The EIBE family

UNIT 18

European Initiative for Biotechnology Education

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The European Initiative for Biotechnology Education (EIBE) seeks to promote skills, enhance understanding and facilitate informed public debate through improved biotechnology education in schools and colleges throughout the European Union (EU).

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The EIBE family

UNIT 18

European Initiative for Biotechnology Education

MATERIALS

Contents

World Wide Web

Few areas are developing as rapidly as biotechnology. So that they can be revised and kept up-to-date then distributed at minimum cost, the EIBE Units are published electronically.

These pages (and the other EIBE Units) are available throughout Europe and the rest of the world on the World Wide Web. They can be found at:

http://www.eibe.reading.ac.uk:8001/

All of the EIBE Units on the World Wide Web are Portable Document Format (PDF) files. This means that the high-quality illustrations, colour, typefaces and layout of these documents will be maintained, whatever computer you have (Macintosh - including Power PC, Windows, DOS or Unix platforms).

PDF files are also smaller than the files from which they were created, so that it will take less time to download documents. However, to view the EIBE Units you will need a suitable copy of the Adobe Acrobat ® Reader programme.

The Acrobat ® Reader 3 programme is available free-of-charge. It can be downloaded from:

http://www.adobe.com/

With this software, you can view or print the EIBE Units. In addition, you will be able to 'navigate' around and search the documents with ease.

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Development

The authors of the unit would like to thank the numerous classes of students and teachers that attended the "Festival International des Sciences ''97' and allowing them to trial and test the activity with them. The authors found it a most challenging and rewarding experience, especially as the sessions were introduced in French and them run in English. With the occasional translation into French to ensure clarity of the idea or procedure.

The authors would also like to extend their thanks to the staff of the museum and organisers of the international festival for their help and goodwill.

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INTRODUCTION

About this Unit

These materials have been devised by practising teachers and educationalists from several European countries, brought together with financial support and encouragement from DGXII of the European Commission, under the auspices of *EIBE, the European Initiative for Biotechnology Education.*

The views expressed in this Unit and the activities suggested herein are those of the authors and not of the European Commission.

Particular attention should be paid to the general safety guidelines given in the introduction to this Unit.

The activities and ideas presented in this unit were exstensively tested by students and teachers from twelve different Luxembourg schools and colleges. They were attending the 'Festival International des Sciences '97'at the Natur Musee in Letzebuerg.

This unit outlines a practical investigation and problem solving activity centred round a fictitious EIBE family. It aims to enliven the teaching of genetics and to integrate theory, technology and some ethical issues in one practical exercise. Topics covered are:

- autosomal and sex linked inheritance;
- the use of restriction enzymes (in practice);
- gel electrophoresis (in practice); and
- genetic screening (a practical simulation).

⚠ Safety

In all of the EIBE Units, we have tried to check that all recognized hazards have been identified and that suitable precautions are suggested.

Where possible, the proposed procedures are in accordance with commonly-adopted general risk assessments. If a special risk assessment may be necessary, this has been indicated.

However, users should be aware that errors and omissions can be made, and that different employers and educational authorities adopt different standards. Therefore, before doing any activity, users should always carry out their own risk assessment. In particular, any local rules issued by employers or educational authorities MUST be obeyed, whatever is suggested in the EIBE Unit.

Unless the context dictates otherwise, it is assumed that:

- practical work is carried out in a properly equipped and maintained science laboratory;
- any mains-operated equipment is properly maintained;
- care is taken with normal laboratory operations such as heating substances;
- good laboratory practice is observed when chemicals or living organisms are used;
- eye protection is worn whenever there is any recognised risk to the eyes;
- pupils and/or students are taught safe techniques for activities such as handling chemicals and microorganisms.

Introduction

The EIBE family provides an inexpensive, practical, laboratory-based simulation of genetic screening. Students are asked to determine the mode of inheritance of a ficticious genetic condition by analysing DNA samples.

It can be used as an introduction to a structured discussion of some of the issues that arise from genetic screening, and to emphasise the importance of this being accompanied by genetic counselling.

Selection of a condition

There are two ways in which the family tree may be used. It can illustrate a true medical condition and its inheritance; such as the single base change (point mutation) that gives rise to sickle cell anaemia. The use of a restriction enzyme to identify the altered DNA has parallels with techniques used to identify of the true condition.

Secondly it can be used to consider a fictitious condition and to explore its inheritance. For example:

	Trait
1	*happy / sad*
2	*hard working / lazy*
3	*generous / miserly*
4	
5	

There are advantages and disadvantages to both approaches. Taking a fictitious condition in this way might be thought of as making light of serious medical issues and might lead students to assume that many traits are inherited in a Mendelian way (i.e. ignoring the idea of multifactorial inheritance).

It should be remembered that this activity is intended as part of a teaching programme about genetics and/or DNA technology and aims to introduce issues which can then be explored in further discussion.

The EIBE family tree

The family tree on page 9 shows the inheritance of an autosomal dominant condition. There are a number of teaching points to be made:

The mother (*Caroline*) is homozygous for the dominant allele while the father (*Paul*) is homozygous for the recessive allele. Students will therefore predict that the children will be heterozygotes.

Gerard and *Cecily*, being heterozygotes, will produce gametes carrying either dominant or recessive alleles (in a 50:50 ratio). The children from *Gerard* and *Cecily* show only two of the three possible genotypes for this locus. This should lead to a discussion about probability and reality (avoiding possible misconceptions that every fourth child must be of a particular genotype if the previous three were of another type).

There is a deliberate problem posed by the analysis of the gels. *Uncle John* and *Aunty Stephania* have two children, one of whom turns out not to follow the expected pedigree. As *Aunty Stephania* is heterozygous and *Uncle John* is homozygous for the dominant allele, the second child presents no problems; however, the first is unexpectedly found to be homozygous for the recessive allele. Questioning the students usually yields a number of possible explanations:

- the child may be adopted and therefore the parents are not his biological parents;
- there was a mix up in the samples and the tests should be repeated;
- there was an unexpected mutation (after some thought the students may realise that this must have occurred during the formation of *Uncle John's* sperm);

- finally, no doubt, some students will come up with the suggestion of an extra marital affair between *Aunty Stephania* and an unknown man!

The EIBE family tree to show sex linkage

The family tree on page 10 illutrates the inheritance of a sex linked recessive condition.

It has been designed to ask the question *'Is the inheritance of the condition a normal recessive inheritance pattern or is it sex linked ?'* Students have to examine the gel electrophoresis results and the family tree in order to make deductions about the mode of inheritance.

The story (to present to the students)

A particular gene is being investigated. Samples have been taken from the members of a large family and from these DNA has been extracted and amplified using the polymerase chain reaction (PCR). For the single locus being investigated there are two different genes (i.e. types of DNA) possible.

A family members who is homozygous for the normal allele (genotype *NN*) will only have DNA of type N. A family member who is homozygous for the allele giving rise to the condition (genotype *nn*) will only have DNA of type n. Heterozygous individuals (genotype *Nn)* will have DNA of both types. Amplification of the DNA region of interest using PCR gives fragments of the same size for both alleles, in this case 6,500 base pairs. (*See EIBE Units 2 and 12 for more details of PCR*.)

The 'samples' contain the amplified DNA. The task is to detect which forms of DNA are present in each sample by treating the DNA with a restriction enzyme (*Bam*H1) and examining the resulting DNA fragments by means of gel electrophoresis.

The difference in the DNA sequence of the alleles *N* and *n* is such that, in the allele *n* there is a base sequence that can be recognised and cut by the restriction enzyme *Bam*H1(*see OHP 14, page 30*). The allele *N* has no restriction site for *Bam*H1 and thus will not be cut by the enzyme.

Individuals who are homozygous for the normal allele (*NN*) only have DNA of the type N which is not affected by treatment with *Bam*H1. Gel electrophoresis separates DNA fragments by size, so in these samples all the amplified fragments of DNA will move together to produce a single band on the gel (*see Fig 1, samples 2 and 5*).

Individuals who are homozygous for the affected allele (*nn*) only have DNA of the type n, so all the DNA will be cut by the restriction enzyme. The action of *Bam*H1 results in two smaller fragments, of different sizes, which will produce two separate bands on the gel after electrophoresis (*see Fig 1, samples 4 and 9*).

Inherited conditions

Single factor (gene)
- Clear path of inheritance
- High chance of inheritance
- Rare in the population
- Many known examples (single gene traits)

Multifactorial
- No clear path of inheritance
- Lower chance of transmission
- More widespread in the population

Examples

Automsomal dominant
- *Huntington's disease*

Automsomal recessive
- *Sickle cell disease*
- *Tay-Sachs disease*
- *Thalassaemia*
- *Cystic fibrosis*

X linked recessive
- *Duchenne muscular dystrophy*
- *Haemophilia A, B*

Y linked
- *Webbed toes*

Heterozygous individuals (*Nn*) will have DNA of both types. Half the DNA fragments (type N) will not be cut by the restriction enzyme and half the DNA fragments (type n) will be cut into two smaller fragments by the action of *Bam*H1. Samples from the heterozygous individual will thus, after incubation with *Bam*H1, contain DNA fragments of three different sizes and will produce three bands on the electrophoresis gel (*see Fig. 1, samples 1 and 3*).

If some DNA fragments of known sizes (a 'DNA ladder') are run alongside the samples it can be demonstrated that the two small fragments would combine to the size of the large piece (4,000 + 2,500 = 6.500). *(For a more detailed explanation of how gel electrophoresis works see EIBE Unit 1.)*

The truth (for the teacher)

The following bacterial plasmids are used:

- pUC19, produced for research by University College (London);
- pEMBL, produced for research by the European Molecular Biology Laboratory (Heidellberg);
- pEIBE, produced for the NCBE by Mark Stevens, AMS, The University of Reading.

The three plasmids are used to create 'samples' that will give one, two or three bands on gel electrophoresis.

Plasmids are circular pieces of DNA, but a plasmid preparation may contain the plasmid in a number of different states; supercoiled, nicked and thus not supercoiled or linear. The different forms would run in an agarose gel at different rates giving multiple bands for a single plasmid. During this simulation each plasmid is treated with a restriction enzyme first. The plasmids chosen have only a single site for *Bam*H1, so that treatment with this restriction enzyme will cut the circular DNA to form a linear piece of DNA that will give a single band after electrophoresis.

Reference

ABC of Clinical Genetics by Helen M. Kingston, BMJ Publishing Group, revised second edition 1997, ISBN 0-7279-1101-5.

Fig. 1 The expected pattern of DNA bands on the stained gels (autosomal inheritance)

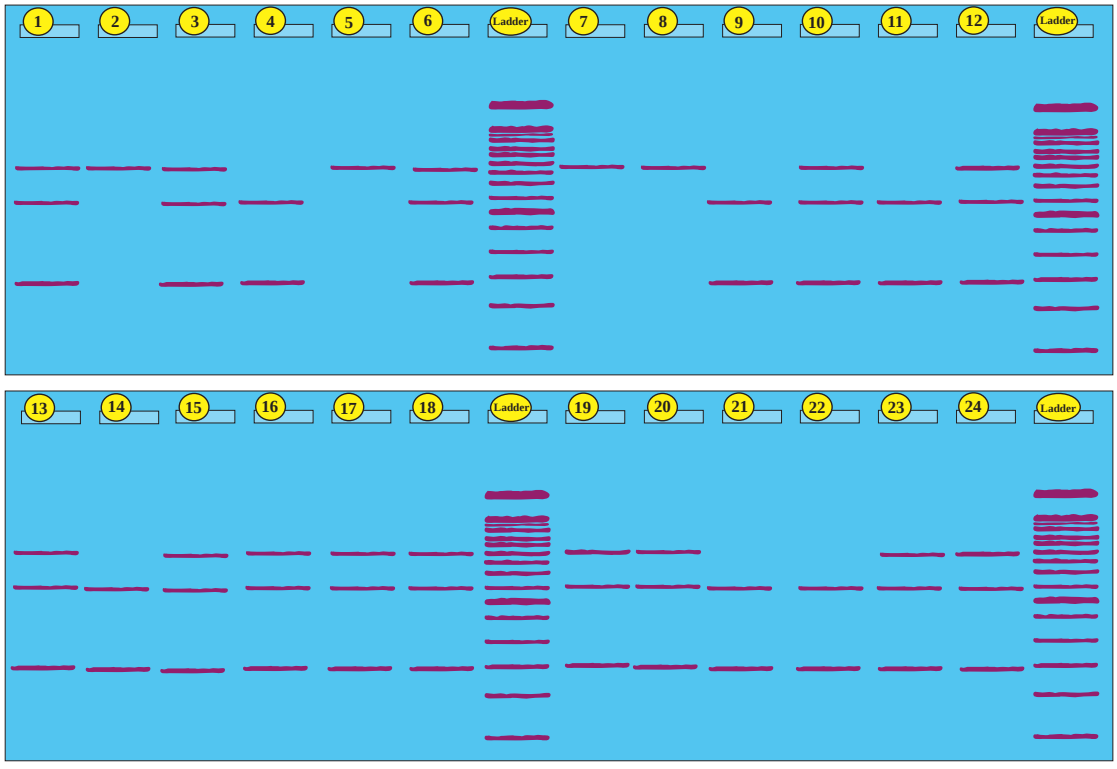


Fig.2 *The EIBE family tree*

(showing DNA bands illustrating an autosomal inheritance pattern)

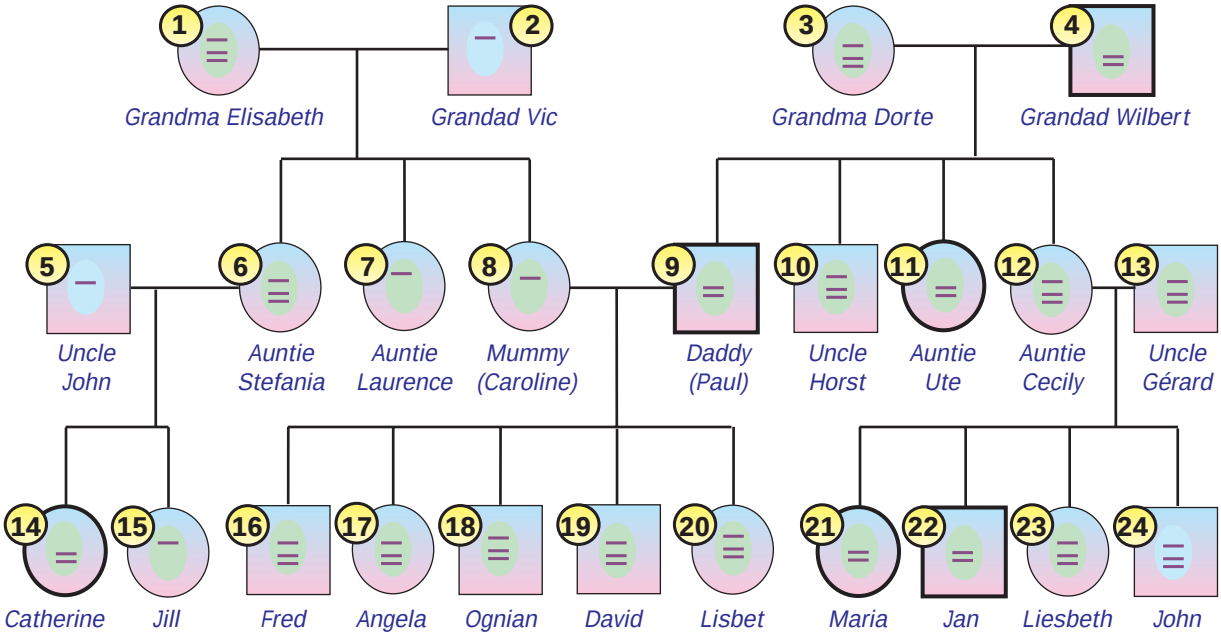


Fig.3 *The extended EIBE family tree*

(showing DNA bands illustrating an autosomal inheritance pattern)

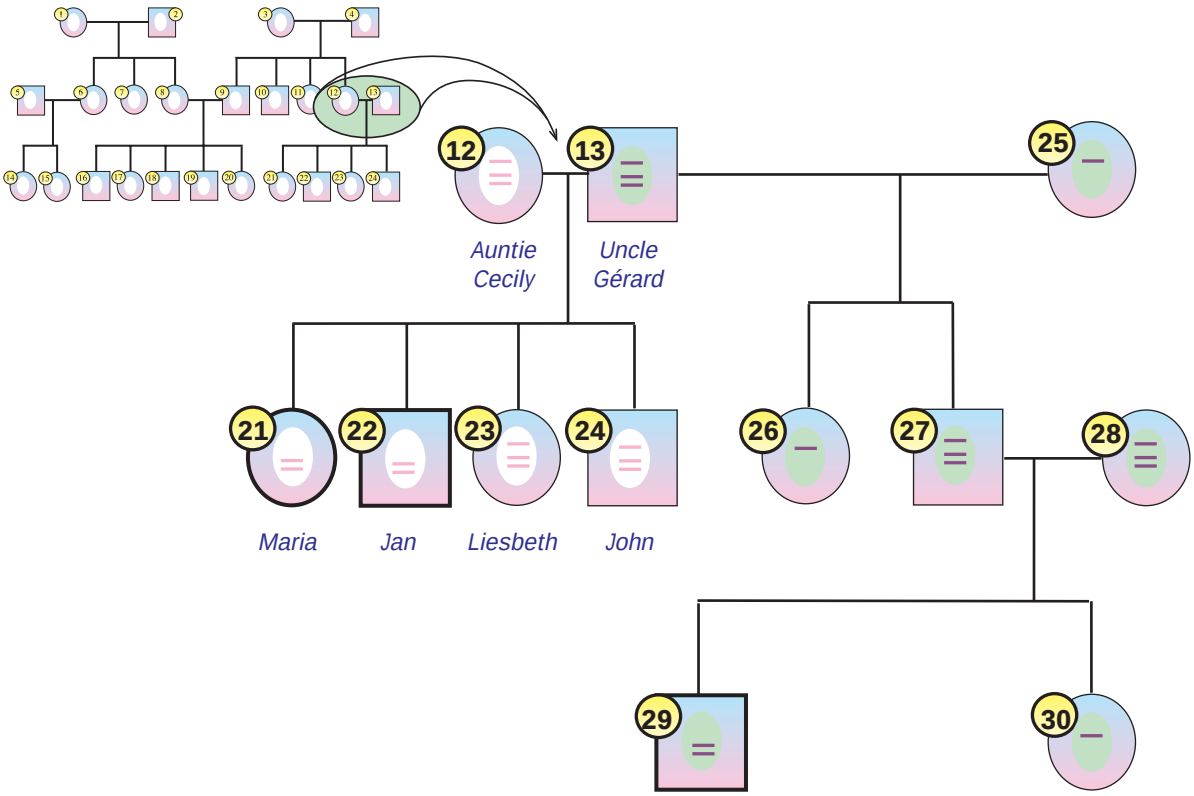


Fig. 4 The EIBE family tree

(showing DNA bands illustrating a sex linked inheritance pattern)

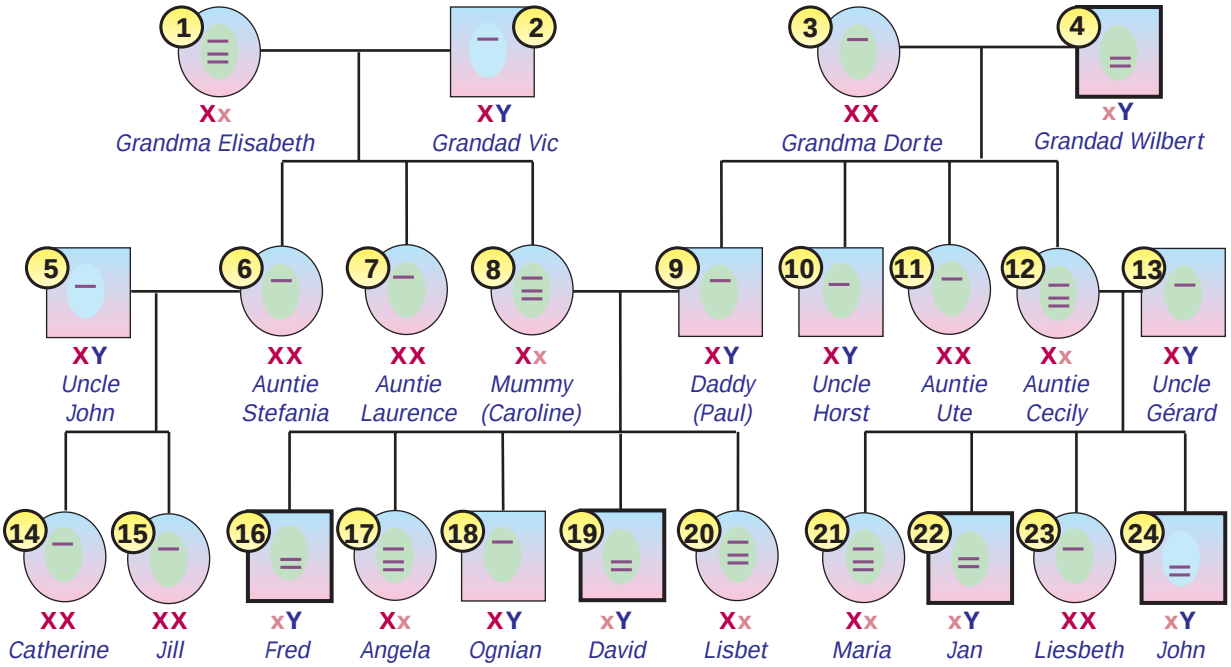
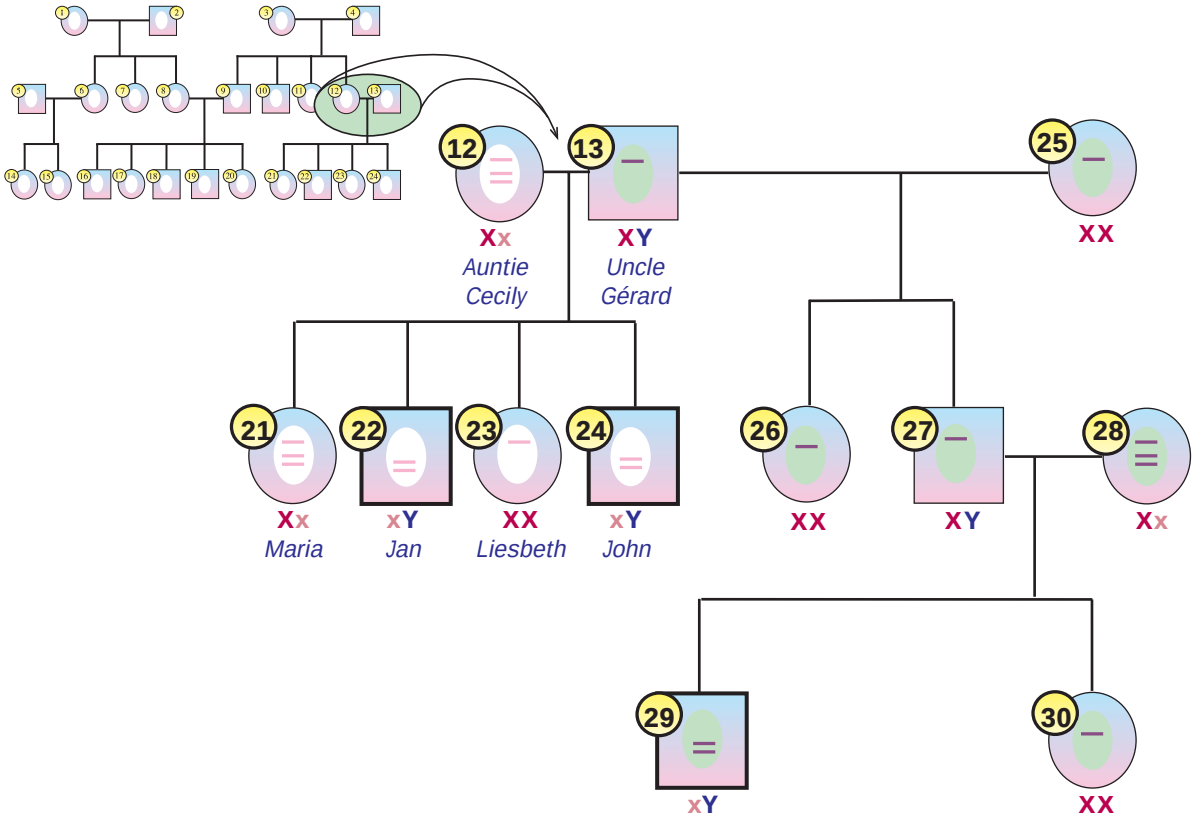


Fig. 5 The extended EIBE family tree

(showing DNA bands illustrating a sex linked inheritance pattern)



PLANNING

The Activity

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Materials

This activity requires equipment, materials and a good protocol for restriction enzyme digests of DNA samples and subsequent separation and visualisation of the DNA fragments using gel electrophoresis. A kit, designed for use in schools, providing such equipment and materials is available from:

NCBE
The University of Reading
Whiteknights
POBox 228
Reading RG6 6AJ

For further details see:

www.rdg.ac.uk/NCBE

Three plasmids are also needed for the DNA 'samples':

pUC18 or pUC19

(a double stranded plasmid of 2,600 base pairs)

This plasmid can be obtained commercially from biological supply companies.

pEMBL

(a double stranded plasmid of 4,000 base pairs)

The plasmid is one that was produced by the European Molecular Biology Laboratory in Munich.

pEIBE

(a double stranded plasmid of 6,500 base pairs)

This particular plasmid can be obtained from the NCBE at the University of Reading.

Organisation

The time needed for this activity depends on the type of electrophoresis apparatus available. It could be completed in three hours plus a half hour break while the gels are running (this would need tanks that could be run at 90 volts). Alternatively the activity can also be performed over a number of sessions that are spread over several days, in which case low voltage apparatus designed for the classroom can used.

Session 1 *40/50mins*

Digestion of the DNA with restriction enzyme *Bam*HI.

Students place the DNA samples into the enzyme tubes and mix the two together. The tubes must immediately be incubated for half an hour. If time allows, loading dye can be added to the samples immediately after incubation (alternatively, it can be added just prior to loading). Incubation must follow the mixing, but the digested samples may be stored in a fridge (5 °C) for a few days.

Session 2 *40/50mins*

Preparation of the agarose gels for DNA separation.

Providing the agarose solution has been prepared and is held melted in a water bath at 50 °C, students can pour the gels and use them in a single session. Gels (properly covered) may be stored in a fridge (5 °C) for several days.

The gel tank should be labelled: both with the group name and with the sample numbers.

Session 3 *40/50mins*

Loading, running and staining the agarose gel.

The time taken to run the gel is determined by the voltage and apparatus used. As soon as the run is finished, the gel should be stained or the DNA will start to diffuse out of the gel into the buffer.

After staining, the gels may be kept in the fridge (or a cold, dark place) for up to a year. They need to be sealed in a small plastic bag to prevent them drying out.

Session 4 *40/50mins*

Interpretation of the gel.

The last session is needed for the students to interpret the results, work out the genotypes of the individual members of the EIBE family and understand the pattern of inheritance.

ADVANCE PREPARATION

Preparation of the DNA 'samples'

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The plasmid solutions

For the equipment and protocols supplied by the NCBE (see above), where 9 mm wide wells are used and the DNA is stained with Azure A, 0.3 µg of DNA is required for each visible band - in a loading volume of 20 µl per well.

Plasmid solutions are normally provided at a concentration of 1 µg/µl, each needs to be diluted to a working concentration of 0.06 µg/µl (*see Table 1*). 5 µl of these solutions contain 0.3 µg of DNA.

These working strength solutions are used to prepare three different plasmid mixes that will be used for the EIBE family DNA samples. Tables 2 and 3 outline the quantities of each mix that are needed for the two practical options. The DNA samples will contain 20 µl of one mix, so the amount of DNA in each sample will be 0.3 µg, 0.6 µg, or 0.9 µg, depending on whether they contain mix A (1 plasmid), mix B (2 plasmids) or mix C (3 plasmids).

Table 1. Preparation of plasmid solutions

plasmid (1 µg/µl)	water	final volume (0.06 µg/µl)
3 µl	47 µl	50 µl
6 µl	94 µl	100 µl
9 µl	141 µl	150 µl
15 µl	235 µl	250 µl

The 'sample' tubes

Prepare tubes 1- 24 (or 30) of the EIBE family EITHER to show autosomal inheritance OR to show a sex linked inheritance by adding 22 µl of plasmid mix A, B or C to each tube according to figures 6 and 7 (also summarised below). Allowing 22 µl per tube makes it possible for students to take an accurate 20 µl sample for analysis.

For autosomal inheritance:
Use mix A for tubes: 2, 5, 7, 8, (25, 26, 30).
Use mix B for tubes: 4, 9, 11, 14, 21, 22, (29).
Use mix C for tubes: 1, 3, 6, 10, 12, 13, 15, 16, 17, 18, 19, 20, 23, 24, (27, 28).

For sex linked inheritance:
Use mix A for tubes: 2, 3, 5, 6, 7, 9, 10, 11, 13, 14, 15, 18, 23, (25, 26, 27, 30).
Use mix B for tubes: 4, 16, 19, 22, 24, (29).
Use mix C for tubes: 1, 8, 12, 17, 20, 21, (28).

Table 2. Preparation of plasmid mixes to show autosomal inheritance in the EIBE family

	Mix **A**	Mix **B**	Mix **C**	volume of plasmid needed
pUC19	-	40 µl	90 µl	130 µl
pEMBL	-	40 µl	90 µl	130 µl
pEIBE	40 µl	-	90 µl	130 µl
water	120 µl	80 µl	90 µl	
Total volume	**160** µl	**160** µl	**360** µl	
No. of 22 µl samples needed	4 (7)*	6 (7)*	14 (16)*	*(optional extension)

Table 3. Preparation of plasmid mixes to show sex linked inheritance in the EIBE family

	Mix **A**	Mix **B**	Mix **C**	volume of plasmid needed
pUC19	-	35 µl	40 µl	75 µl
pEMBL	-	35 µl	40 µl	75 µl
pEIBE	100 µl	-	40 µl	130 µl
water	300 µl	70 µl	40 µl	
Total volume	**400** µl	**140** µl	**160** µl	
No. of 22 µl samples needed	13 (17)*	5 (6)*	6 (7)*	*(optional extension)

Fig. 6 Preparation of 'sample' tubes to show autosomal inheritance in the EIBE family

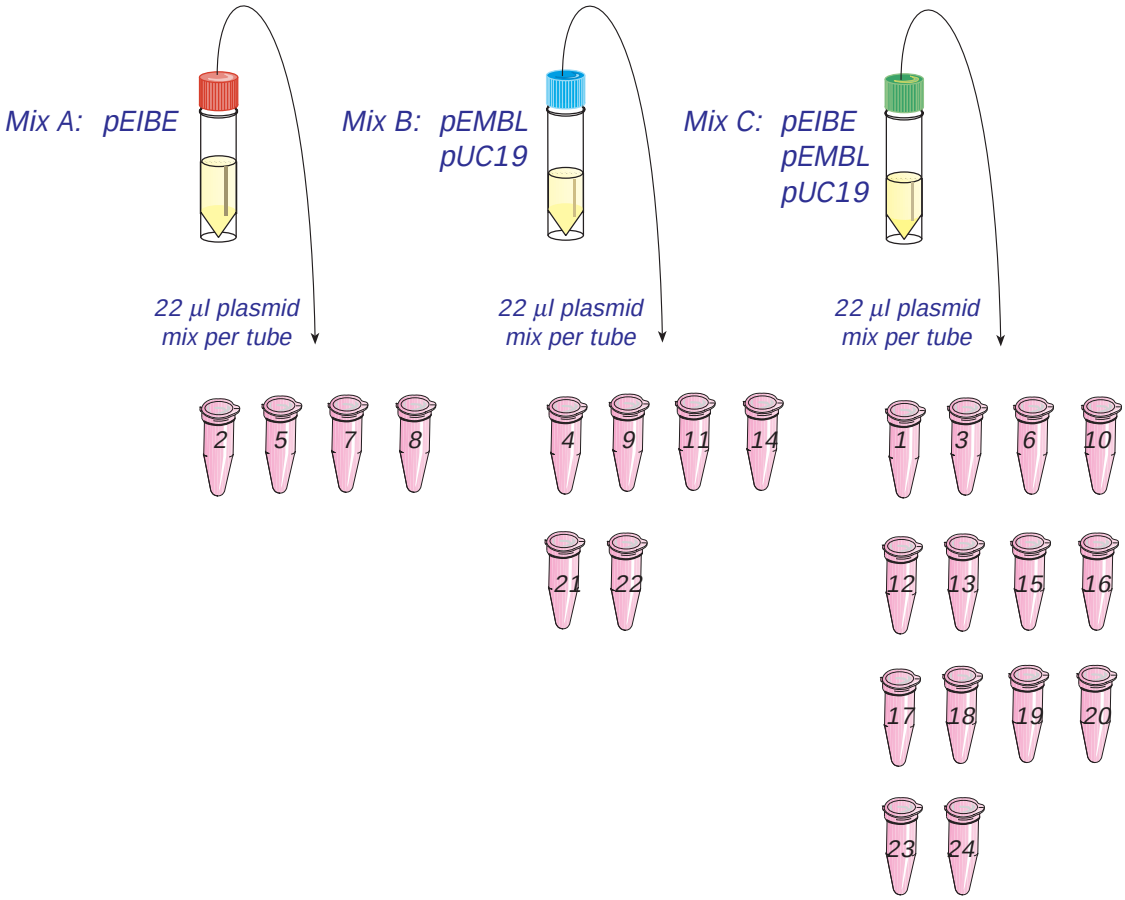
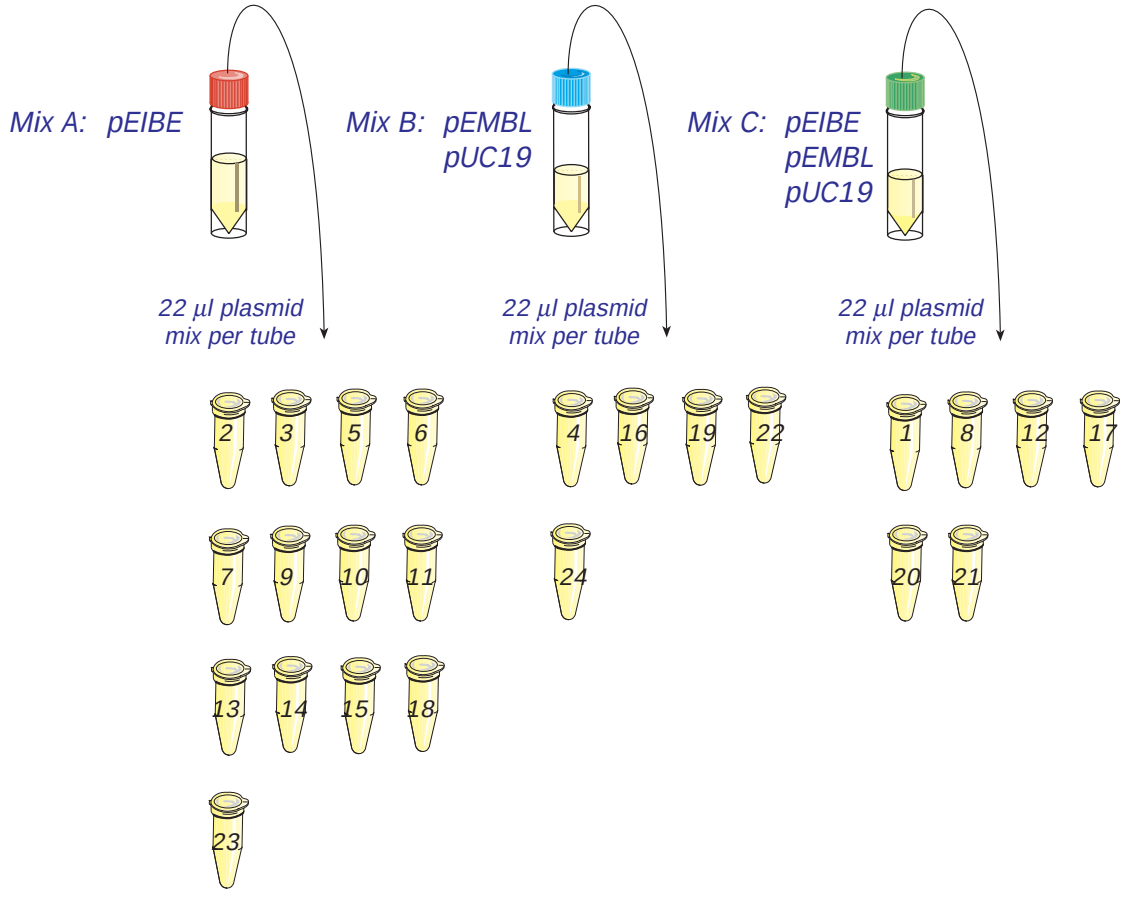


Fig. 7 Preparation of 'sample' tubes to show sex linked inheritance in the EIBE family



STUDENT INFORMATION

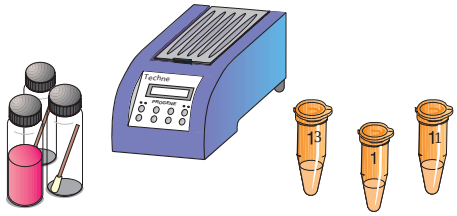
DNA Analysis of family samples

The polymerase chain reaction (PCR) is used to amplify a target sequence of DNA from a very small sample such as a few buccal cells or even a single foetal cell (in pre-implantation diagnosis). PCR is used to screen for the four most common alleles of the Cystic fibrosis gene and can be used to identify 85% of all carriers.

Cystic fibrosis is one of several genetic diseases known to be caused by base substitution in a gene. If the site of the mutation is the recognition site for a particular restriction enzyme, it can be detected by analysing the size of the DNA fragments present after treatment with that restriction enzyme.

In the EIBE family activity you are given samples to analyse for the presence of two possible alleles of a gene, here is an outline of the task.

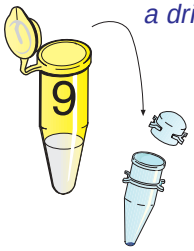
1



Samples have been taken from each member of the EIBE family. DNA was extracted and amplified using PCR. The tubes respresent the amplified DNA extracts from these samples.

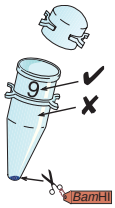
2

20 µl of the DNA sample is transferred to a tube containing a dried restriction enzyme...

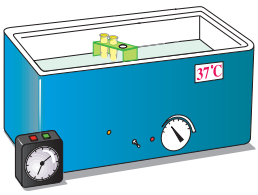


3

...the tube is labelled.

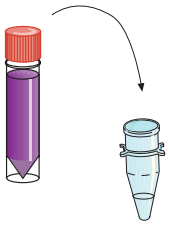


4



The DNA solution and the dried enzyme are mixed thoroughly and then incubated in a water bath at 37 °C for at least 30 minutes.

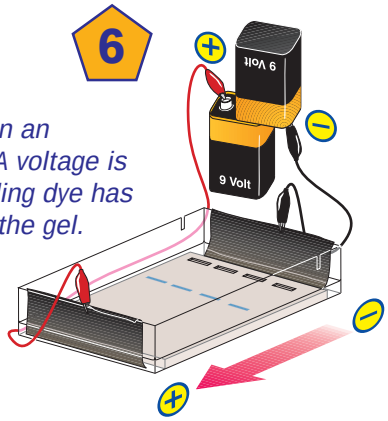
5



2 µl of loading dye is added to each sample, mixed thoroughly and...

6

...loaded into a well in an electrophoresis gel. A voltage is applied until the loading dye has moved to the end of the gel.



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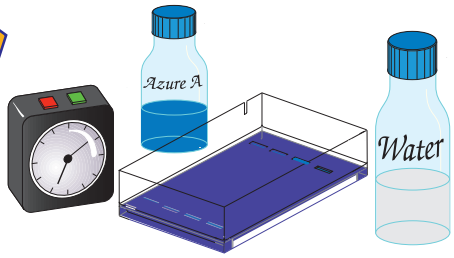
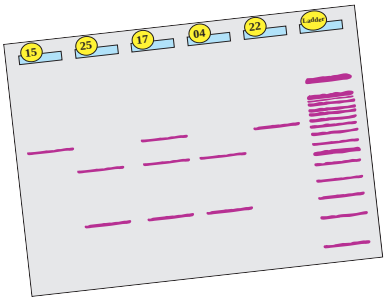


The gels are stained with Azure A to visualise the DNA and...

8

...a record is made of the number and positions of the DNA bands.



9

Interpretation of the pattern of DNA bands found in each sample can indicate the alleles carried by that individual. All the results may then be reviewed taking into consideration the known family relationships (see the EIBE family tree).

OHP list

There follows a collection of over head projector sheets that can be used to help introduce and present the activity .

Some clinical genetics:

DNA analysis of a locus

OHP 18
page 34

DNA fragment sizes

A point mutation creating a new restriction site can lead to the possibility of three different sized fragments of DNA.

OHP 19
page 35

DNA gel bands and genotype

Three possible genotypes and their band patterns after analysis.

OHP 20
page 36

DNA ladder

The band pattern of a typical 1 kb reference ladder

OHP 21
page 37

DNA bands in a gel

The use of a DNA ladder to estimate sizes of DNA fragments on a gel. Bands of 6,500 base pairs, 4,000 base pair and 2,500 base pairs are shown.

Introducing the EIBE Family Activity

OHP 22
page 38

The EIBE family

A family tree for three generations of a hypothetical family.

OHP 23
page 39

The extended EIBE family

An extended family tree (in case more samples are needed for a large class).

OHP 24
page 40

Genotype and phenotype

A reminder that the phenotype of two individual may be the same but the genotype can be different.

OHP 2
page 41

DNA analysis to detect genotype

DNA fragments in a gel after DNA analysis can be used to identify the alleles at a particular locus.

Practical details:

OHP 26
page 42

Enzyme treatment

20µl of the DNA sample (in a microcentrifuge tube) should be transferred to a tube containing the dried (blue stained) restriction enzyme *Bam*H1.

OHP 27
page 43

Labelling

The tube must be labelled at the top where the plastic is frosted, ink easily rubs off the shiny part of the tube.

OHP 28
page 44

Results

The expected pattern of gel bands for samples 1 to 24. Four 1 kb ladders are shown for reference.

Sickle cell disease

OHP 29
page 45

Normal β globin allele

DNA coding for β globin, with three sites for the restriction enzyme *Mst*II.

OHP 30
page 46

The Sickle cell allele

A single base change results in the 'Sickle cell' allele, this has only two sites for the restriction enzyme *Mst*II.

Inherited conditions (diseases)

Single factor (gene)

Clear path of inheritance
High chance of inheritance
Rare in the population
Many known examples (single gene traits)

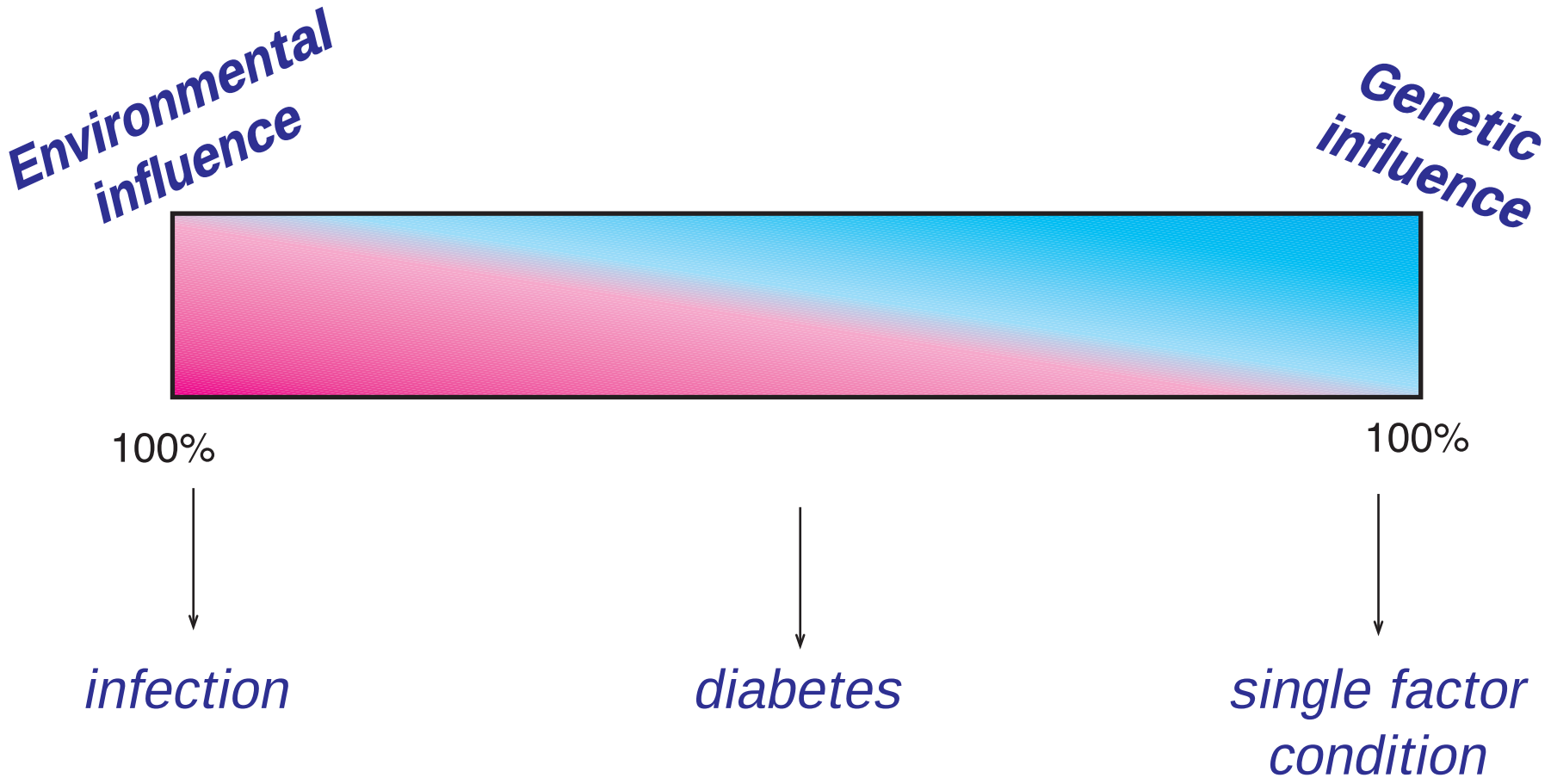
Multifactorial

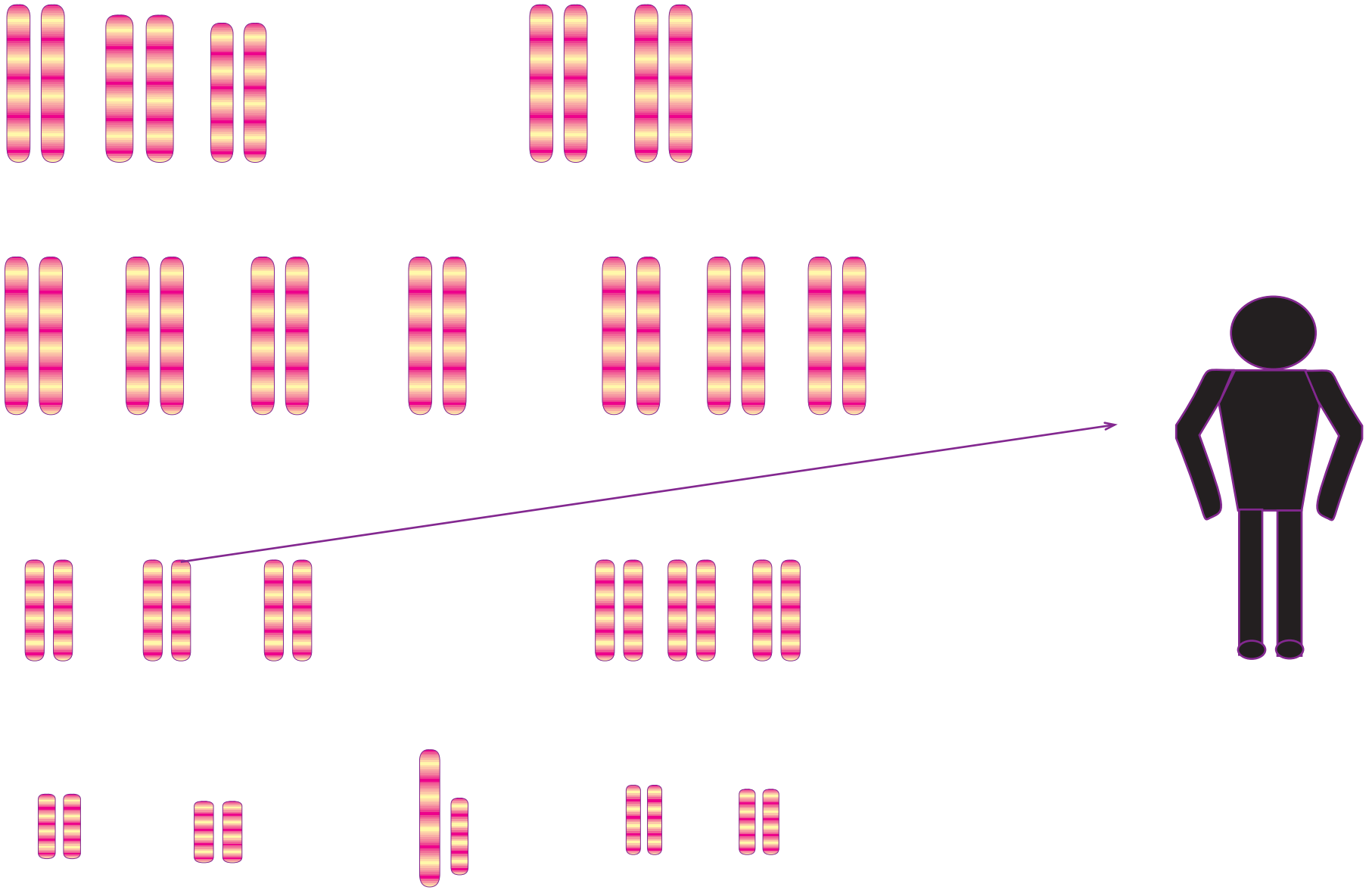
No clear path of inheritance
Lower chance of transmission
More widespread in the population

Single factor conditions (single gene)

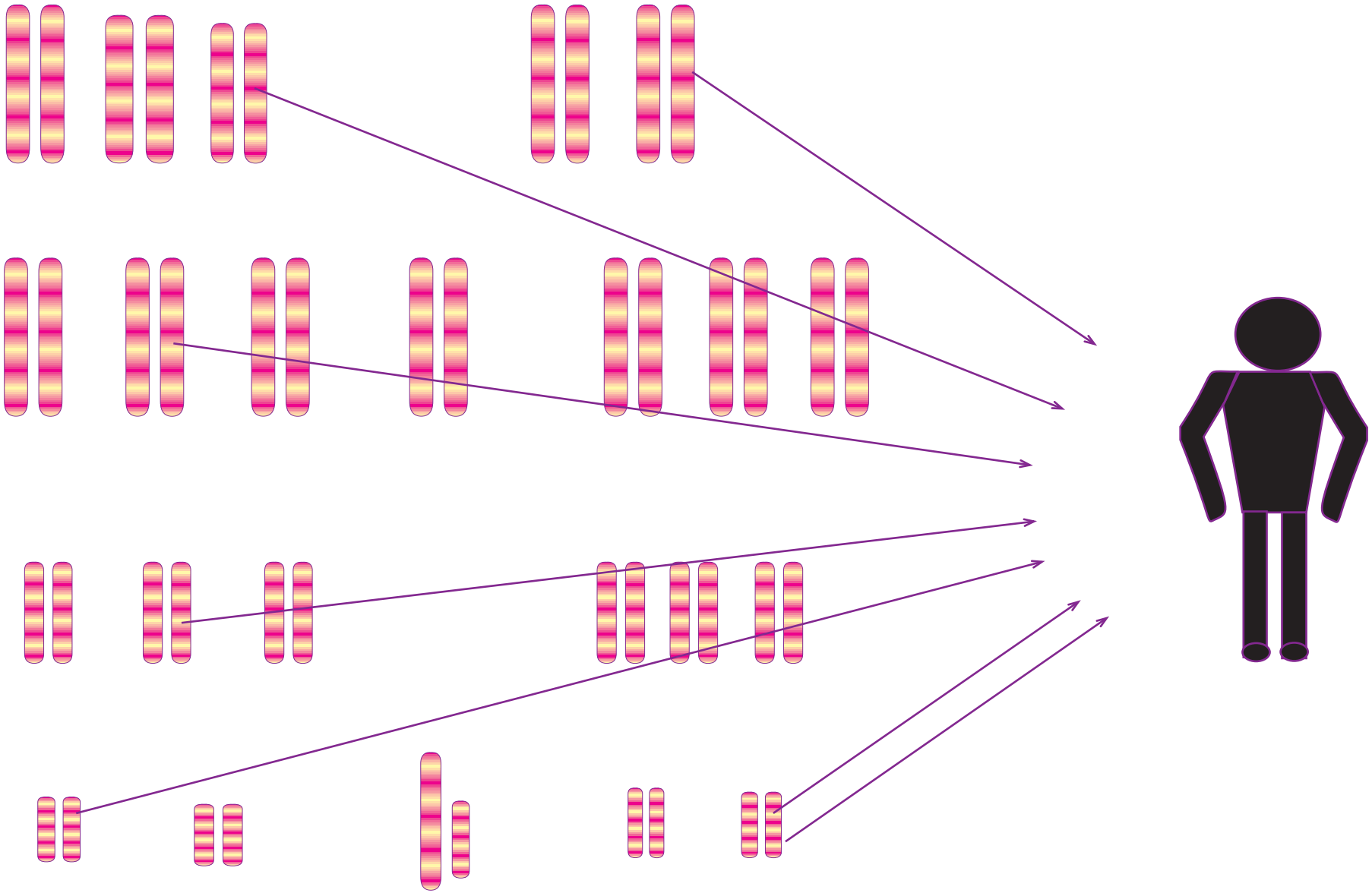
Autosomal dominant	*Huntington's disease*
Autosomal recessive	*Sickle cell disease* *Tay-Sachs disease* *Thalassaemia* *Cystic fibrosis*
X linked recessive	*Duchenne muscular dystrophy* *Haemophilia A, B*
Y linked	*Webbed toes*

Environment and/or genes

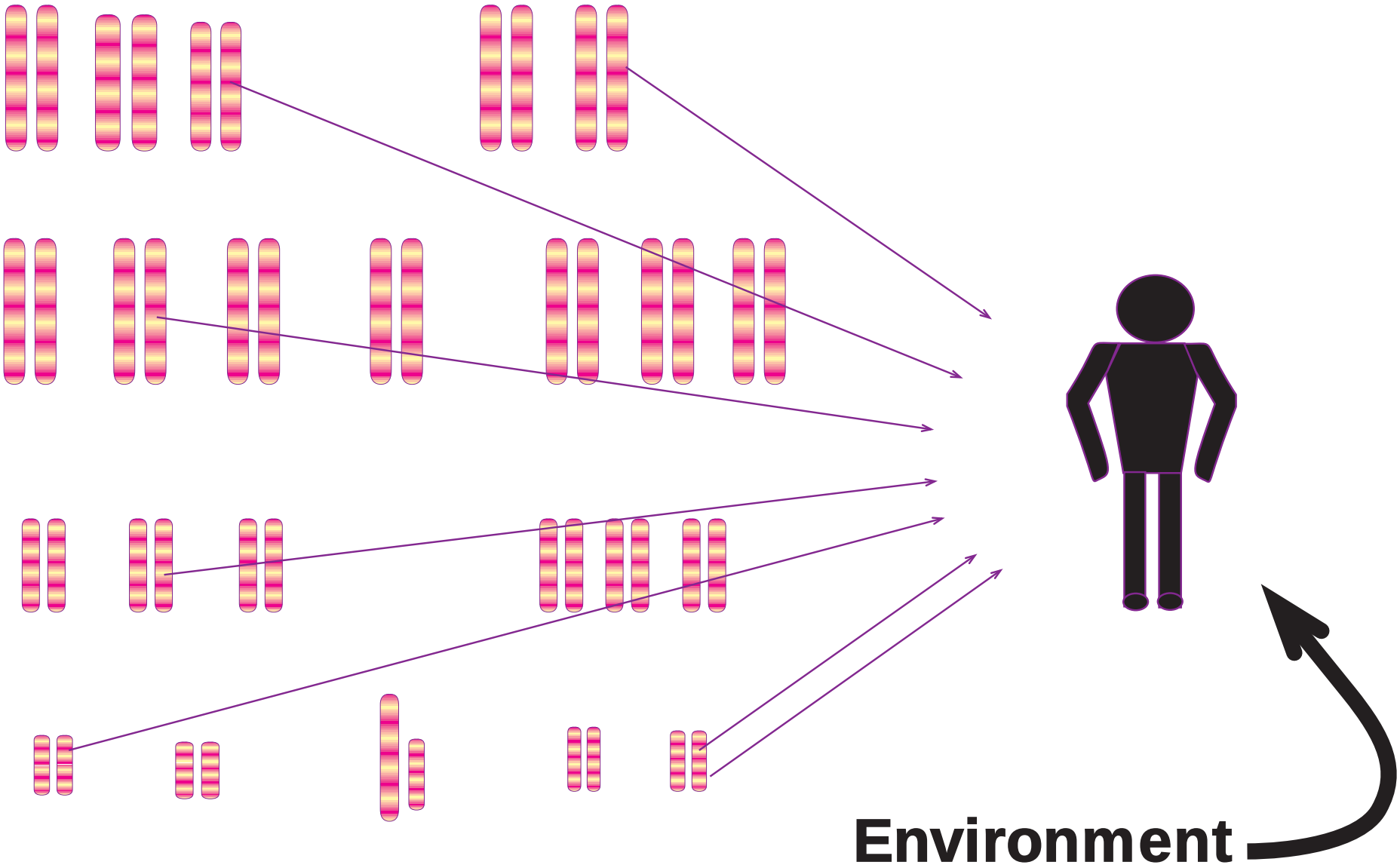




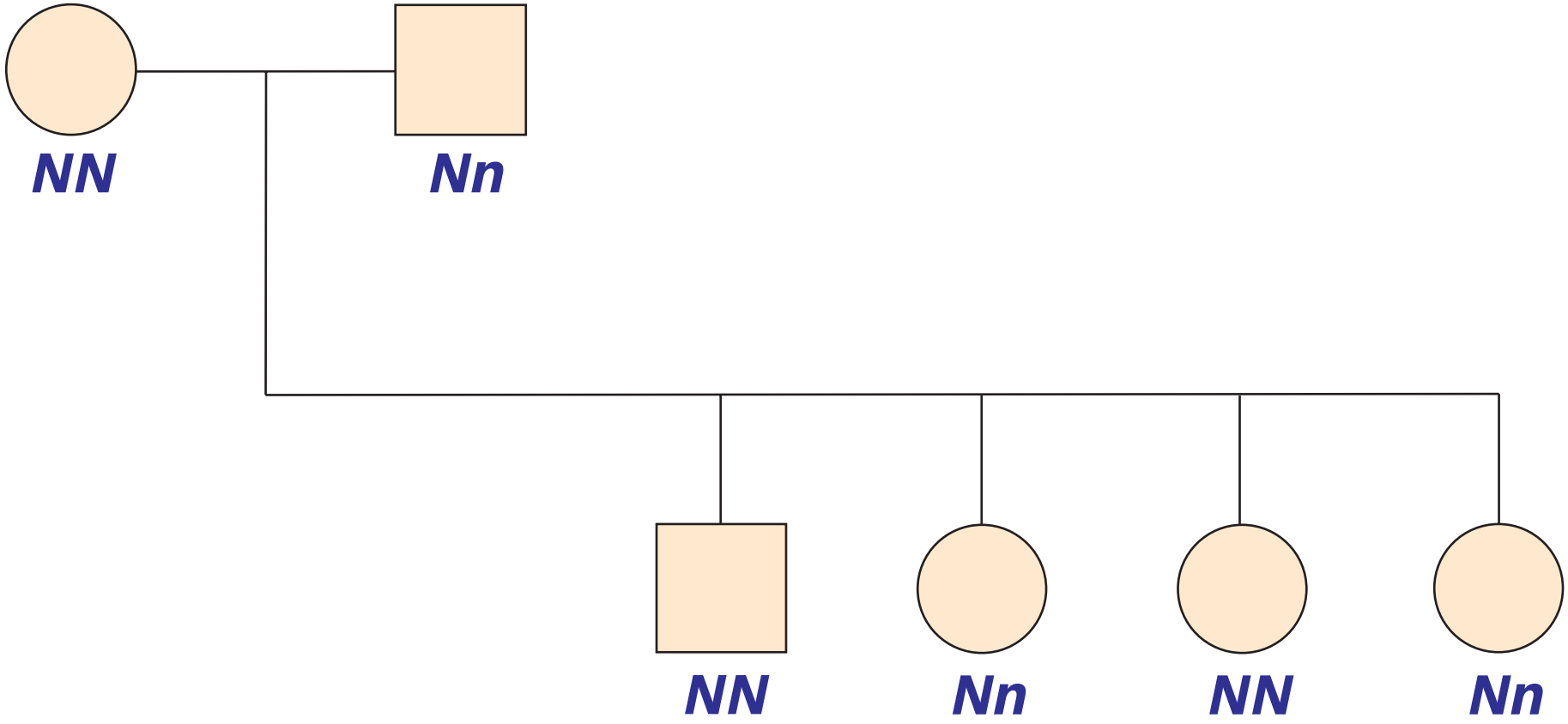
OHP 4



OHP 5



OHP 6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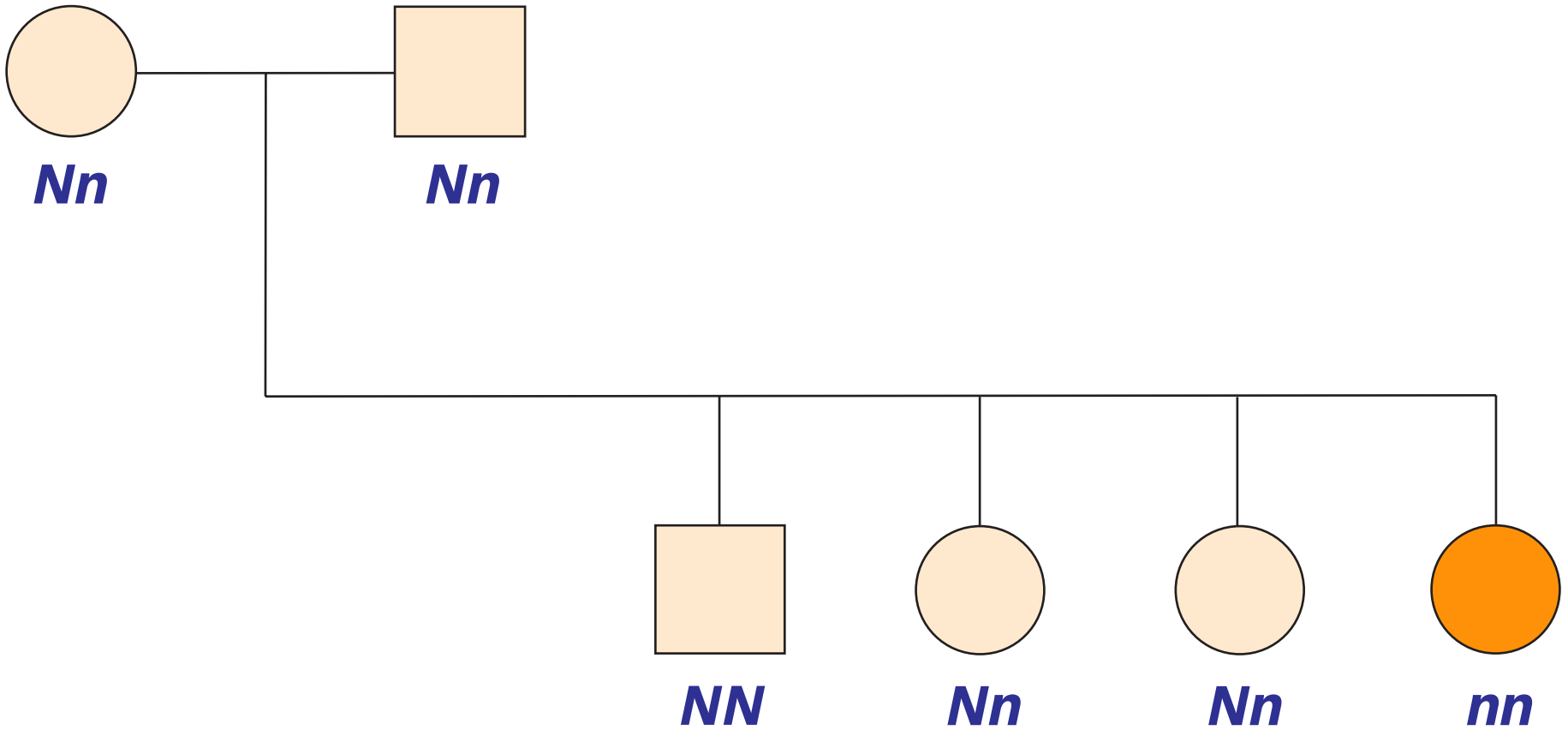
Nn
NN
Nn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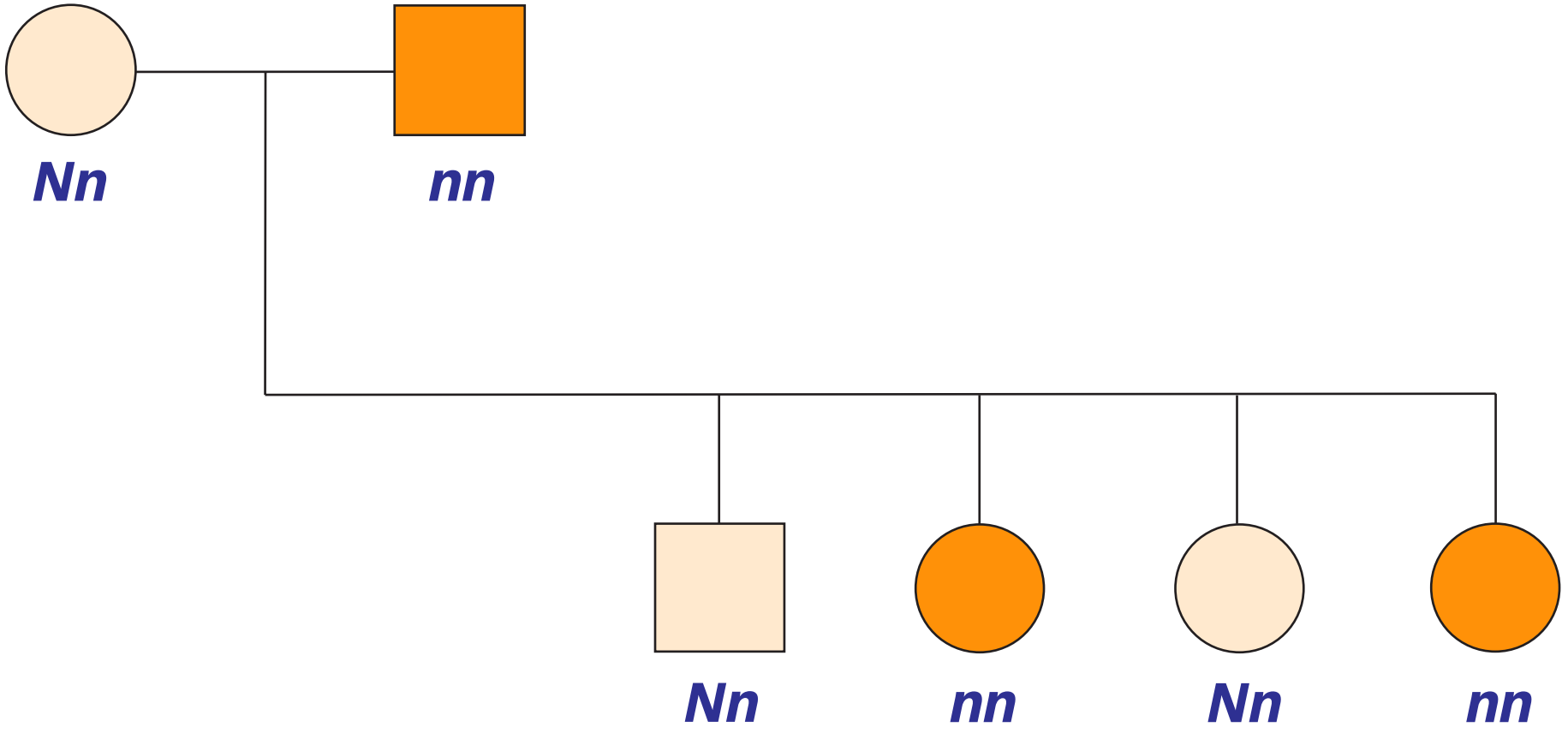
Nn
NN
Nn
Nn
nn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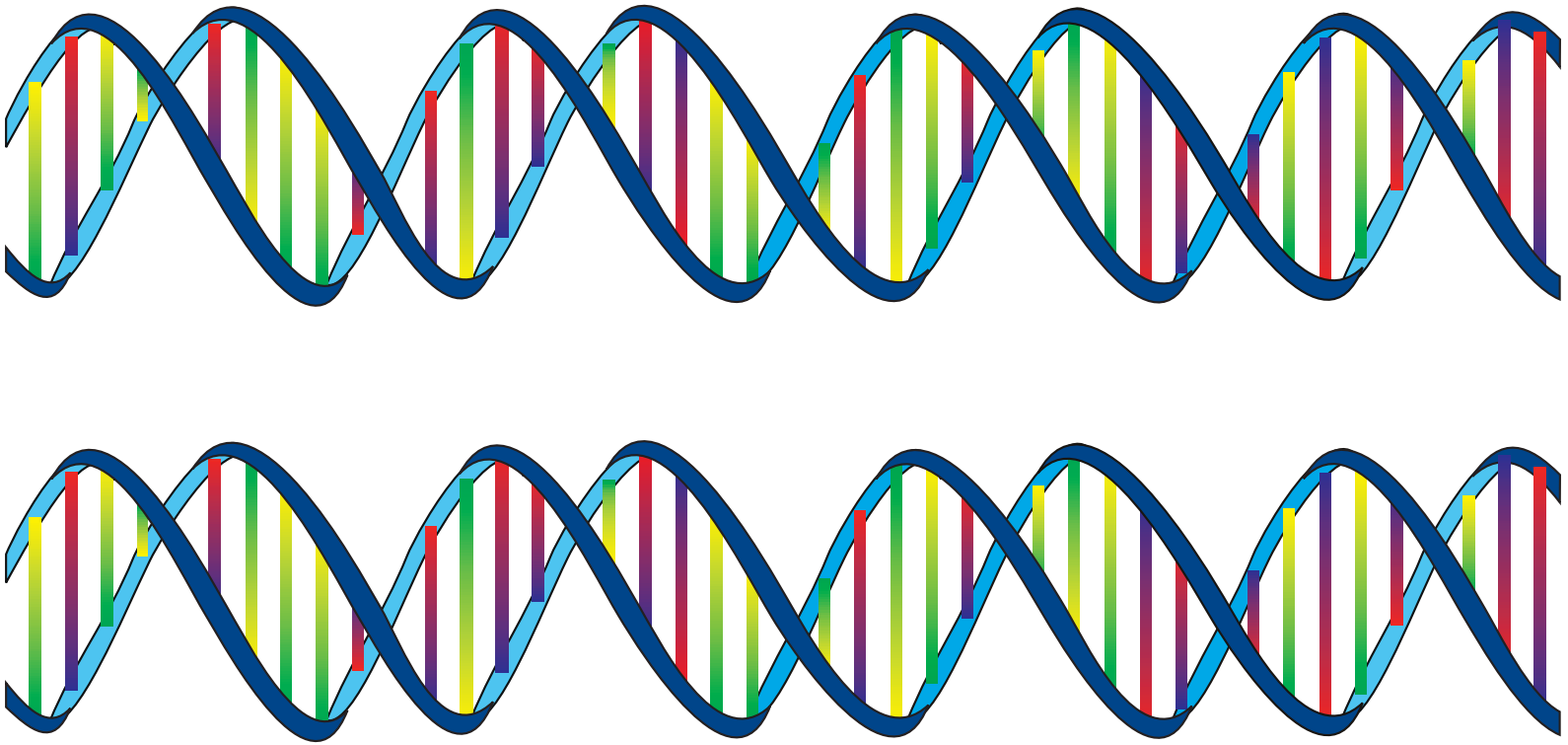
nn
Nn
nn
Nn
nn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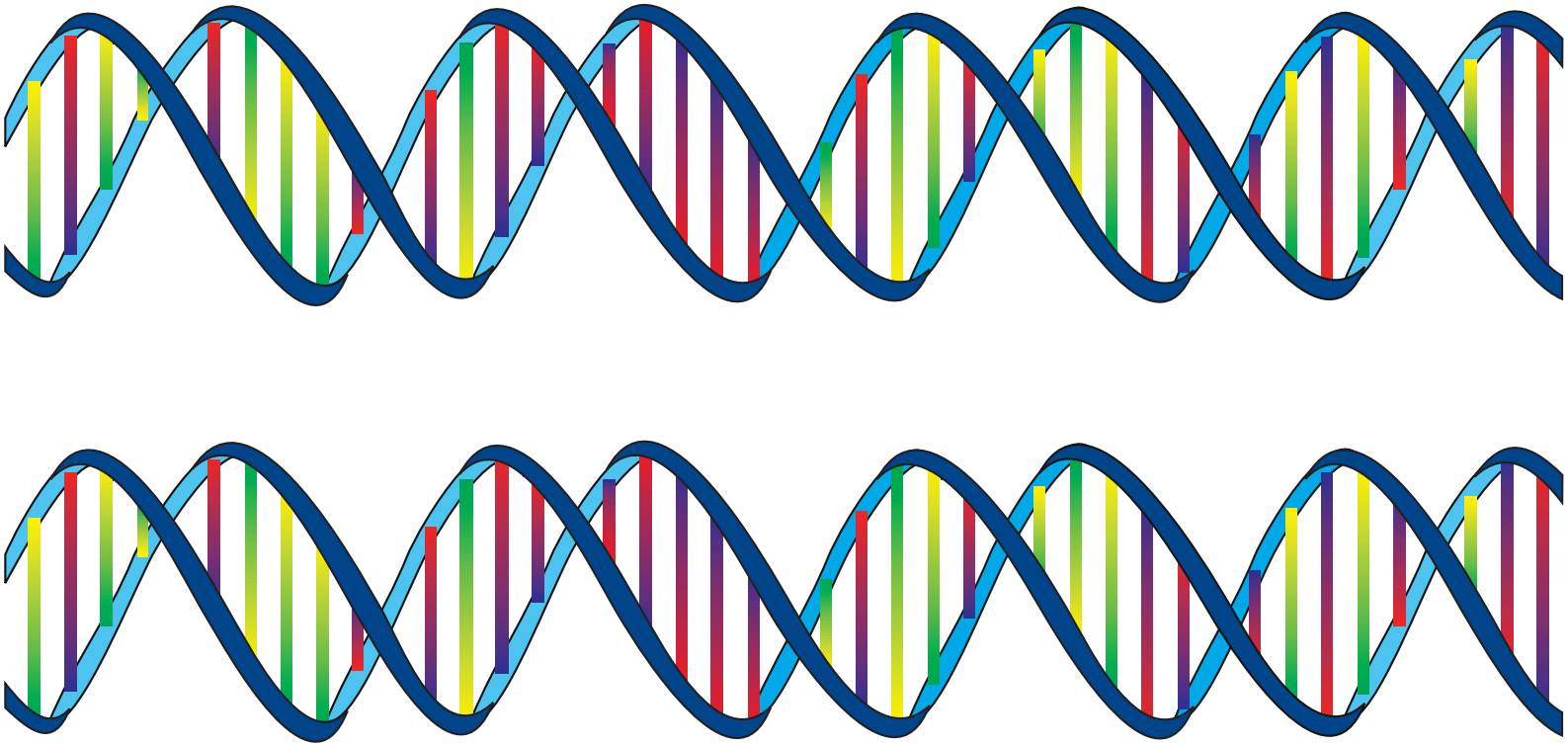
N
NN

N

n

Nn



n

n

nn

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ATCGGTACTGCAGGATCCGCAAACTAATGCA
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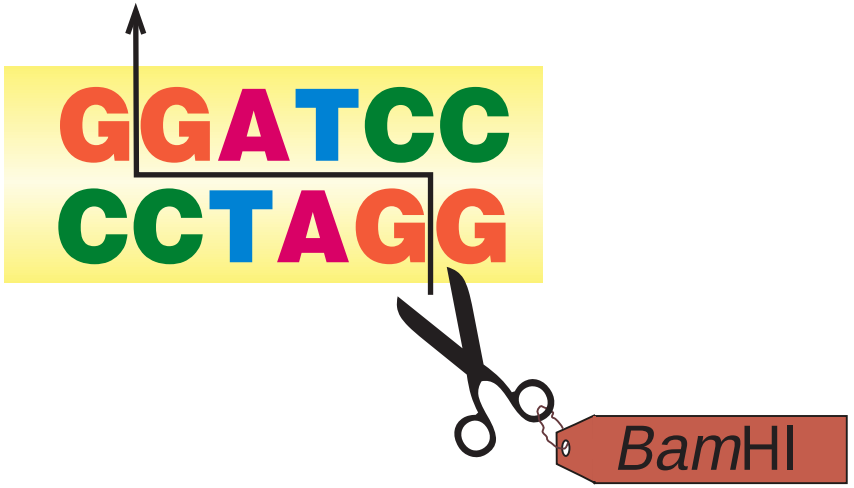

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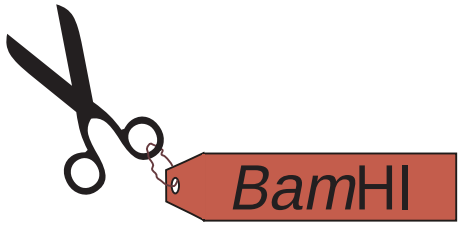
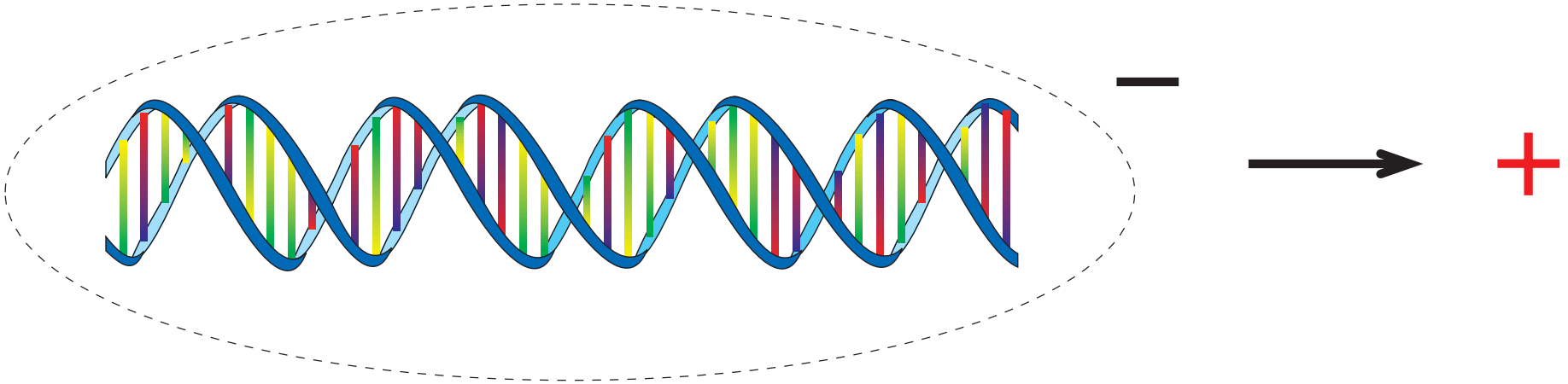
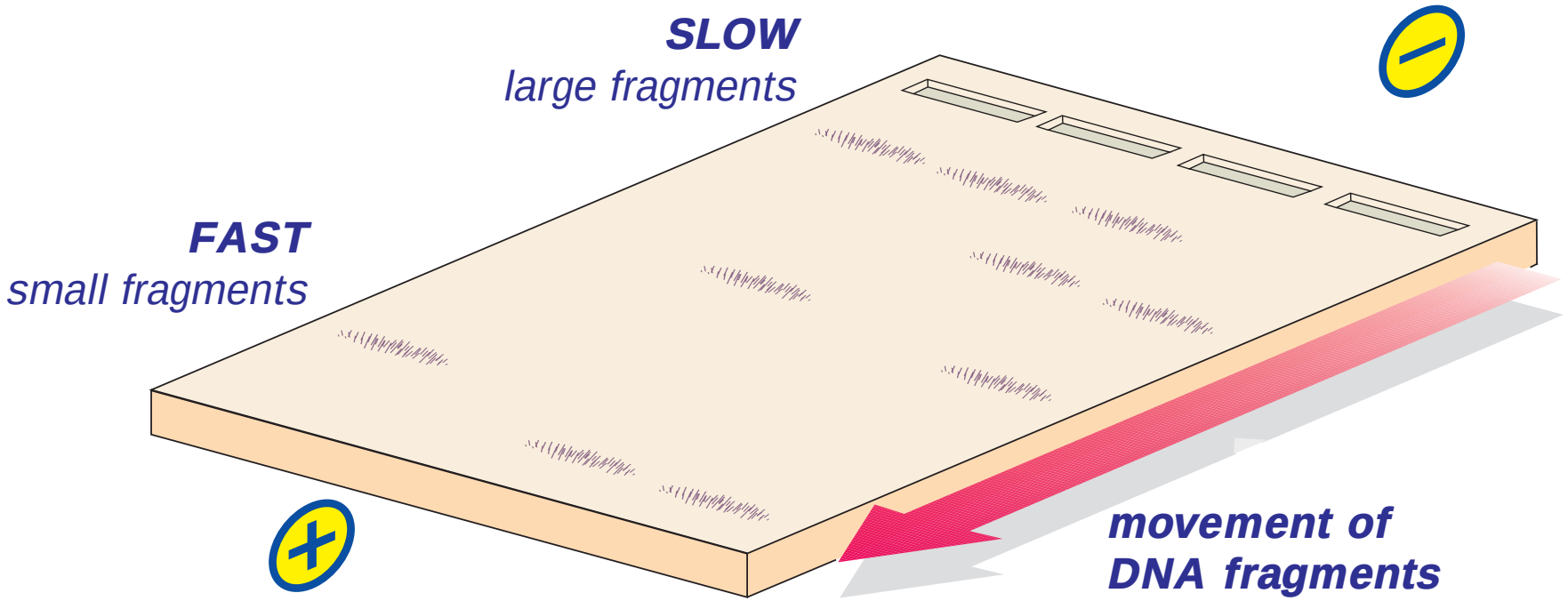


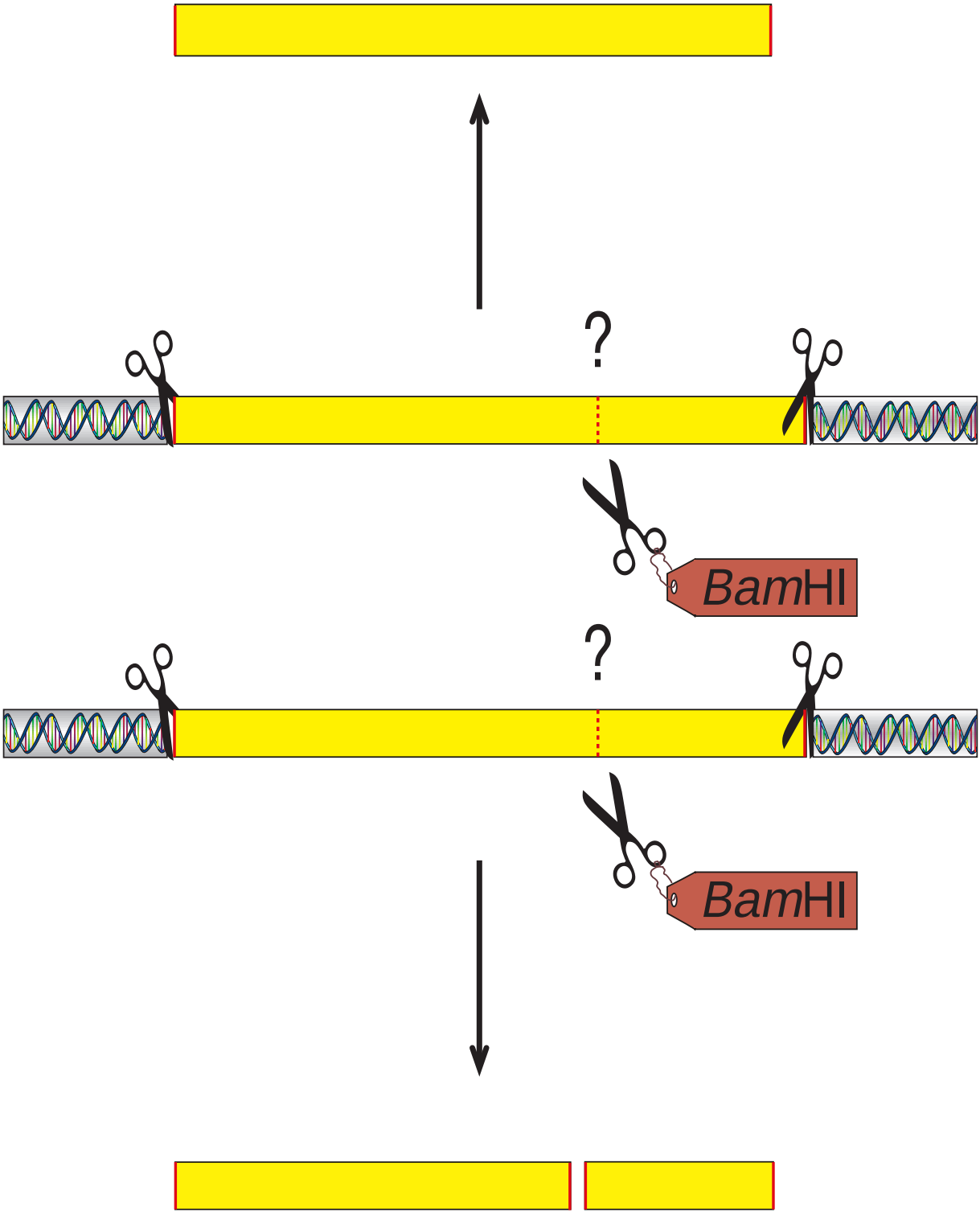
ATCGGTACTGCAG
TAGCCATGACGTCCTAG

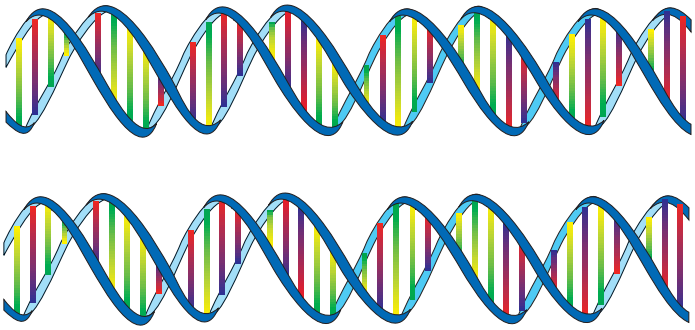
GATCCGCAAACTAATGCA
GCGTTTGATTACGT

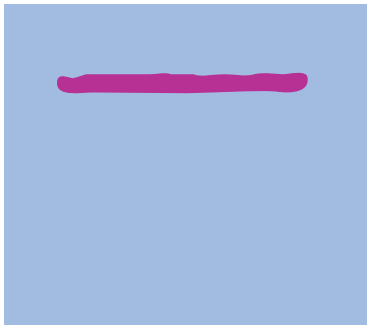


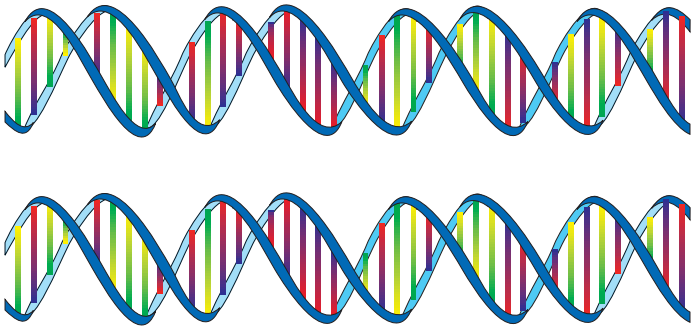
OHP 16

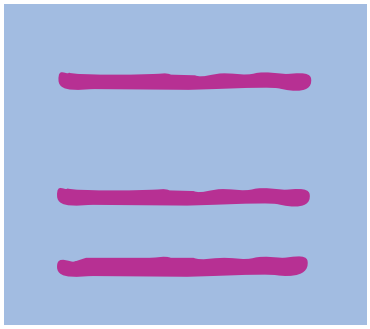

SLOW
large fragments
FAST
small fragments
movement of
DNA fragments


?
BamHI
?
BamHI

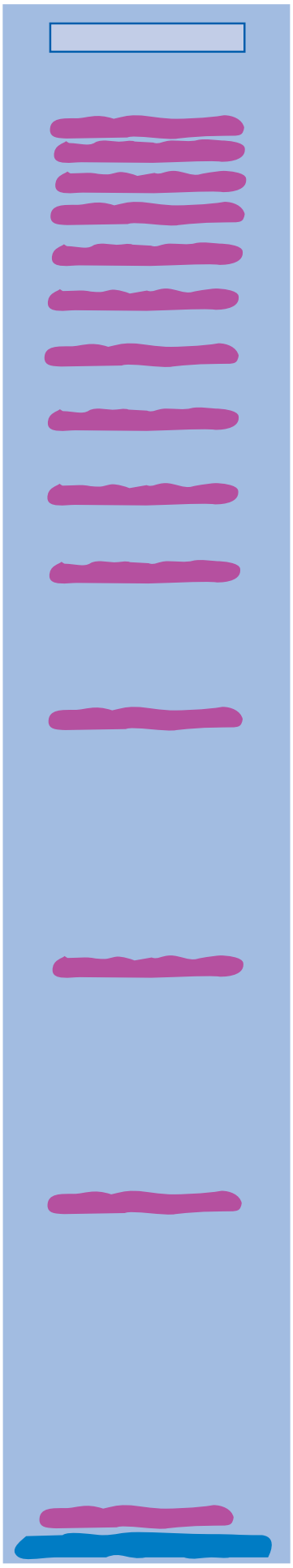


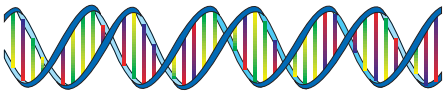


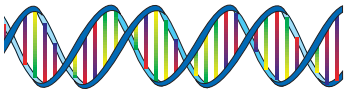




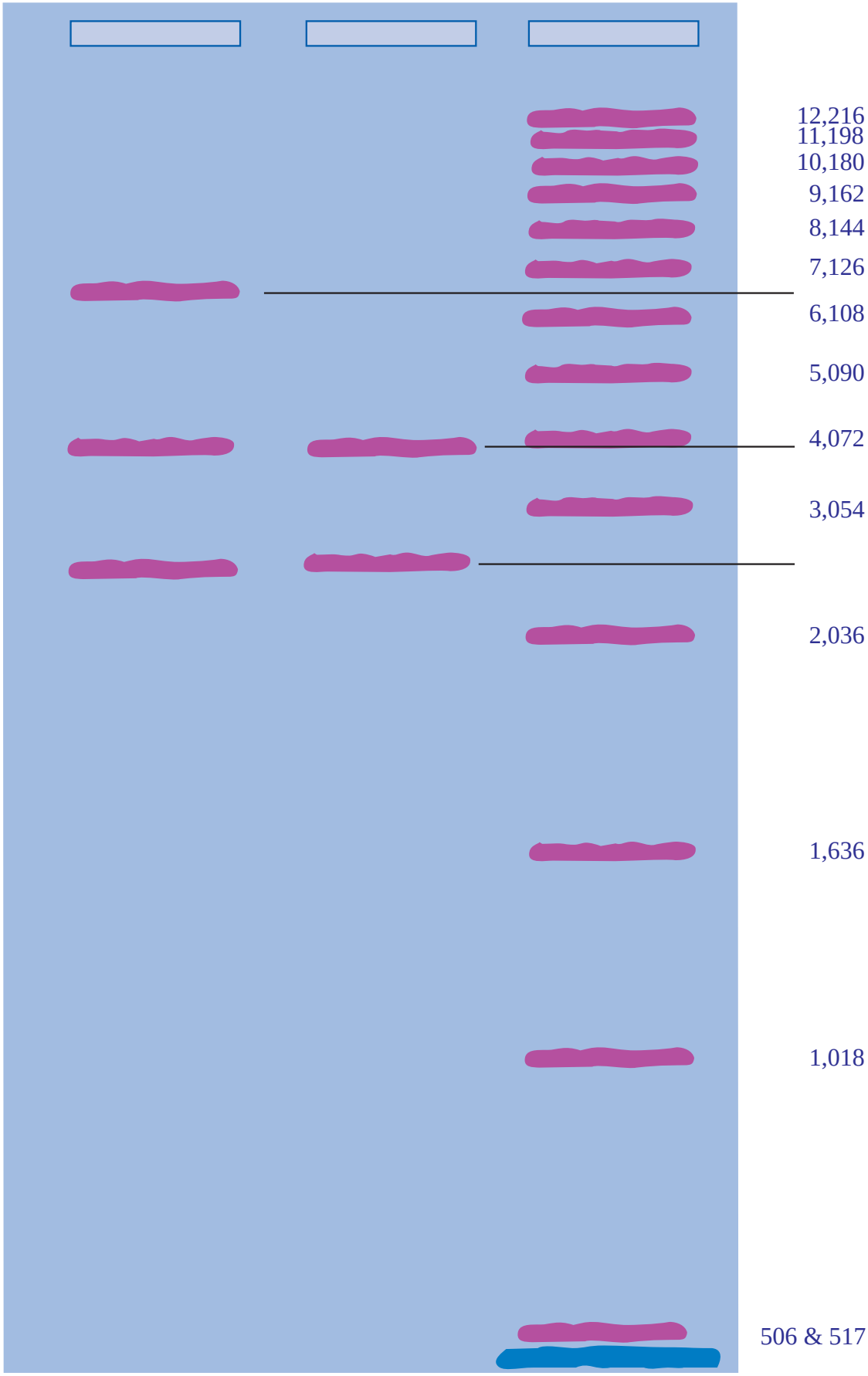
1 kb DNA ladder







OHP 20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11,198
10,180
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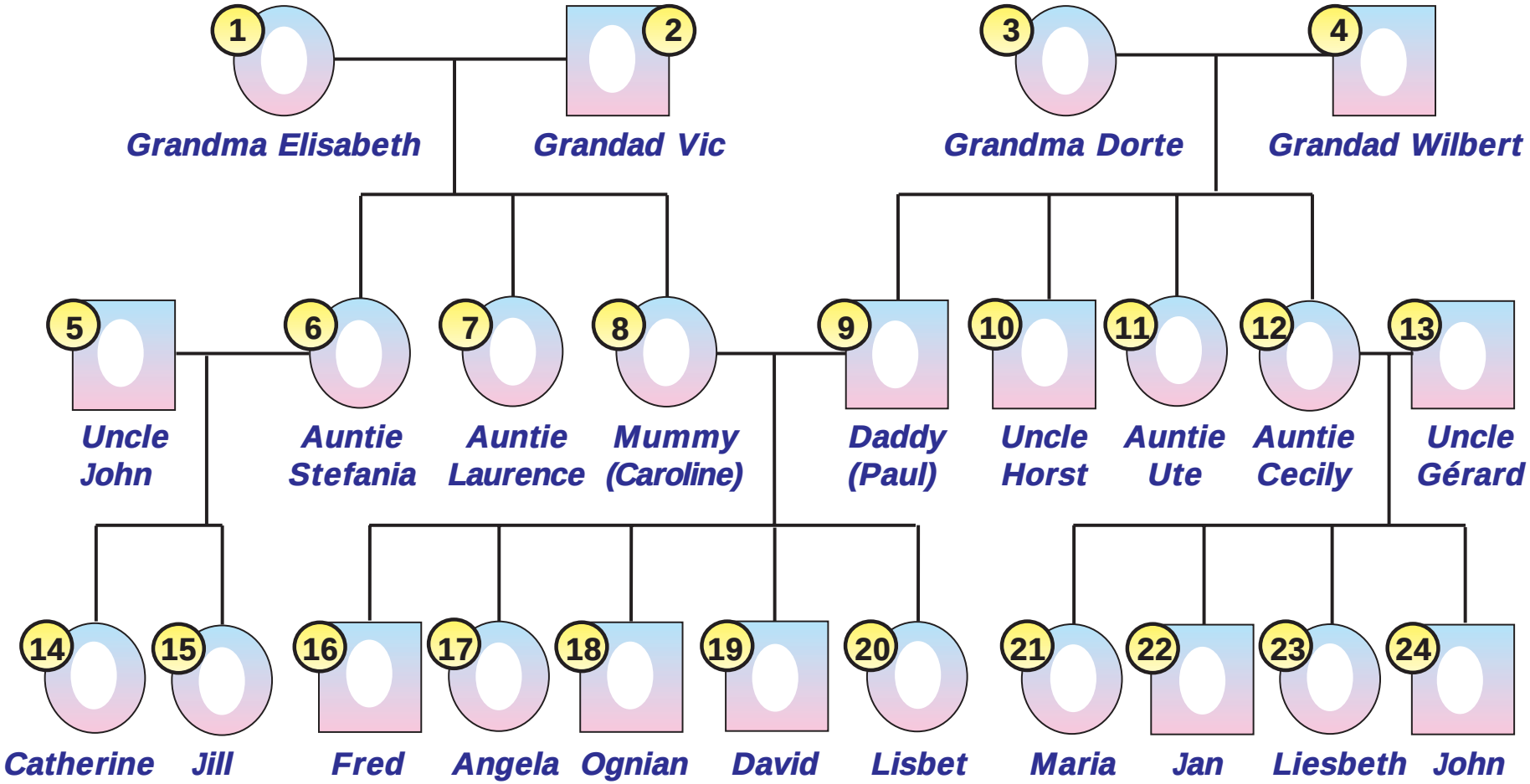
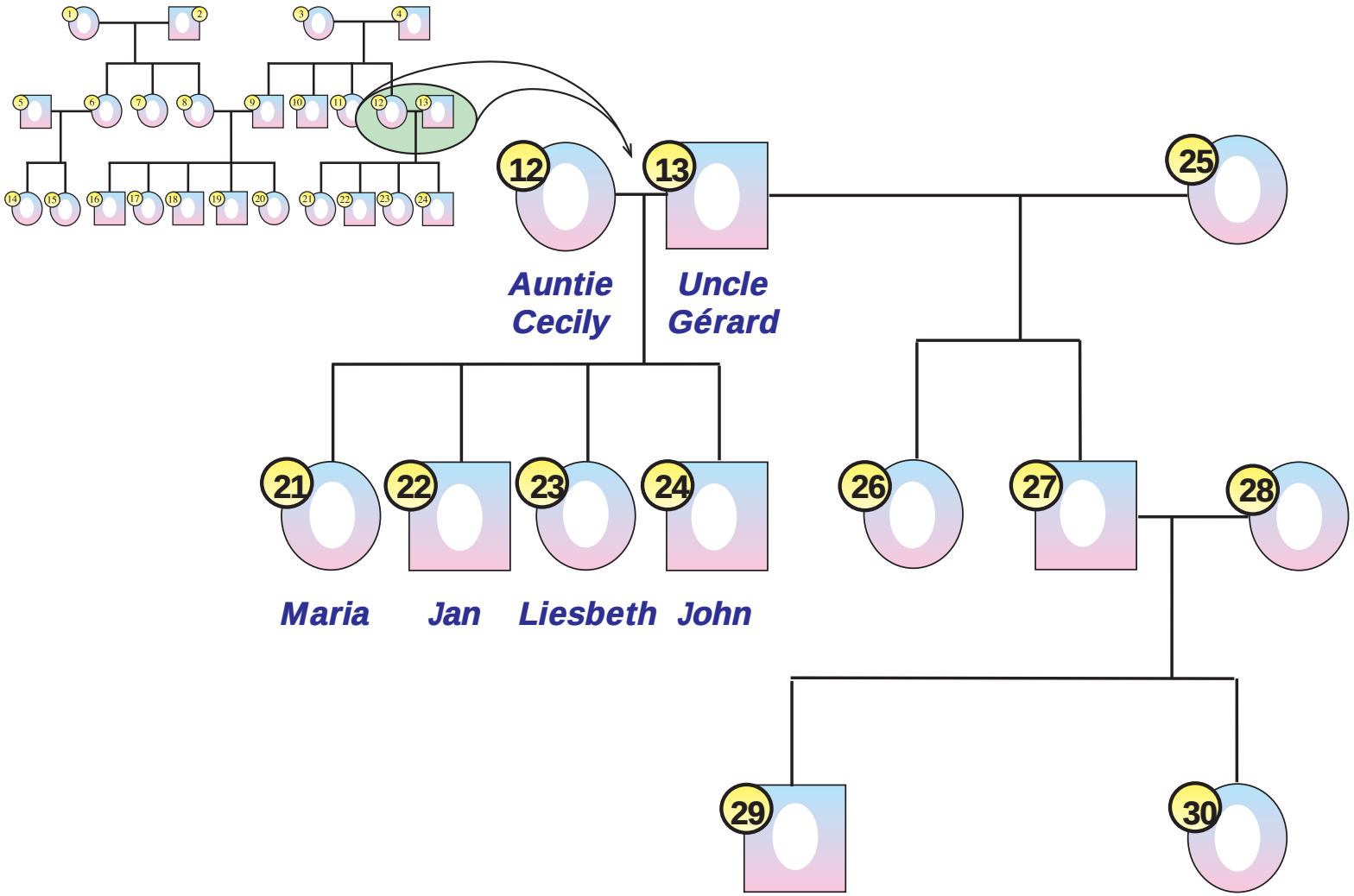
8,144
7,126
6,108
5,090
4,072
3,054
2,036
1,636
1,018
506 & 517


1
2
3
4
Grandma Elisabeth
Grandad Vic
Grandma Dorte
Grandad Wilbert
5
6
7
8
9
10
11
12
13
Uncle John
Auntie Stefania
Auntie Laurence
Mummy (Caroline)
Daddy (Paul)
Uncle Horst
Auntie Ute
Auntie Cecily
Uncle Gérard
14
15
16
17
18
19
20
21
22
23
24
Catherine
Jill
Fred
Angela
Ognian
David
Lisbet
Maria
Jan
Liesbeth
John

OHP 22


12
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Auntie Cecily
Uncle Gérard
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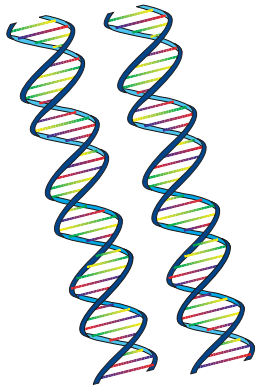
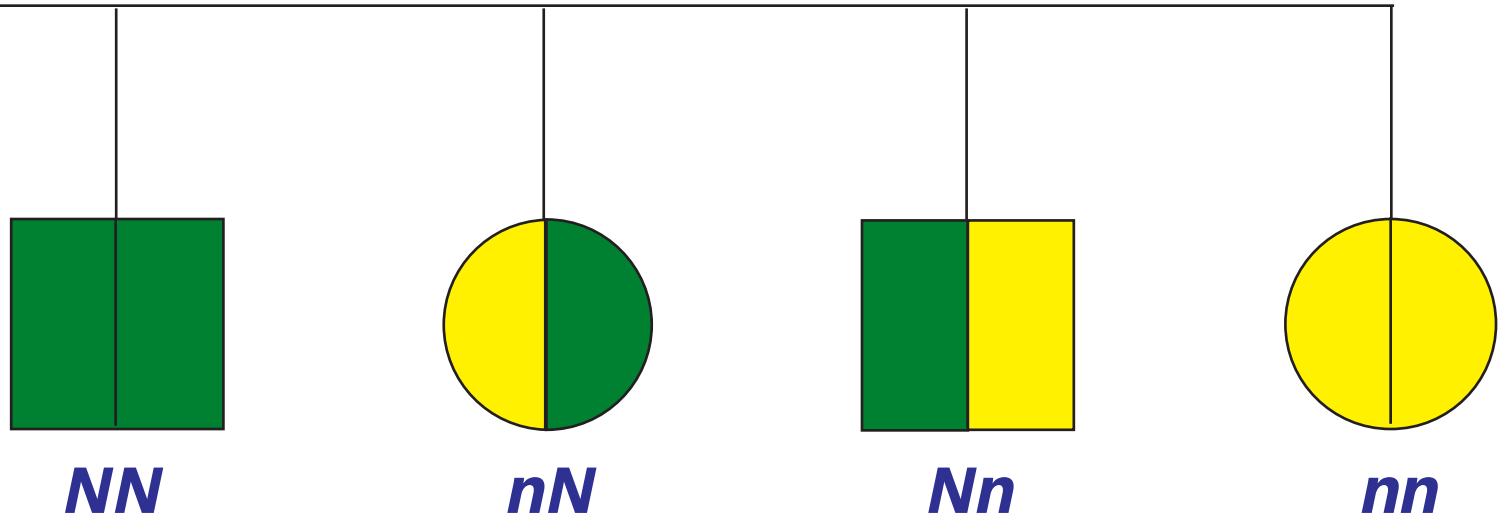
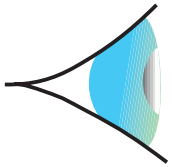
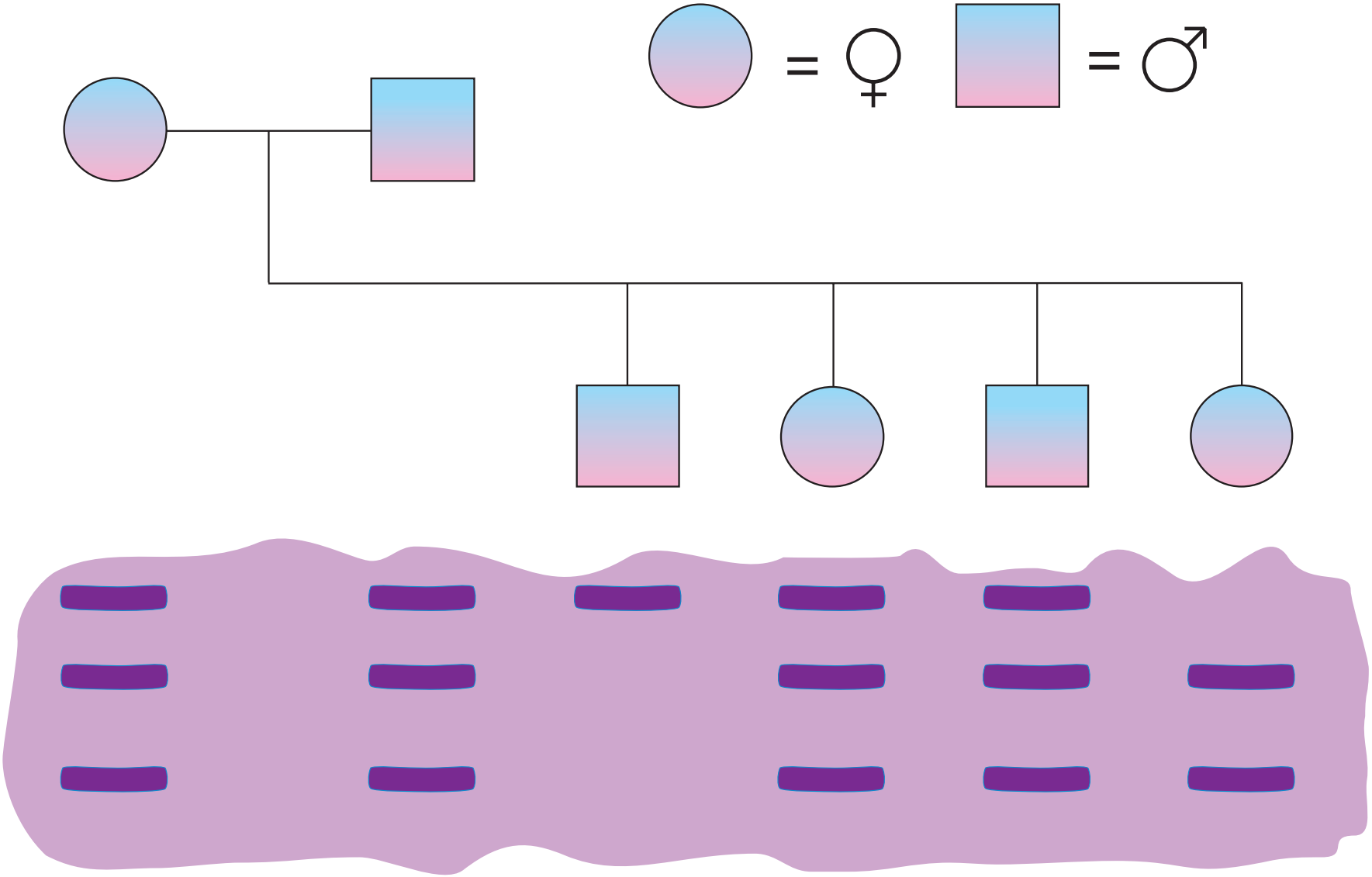
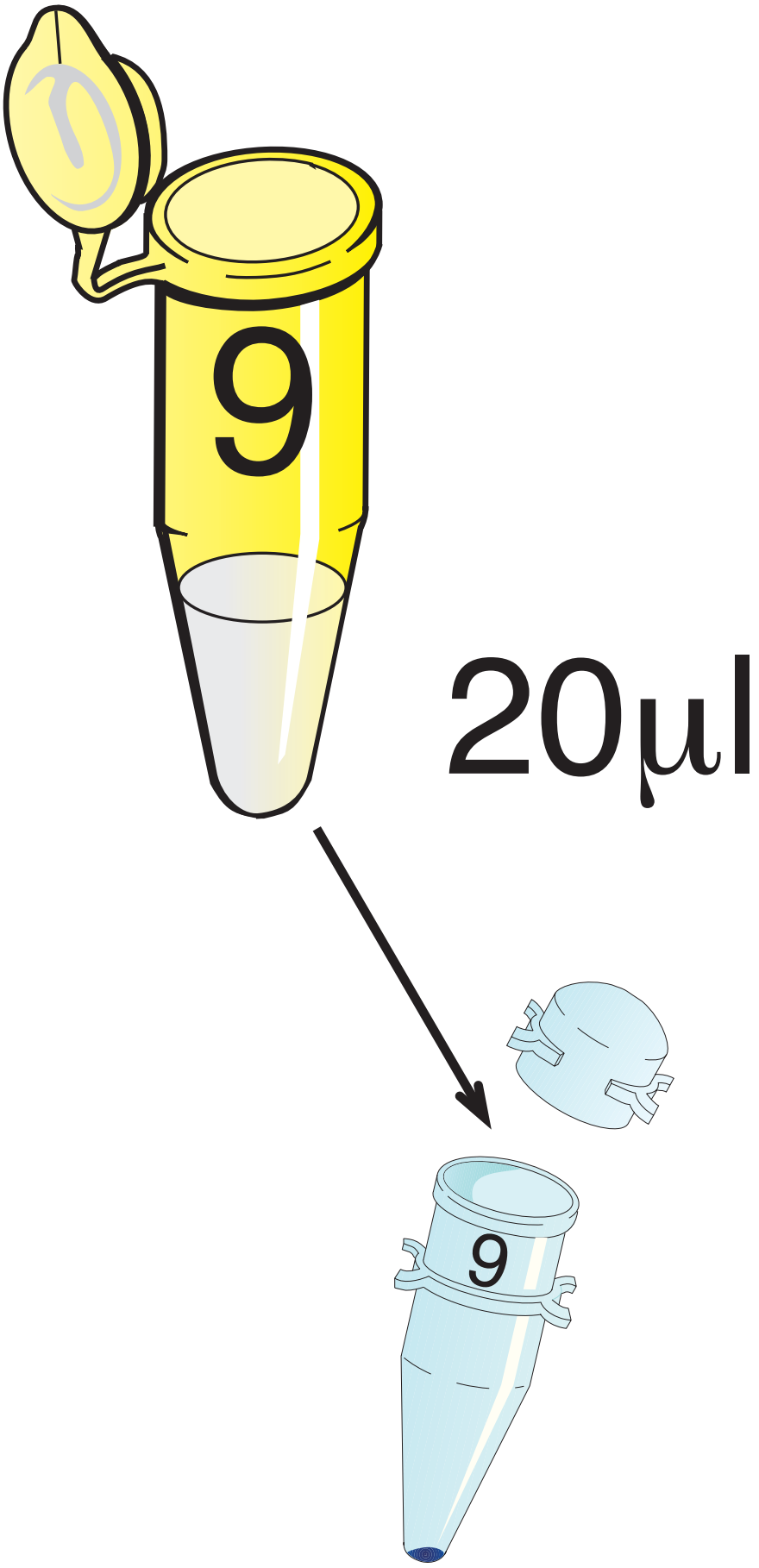
27
28
Maria
Jan
Liesbeth
John
29
30

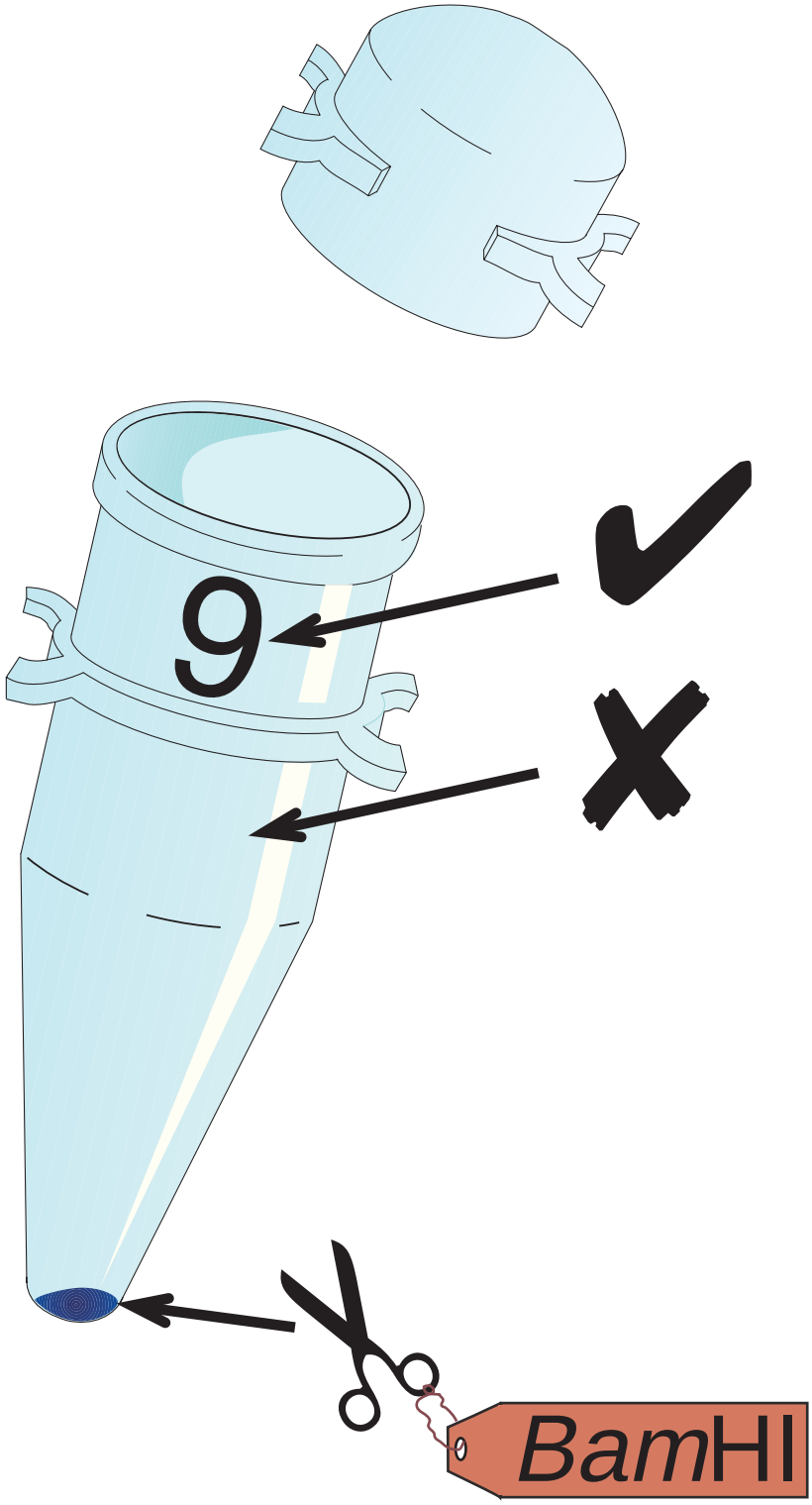


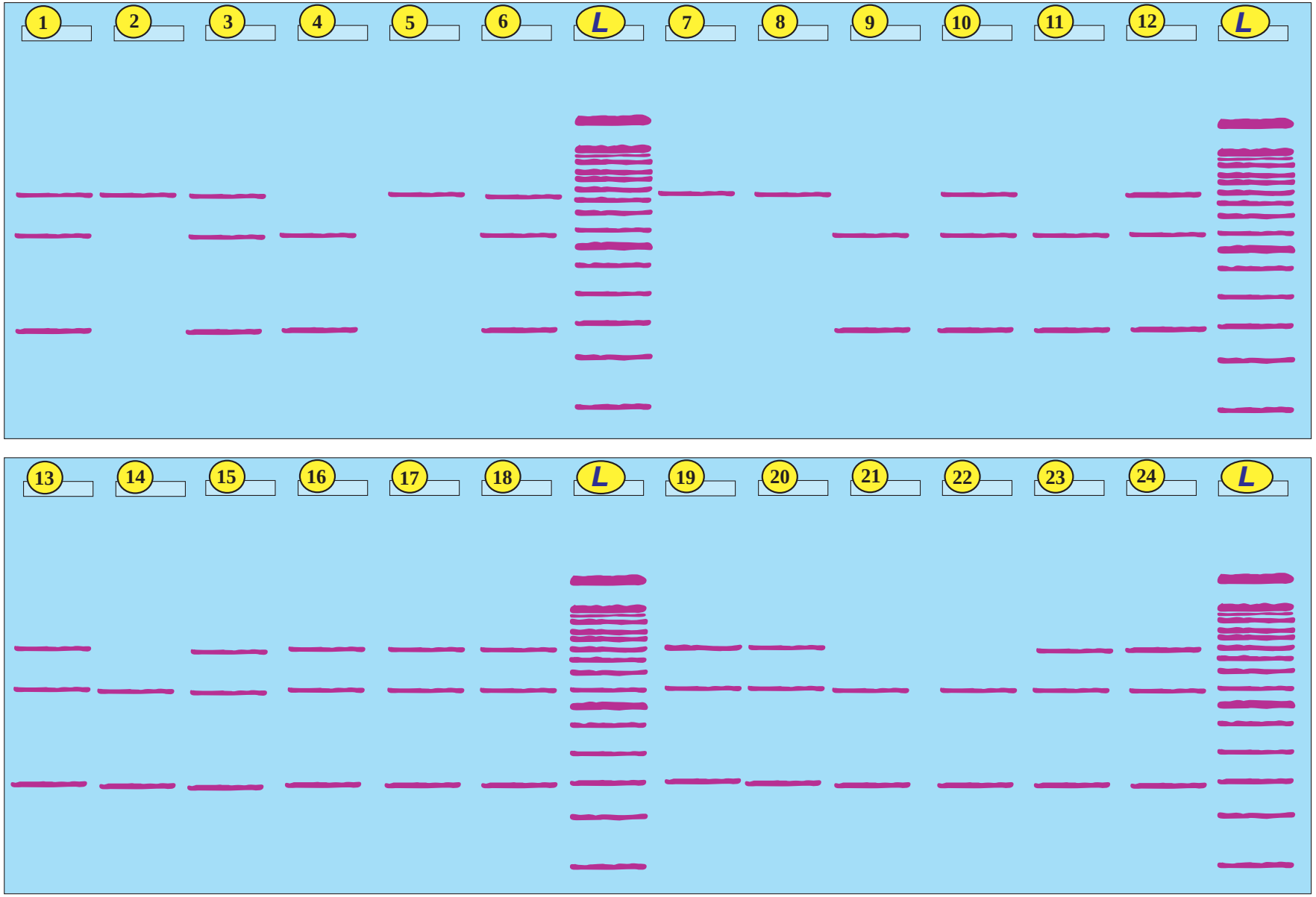

NN
nN
Nn
nn






9
20µl
9

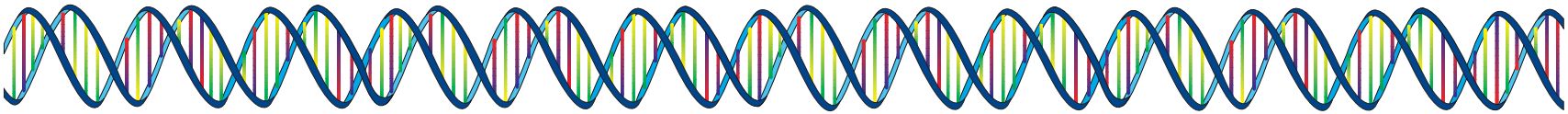

9
BamHI



L - ladder of DNA fragments of known sizes for reference

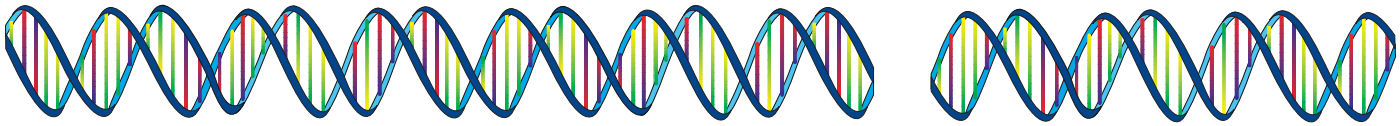
OHP 28

Normal β globin allele

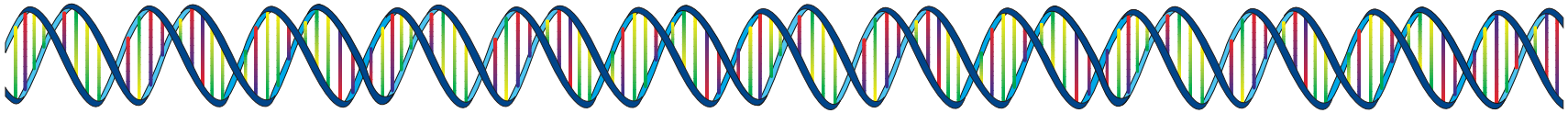


GAG

codes for glutamic acid in the haemoglobin molecule



Sickle cell allele



GTG

codes for valine in the haemoglobin molecule