100µL (0.1mL) to 1000µL (1mL) of samples, in 10µL increments, using a 1mL pipette.
* Micropipettes covering volumes ranging from 2µL to 20µL.
  You can measure from 2µL (0.002mL) to 20µL (0.02mL) of samples, with 0.1µL increments, using a 20µL micropipette.

CAUTION! Do not exceed the recommended volume range. If exceeded, it may damage the pipette or affect its accuracy.

2. Attaching a new disposable tip to the pipette shaft

* Hold a micropipette vertically in one hand and using your other hand, steady the tip holder. Carefully place the pipette into the tip and press gently to make a positive airtight seal.

3. Pipetting a sample

i. Press the plunger to the FIRST STOP. Then holding the pipette vertically, immerse the tip into the sample.

CAUTION! Only immerse the tip in the sample to a depth of approximately 1-2 mm. If immersed too deeply, the volume measured will be inaccurate.

ii. Allow the plunger to return slowly to the UP position.

CAUTION! Never let it snap up! If allowed to snap up, the volume measured will be inaccurate due to the presence of air bubbles.

4. Dispensing the sample

i. Withdraw the tip from the sample liquid.

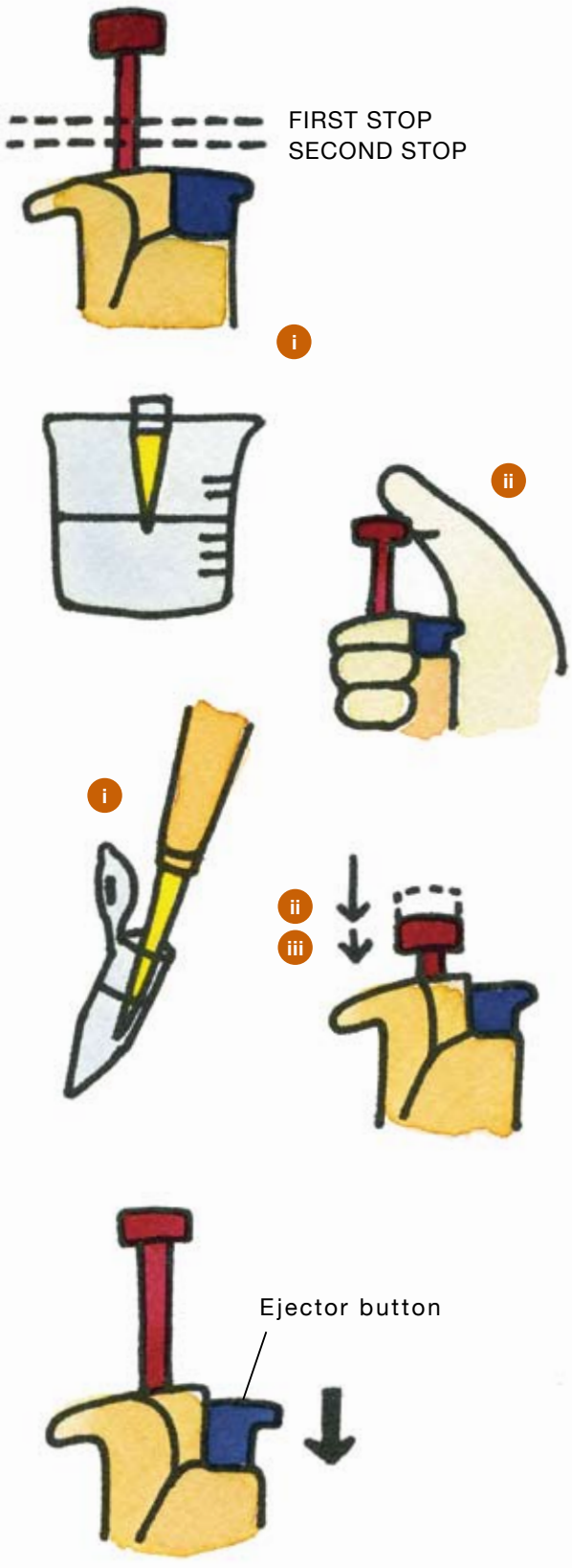
ii. Press the tip gently against the side wall of the receiving vessel and slowly depress the plunger to the FIRST STOP.

iii. Wait 1-2 seconds and press the plunger to the SECOND STOP, expelling any residual liquid in the tip.

iv. With the plunger fully pressed, withdraw the pipette from the vessel carefully.

5. Discarding the tip

Discard the tip by depressing the tip ejector button.

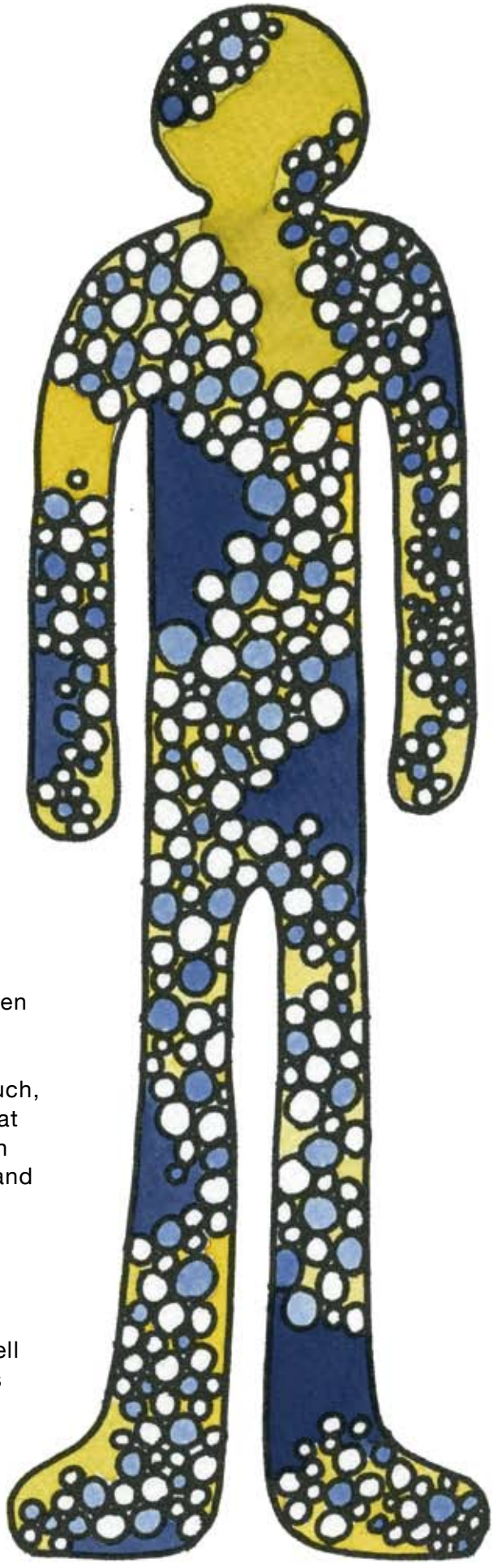


Practice

Let's try to divide 20µL of the sample liquid into four 5µL samples.
If done successfully, the four droplets will be the same size.

Part 3

Human bodies are composed of a variety of cells



60,000,000,000,000

That's the number of the cells that make up a human body. These 60 trillion cells are categorized into 210 types of cells, including red blood cells, nail keratinocytes, fibroblasts (skin cells), cardiac muscle and neuronal cells.

Sorry, Yellow-Green is NOT Available.

Color names, such as Red or Green, are labels for parts of a continuum that has been subdivided arbitrarily. However, the names of cells are not. Different names of cells represent the different types of cells. As such, although there is a color "yellow-green" that exists between yellow and green, cells with qualities lying between nail keratinocytes and fibroblasts do not exist.

Can you tell a book by its cover?

The traditional classification has been based on the shape and structure of the cell as seen in the microscope and its affinities for various stains. Today, it is noted that the difference in the genome-expression patterns of cells determines the cell type.

Part 4

What is a genome?

The information that makes you; that is your genome

The human genome is the entire genetic information encoding a human being; all genes and non-gene regions of full-length DNA.

Nuclear shelter!?

The genome DNA is enclosed in the “nucleus” of the cell, as if they were protected by a nuclear shelter.

Every cell contains the entire information that makes you

Each cell in your body, with a few exceptions*, has a nucleus. That means that all of your 60 trillion cells have your genome.

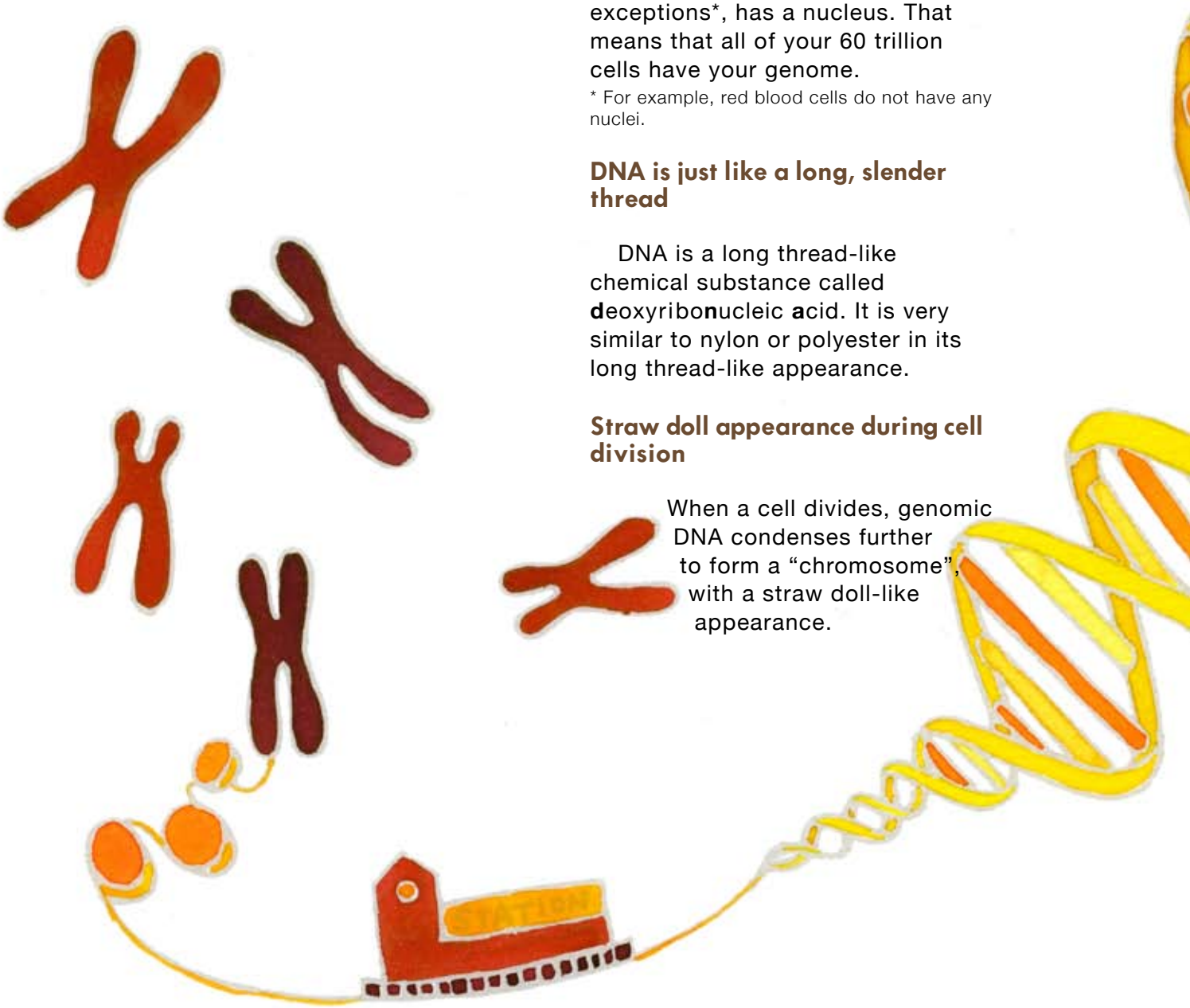
* For example, red blood cells do not have any nuclei.

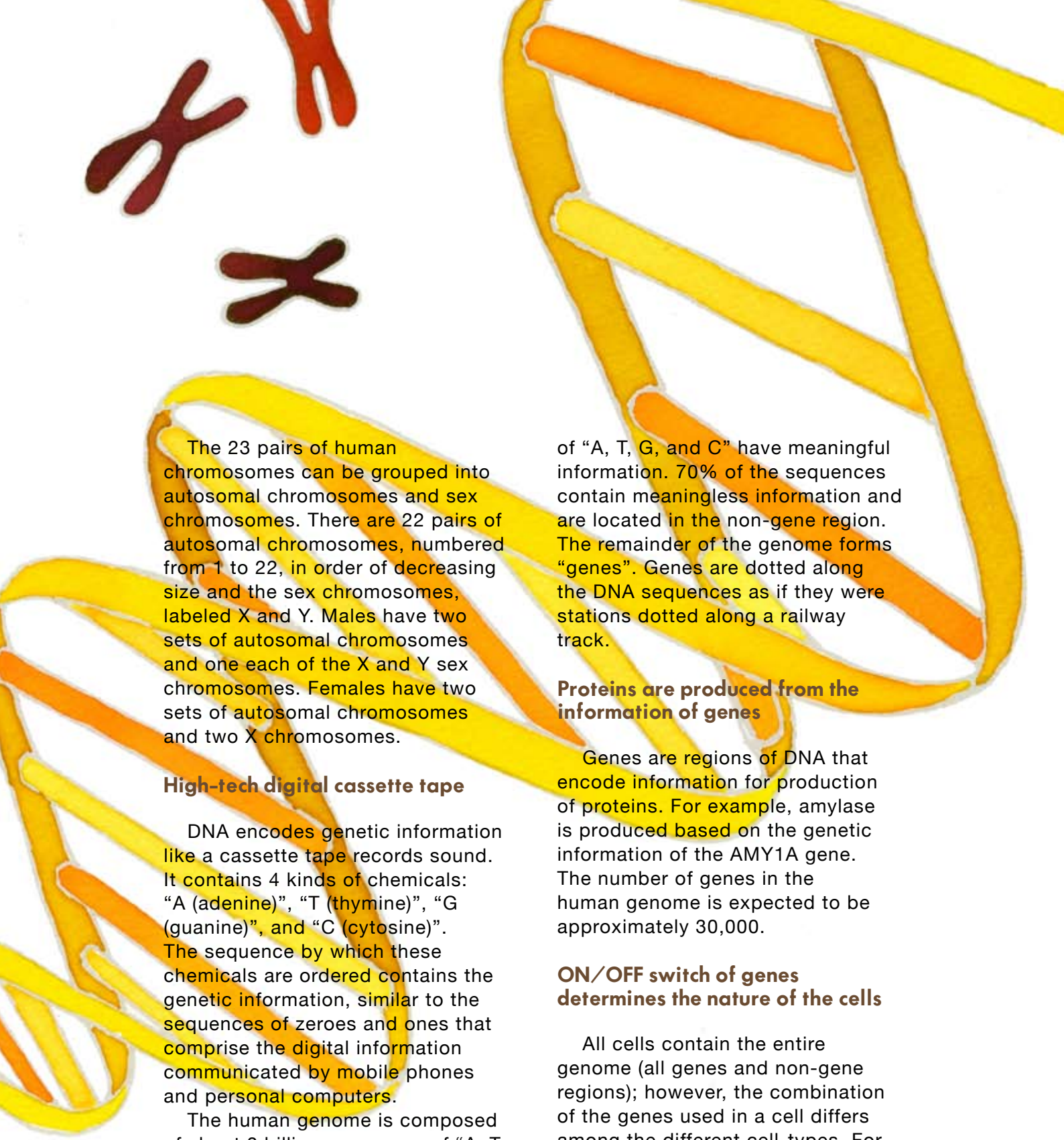
DNA is just like a long, slender thread

DNA is a long thread-like chemical substance called **d**eoxyribo**n**ucleic **a**cid. It is very similar to nylon or polyester in its long thread-like appearance.

Straw doll appearance during cell division

When a cell divides, genomic DNA condenses further to form a “chromosome”, with a straw doll-like appearance.





The 23 pairs of human chromosomes can be grouped into autosomal chromosomes and sex chromosomes. There are 22 pairs of autosomal chromosomes, numbered from 1 to 22, in order of decreasing size and the sex chromosomes, labeled X and Y. Males have two sets of autosomal chromosomes and one each of the X and Y sex chromosomes. Females have two sets of autosomal chromosomes and two X chromosomes.

High-tech digital cassette tape

DNA encodes genetic information like a cassette tape records sound. It contains 4 kinds of chemicals: “A (adenine)”, “T (thymine)”, “G (guanine)”, and “C (cytosine)”. The sequence by which these chemicals are ordered contains the genetic information, similar to the sequences of zeroes and ones that comprise the digital information communicated by mobile phones and personal computers.

The human genome is composed of about 3 billion sequences of “A, T, G, and C”. In recent years, all of the sequences in the human genome have been precisely decoded.

Stations dotted on a looooooooooong railway track

Not all of the 3 billion sequences of “A, T, G, and C” have meaningful information. 70% of the sequences contain meaningless information and are located in the non-gene region. The remainder of the genome forms “genes”. Genes are dotted along the DNA sequences as if they were stations dotted along a railway track.

Proteins are produced from the information of genes

Genes are regions of DNA that encode information for production of proteins. For example, amylase is produced based on the genetic information of the AMY1A gene. The number of genes in the human genome is expected to be approximately 30,000.

ON/OFF switch of genes determines the nature of the cells

All cells contain the entire genome (all genes and non-gene regions); however, the combination of the genes used in a cell differs among the different cell-types. For example, gene A may be switched “ON”, gene B “OFF”, gene C “ON”, and gene D “OFF”, etc.

The pattern of the genome expression, or the combination of the used/unused genes, determines the varieties and quantities of the proteins produced, eventually giving rise to different cell types.

Part 5

What are stem cells?

It all started from a fertilized egg

All of your 60 trillion cells originated from a fertilized egg. A fertilized egg divided over and over again, developing from an embryo into a fetus, eventually growing into YOU.

The future path is narrowing

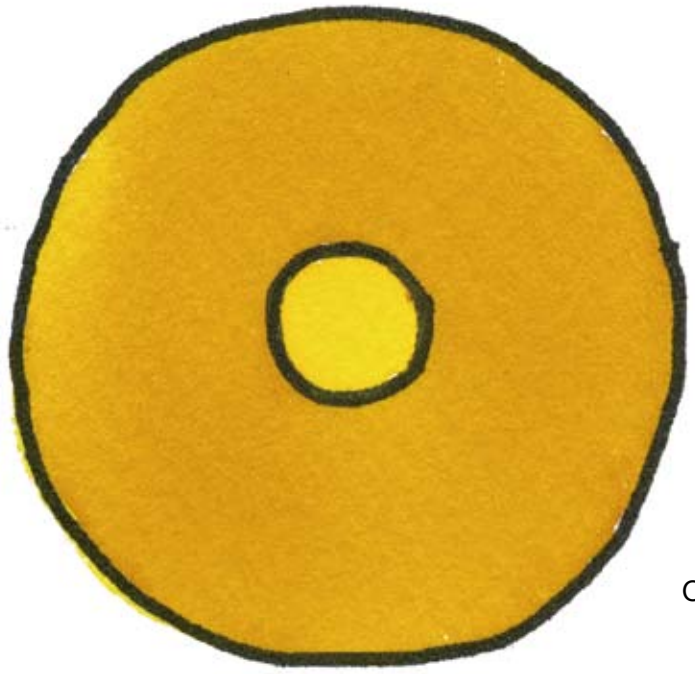
As the development of a fertilized egg progresses, the roles of cells become gradually restricted. Mesoderm cells can never become endoderm or ectoderm cells.

The wait is over

Your body contains a type of cell that can infinitely produce each cell of a particular tissue or organ. These cells are known as “stem cells”.

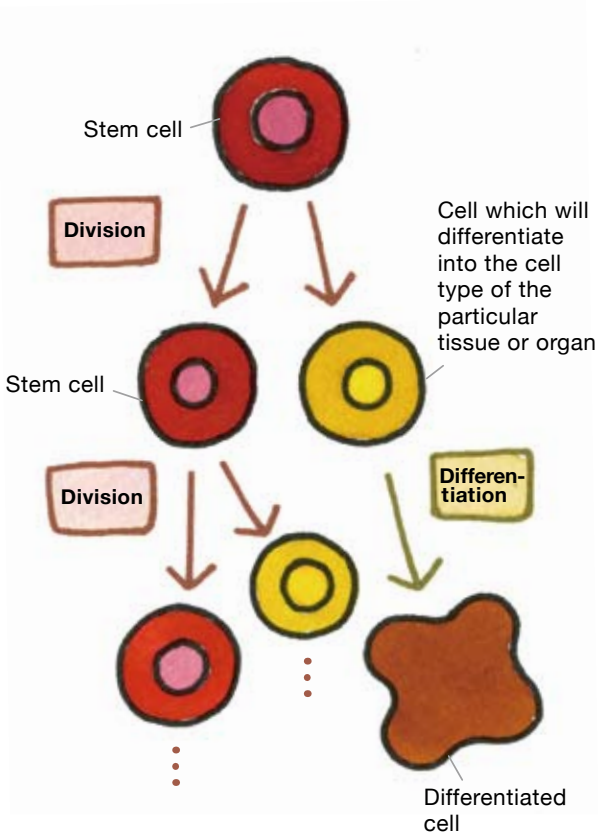
Prior to becoming a specific cell

A stem cell simultaneously produces a copy of itself and a cell which will differentiate into the cell type of the particular tissue or organ. That is, they have the potential for self-renewal.



The result of choosing the future path

Differentiation is the process by which a cell transforms into another cell, with a defining shape and function. Once cells have differentiated,



they cannot further differentiate into another cell type.

Determinants of the path

The chemical substances which cause neighbor cells to differentiate are known as “inducers”. Inducers act on stem cells, causing changes in their shape and function to that of a particular cell type.

Representative examples of stem cells: Tissue stem cells, Pluripotent stem cells

Tissue stem cells (adult stem cells) and pluripotent stem cells are two of the main categories of stem cells. In general, different categories of stem cells differ in their potential for division and/or differentiation.

	Tissue stem cells	Pluripotent stem cells
Division potential	If necessary	Infinite
Differentiation potential	Restricted	Unrestricted
Examples	Neural stem cells, hematopoietic stem cell	ES cells, iPS cells

The difference between pluripotency and totipotency

There are differences in the differentiation potential between fertilized eggs and pluripotent stem cells. Fertilized eggs can differentiate into all of the cells required for embryonic development, including cells of the placenta and each organ of the human body. Alternatively, it is difficult for pluripotent stem cells to develop into the embryo as they are unable to differentiate into placenta cells or plan the body shape of the embryo, though they can form all cell types of the adult body.

"Pluripotency" indicates the ability to differentiate into all cell types of the adult body, whereas "totipotency" indicates the ability to develop into the embryo.

Representative examples of pluripotent stem cells: ES cells, iPS cells

Embryonic stem cells (ES cells) and induced pluripotent stem cells (iPS cells) are well-known pluripotent stem cells; their origin being the distinguishing factor between the two. ES cells originate from the inner cell mass (ICM) of the blastocyst and can be isolated by culturing the ICM under specific conditions.

iPS cells originate from all of the cells in the body. Through the introduction of pluripotency-inducing factors into cells and culturing under certain conditions, we can obtain iPS cells. The first iPS cells were created by introducing 4 genes (Oct3/4, Sox2, Klf4, c-Myc) into fibroblasts using viral vectors. There are some issues for their application in medicine as the c-Myc gene is oncogenic and the use of viruses raises some ethical concerns.

When does life start?

It is not easy to determine when exactly life begins. Some religions believe that life starts at fertilization. Therefore, research using human ES cells derived from human embryos can be controversial in some cultures.

For almost 10 years the Japanese government has employed much stricter reviews and restrictions than the international standards, which are set by The International Society for Stem Cell Research (ISSCR). Therefore, some people believe that Japan is holding back the spread of human ES cell research.

In contrast, human iPS cells, which are derived from somatic cells, avoid the ethical issues associated with the destruction of the human embryo.

Privacy protection

As your iPS cells are derived from your somatic cells, they contain your personal data. Therefore, there have been ethical issues with the protection of genomic information.

Future prospects

Various medical and scientific research projects, using pluripotent stem cells, are currently being undertaken, with great expectations. Stem cell therapies, such as the transplantation of pluripotent stem cell-differentiated islet of Langerhans cells into diabetic patients, are being examined to determine the side effects of iPS cell therapy. The exact mechanisms behind the disease being treated must be determined using disease-model cells to determine whether stem cells can be used for treatment. Furthermore, the mechanisms behind inducing the differentiation/dedifferentiation of these cells must also be determined.

iPS cells are more adapted for basic scientific research, such as the clarification of the mechanisms behind diseases, as they are easily established and avoid the ethical issues associated with the destruction of an embryo.

iPS cells can also avoid the issue of immune rejection as they can be easily established from your own somatic cells. However, iPS cells do have some issues associated with their safety as the genes can randomly insert into the genome or the initialization may be imperfect. Therefore, it is said that ES cells should be first considered for application in regenerative medicine.

Part 6

Bioimaging

2008 Nobel Prize in Chemistry

Dr. Osamu Shimomura, Dr. Martin Chalfie, and Dr. Roger Y. Tsien won the Nobel Prize for Chemistry in 2008 for the discovery and development of the green fluorescent protein, GFP.

GFP plays an important role in the observation of the cells and is one of the most well-known proteins throughout the life science research community.

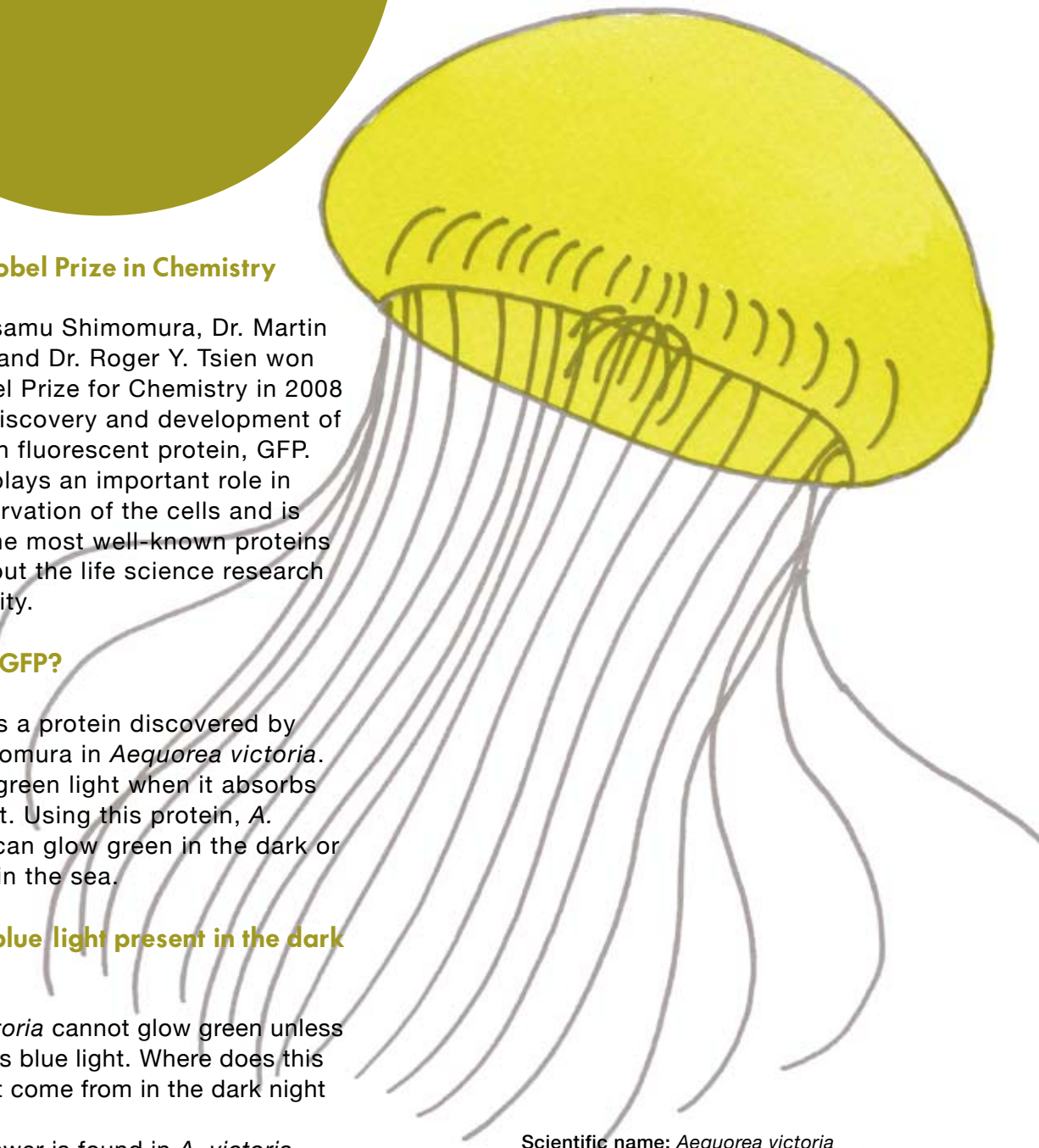
What is GFP?

GFP is a protein discovered by Dr. Shimomura in *Aequorea victoria*. It emits green light when it absorbs blue light. Using this protein, *A. victoria* can glow green in the dark or at night in the sea.

How is blue light present in the dark sea???

A. victoria cannot glow green unless it absorbs blue light. Where does this blue light come from in the dark night at sea?

The answer is found in *A. victoria* itself. A protein called aequorin, which was discovered from *A. victoria*, can emit blue light depending on changes in Ca^{2+} concentration.



Scientific name: *Aequorea victoria* *Aequorea Victoria* is also called the crystal jelly. This species can grow to 20 cm in diameter. These jellyfish are most often seen during the spring and summer periods.

The difference between luminescence and fluorescence

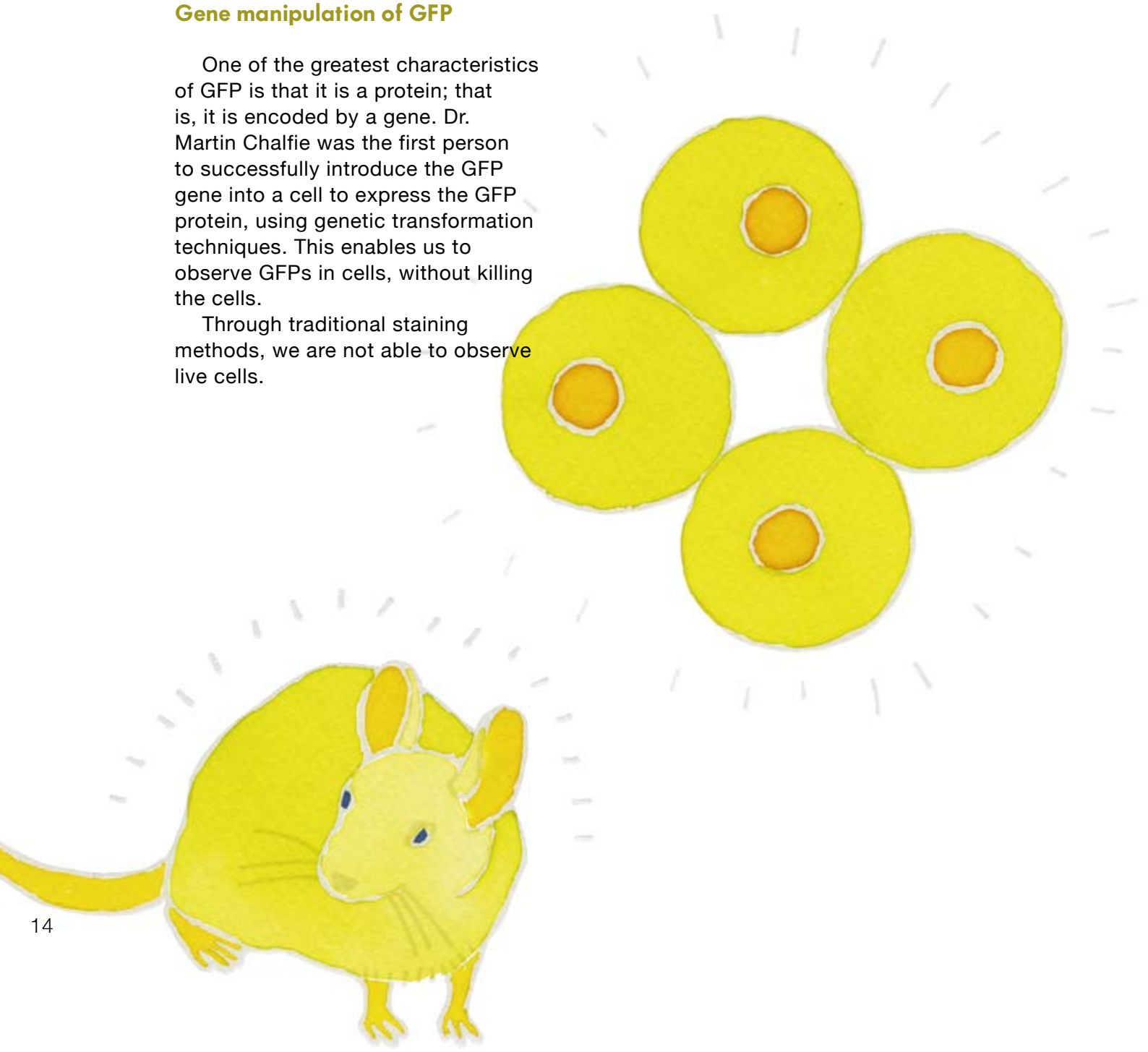
Light-emitting materials like aequorins are known as “luminescent materials”. Luciferin proteins, associated with firefly flashing, are well-known luminescent materials.

In contrast, materials like GFP, which emit a light when they absorb another light, are known as “fluorescent materials”.

Gene manipulation of GFP

One of the greatest characteristics of GFP is that it is a protein; that is, it is encoded by a gene. Dr. Martin Chalfie was the first person to successfully introduce the GFP gene into a cell to express the GFP protein, using genetic transformation techniques. This enables us to observe GFPs in cells, without killing the cells.

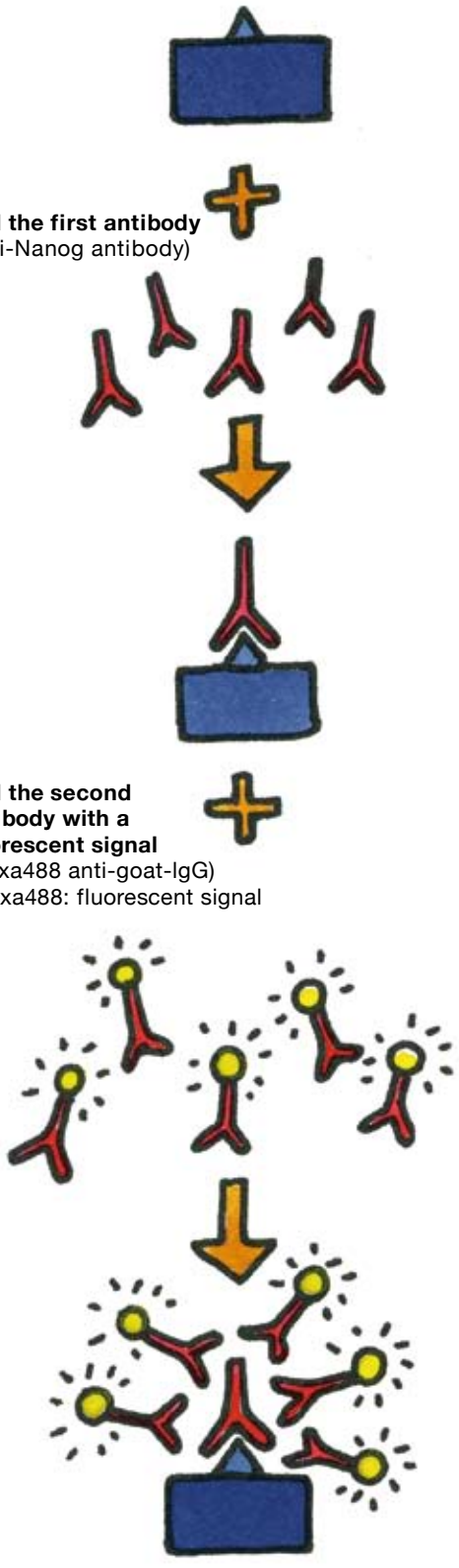
Through traditional staining methods, we are not able to observe live cells.



Target Protein (antigen)
(For example, Nanog protein)

Add the first antibody
(anti-Nanog antibody)

Add the second antibody with a fluorescent signal
(Alexa488 anti-goat-IgG)
*Alexa488: fluorescent signal



Live coverage of a gene turned 'ON'

One of the main applications of GFP is as a 'reporter system'. In this system, GFP reports when and where a gene is turned 'ON'.

The principle is as follows: The GFP gene is inserted into the adjacent region of a gene X which produces the protein X, and both GFP and the protein X are produced when the gene X is turned 'ON'.

As GFP is detectable through the emission of green light, we can indirectly observe protein X.

Other fluorescence methods: Immunofluorescence

Immunofluorescent staining is another method for observing molecules in cells, through the use of luminescent materials. You can detect a protein X by labeling it with a fluorescent material. These cells cannot be observed alive as they must first be fixed. The principle is as follows:

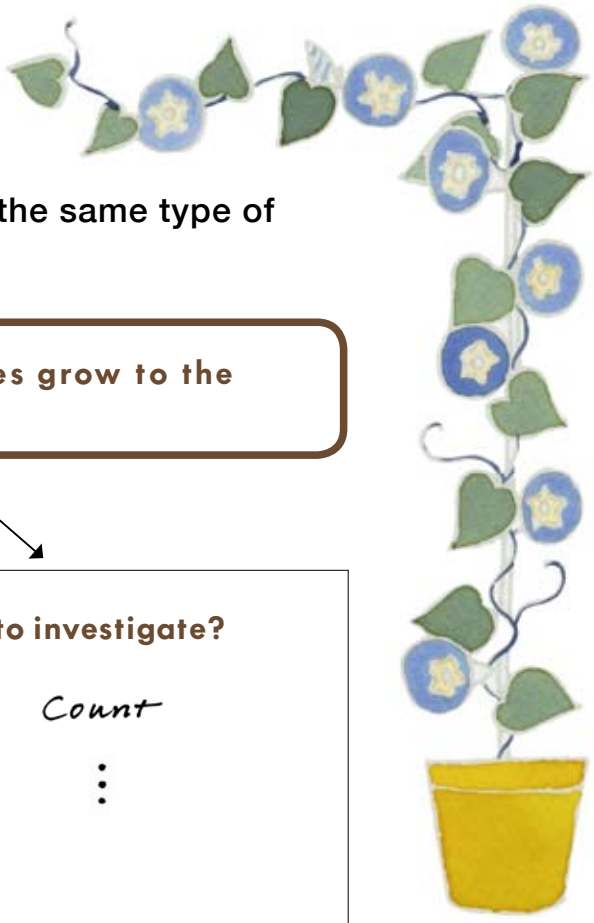
1. Add the first antibody (raised against the protein X) to the cells.
* Antibodies selectively bind to the particular protein.
2. Add the second antibodies (raised against the primary antibody) with a fluorescent signal to the cells.
3. Observe the protein X through a fluorescent microscope.

Scientific activities

Scientific researchers do scientific research every day. It is the process of weaving new knowledge where researchers create a hypothesis about a particular question and then examine that hypothesis through carefully conducted experiments.

In this activity, we would like you to experience the process of creating a hypothesis about a particular question and examining it using some experiments.

First, let's make a hypothesis about the following question:



●Example

Now, you are growing two of the same type of morning glory plants.

Mission! Did both morning glories grow to the same level?

Factors to assume that both morning glories grew to the same level:

1. The number of leaves
2. The number of seeds
3. The number of flowers

⋮

How to investigate?

Count

⋮

List as many as you can. Ask your friends.

Hypothesis

If the numbers of leaves counted are the same, you can assume that both morning glories grew to the same.

Let's TRY!

Now, you are growing two of the same type of pea.

Mission!

Did both pea plants grow to the same level?

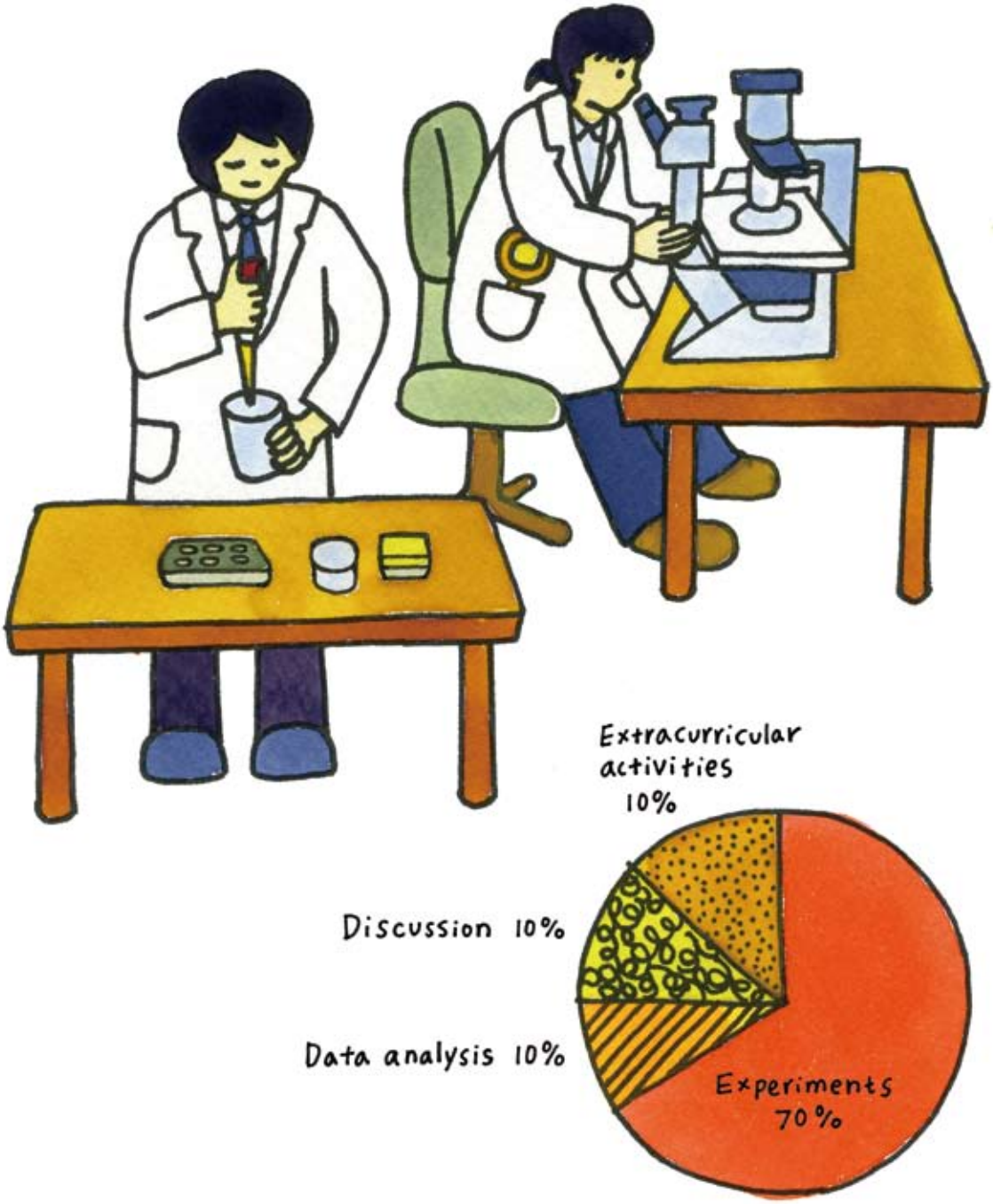
Factors to assume that both pea plants grew to the same level

How to investigate

List as many as you can. Ask your friends.

Hypothesis

The lifestyle of a researcher



Q. How do you spend a usual day?
A. I spend almost all day in the laboratory.

Q. What do you do outside the laboratory?
A. Join academic meetings or boost exchanges with other researchers.

Q. What are the most important things in your research activities?
A. Cooperation, friendship and concentration.

Supplementary material

"A Human Genome Map for Every Home (2nd Edition)", 2008
Editorial supervisor: Ministry of Education, Culture, Sports, Science and technology (MEXT) in Japan
Producers: Kei Kano, Chisato Sakai, Masahiro Kawakami, Kaori Muroi, Kazuto Kato
Downloadable from http://stw.mext.go.jp/20081025/index.html

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Planning Science Communication Group, Institute for Integrated Cell-Material Sciences (iCeMS), Kyoto University: Kei KANO, Eri MIZUMACHI
International Public Relations Office, iCeMS, Kyoto University: Yutaka IIJIMA, Ayumi HAGUSA
International Public Communications Office, Center for iPS Cell Research and Application (CiRA), Kyoto University: Akemi NAKAMURA, Saki TAMURA
Production Science Communication Group, iCeMS, Kyoto University: Kei KANO, Eri MIZUMACHI
Support Norio NAKATSUJI (iCeMS, Kyoto University)
Kohei YAMAMIZU (Institute for Frontier Medical Sciences, Kyoto University)
Koji TANABE (CiRA, Kyoto University)
Kazuhiko NAKAYAMA (Olympus Corporation)
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