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PCR and congenics

Wednesday 28th September 2011

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Aims and Objectives

- Explain the concept of PCR and how to perform a PCR
- The practical: Use PCR to identify different genetically modified strains.
- **Using PCR to answer a biologically relevant question**

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Aims and Objectives

- Explain the concept of PCR and how to perform a PCR
- The practical: Use PCR to identify different genetically modified strains.

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7 minute challenge!

In groups of 6:

- Write a list of reagents you need for a PCR
- Write a programme for a typical PCR
- Describe how you would analyse PCR results

(Suggestion: Delegate! 2 people address each question for 5 mins then go through your answers as a group in the last 5 mins)

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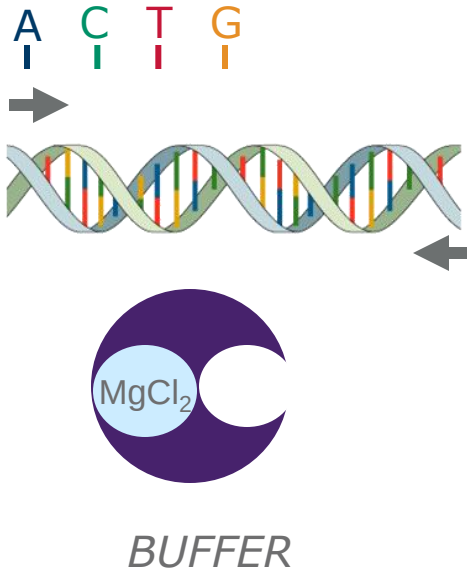
7 minute challenge! Answers.

Write a list of reagents you need for a PCR

- Template DNA!
- Primers – forward and reverse
- dNTPs
- DNA Polymerase
- $MgCl_2$ (Enzyme co-factor)
- Enzyme buffer



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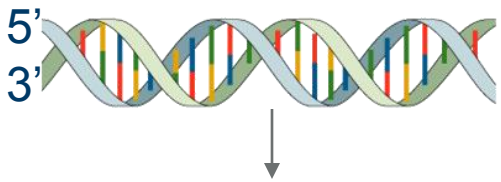
7 minute challenge! Answers.

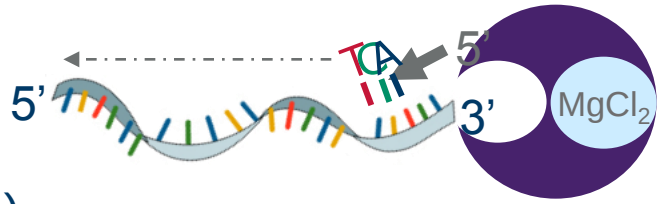
Write a programme for a typical PCR

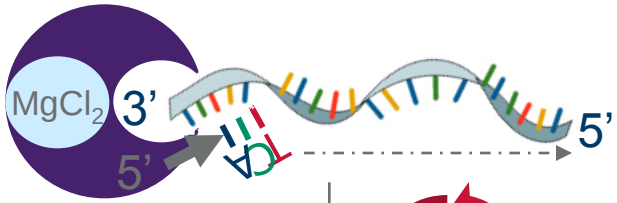
1. Initial denaturation
(ensure all template is denatured)



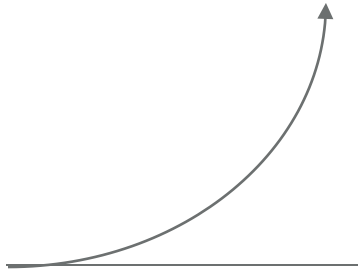
Then cycles of:

2. Denaturation (95^{o}C)
3. Annealing (Tm minus 5-7^{o}C)
4. Elongation (72^{o}C – DNA Polymerase)





20 – 40 cycles.
Gives exponential amplification



5. Then final elongation at 72^{o}C

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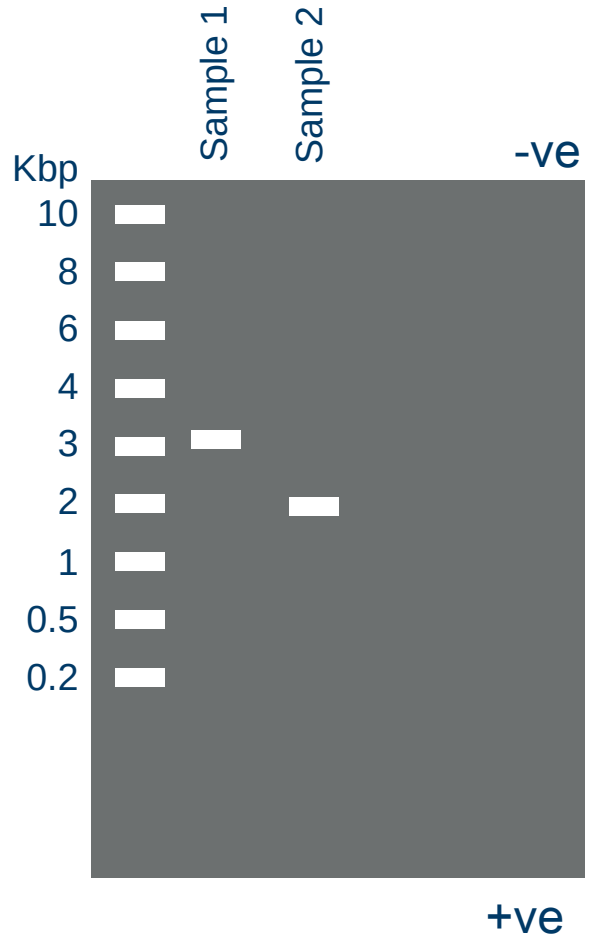
7 minute challenge! Answers.

- Describe how you would analyse PCR results
- Agarose gel electrophoresis
- Molecular weight marker



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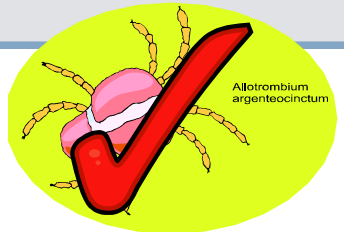
Aims and Objectives



- Explain the concept of PCR and how to perform a PCR
- The practical: Use PCR to identify different genetically modified strains.

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Use PCR data to identify different genetically modified strains.

Transgenic:

Knock-out:

Knock-in:

Inbred:

Recombinant Inbred:

Consomic: A single chromosome from one inbred strain is transferred onto the genetic background of a second strain. Chromosome substitution

Congenic: An interval (1-99%) of a single chromosome from one inbred strain is transferred onto the genetic background of a second strain.

The Practical

The biological question:

Which genes are responsible for SLE?

- Animal model of SLE: BXSB mice
- Breeding has narrowed down the genes responsible for disease
- Some genes linked to disease are on Chr 1.

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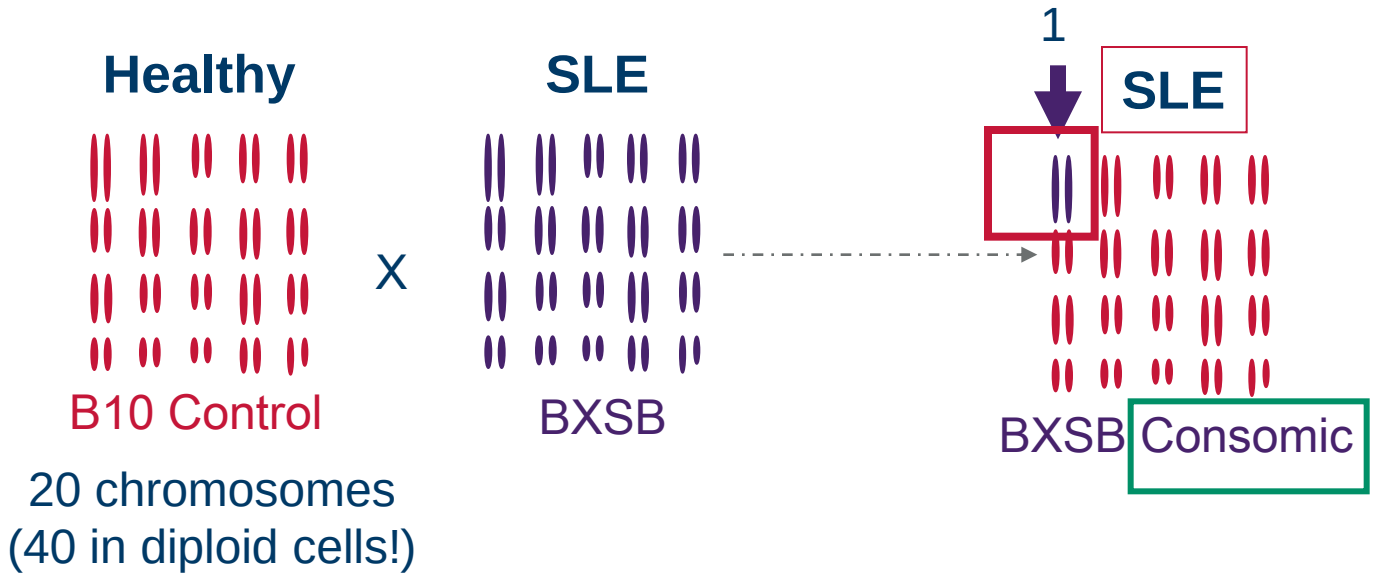
The Practical



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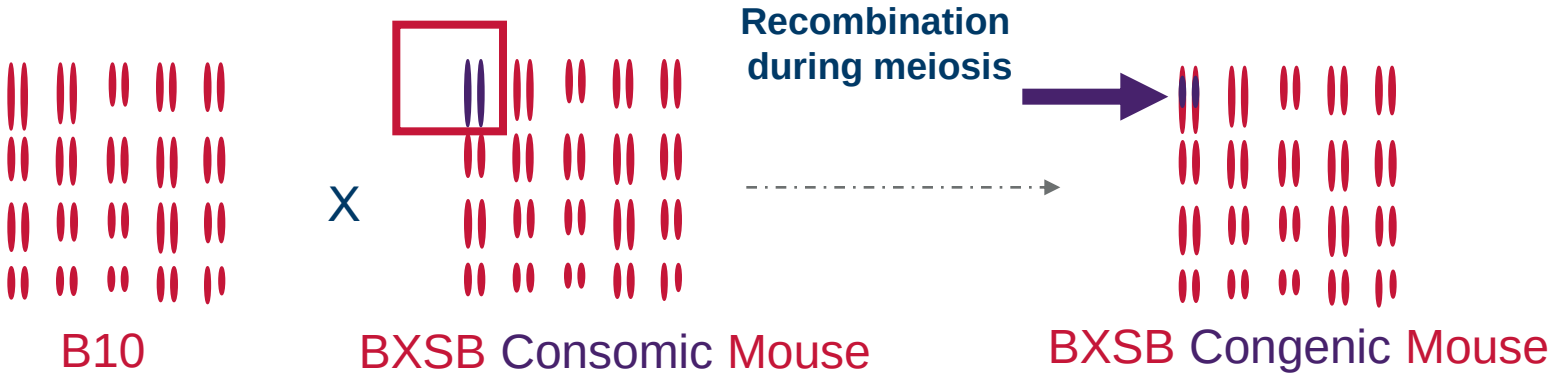
The Practical



- How do we know **if** and **where** recombination has occurred?

The Practical

Microsatellites!

Sequence analysis - Microsatellites

What are microsatellites?

- ✓ Short sequence repeats (2 - 4 bp repeats)
- ✓ Found in most genomes
- ✓ Exceptional variability in humans and mice
- ✓ Bordered by unique DNA sequence
- ✓ Can be examined using PCR

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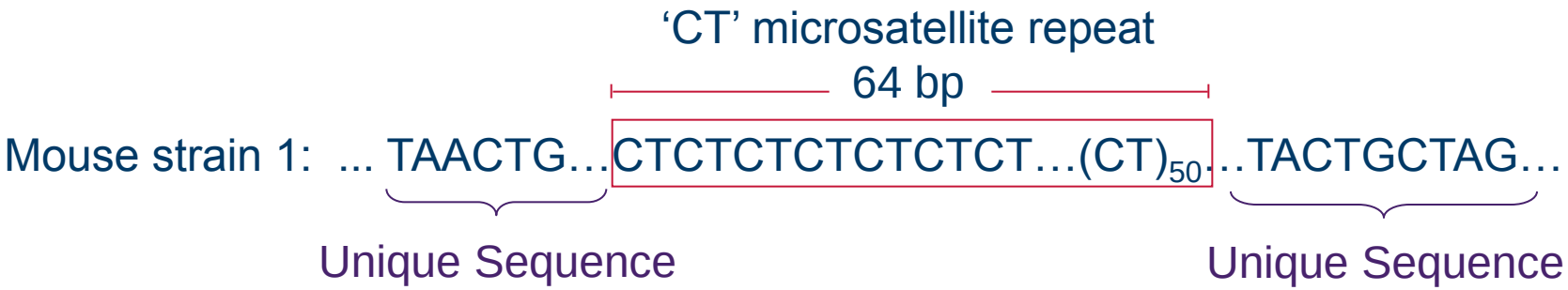
Microsatellites

Example:



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Microsatellites

How can you detect the difference between the strains?

Design primers against the unique sequence either side of the microsatellite
Extract DNA from each mouse strain
Do a PCR (with the DNA as your template) with primers that recognise the unique seq.

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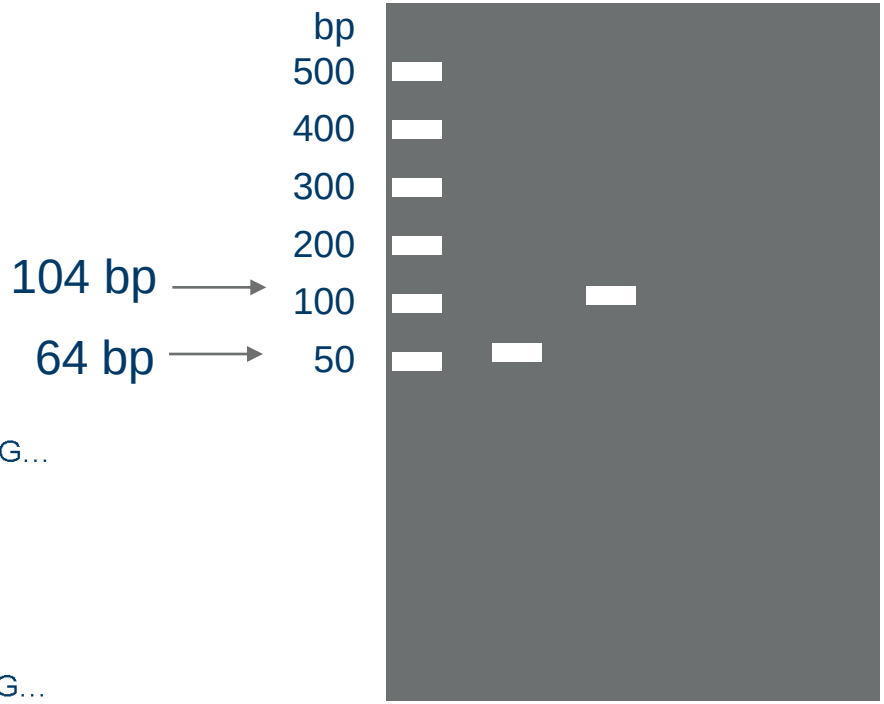
Microsatellites

How can you detect the difference?

- ✓Agarose gel electrophoresis
- ✓Molecular weight marker



64 bp

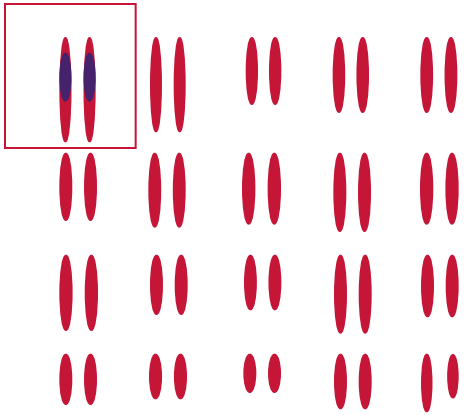
Mouse strain 1: ... TAACTGCTCTCTCTCTCTCT…$(CT)_{25}$TACTGCTAG…

104 bp

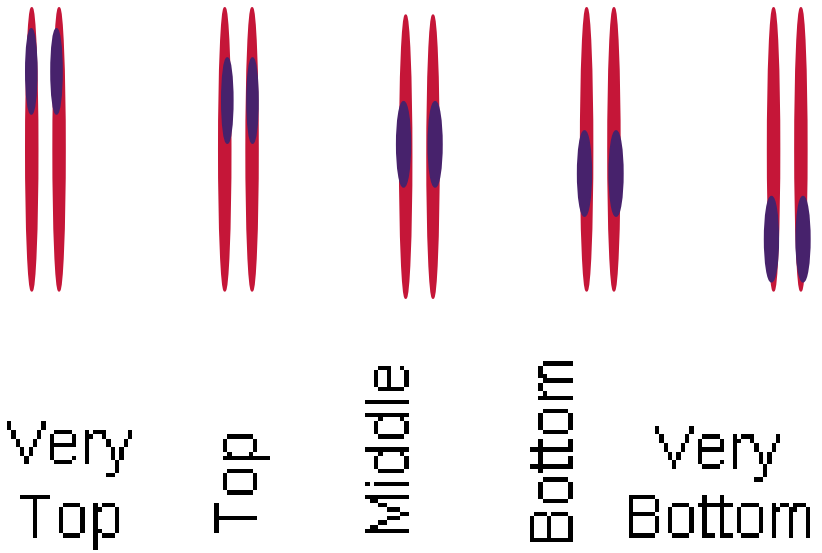
Mouse strain 2: ... TAACTGCTCTCTCTCTCTCT… $(CT)_{45}$TACTGCTAG…

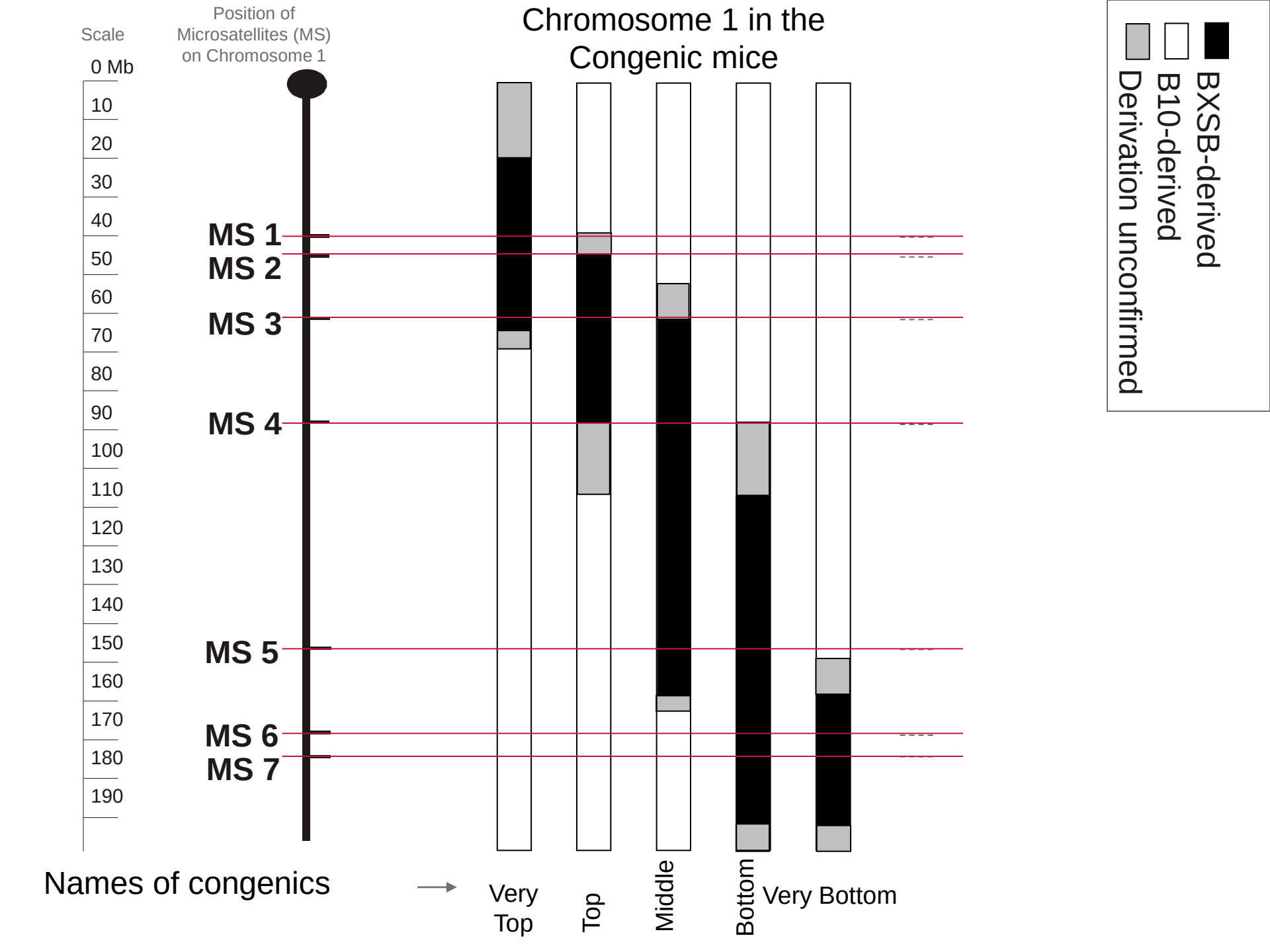
The Practical

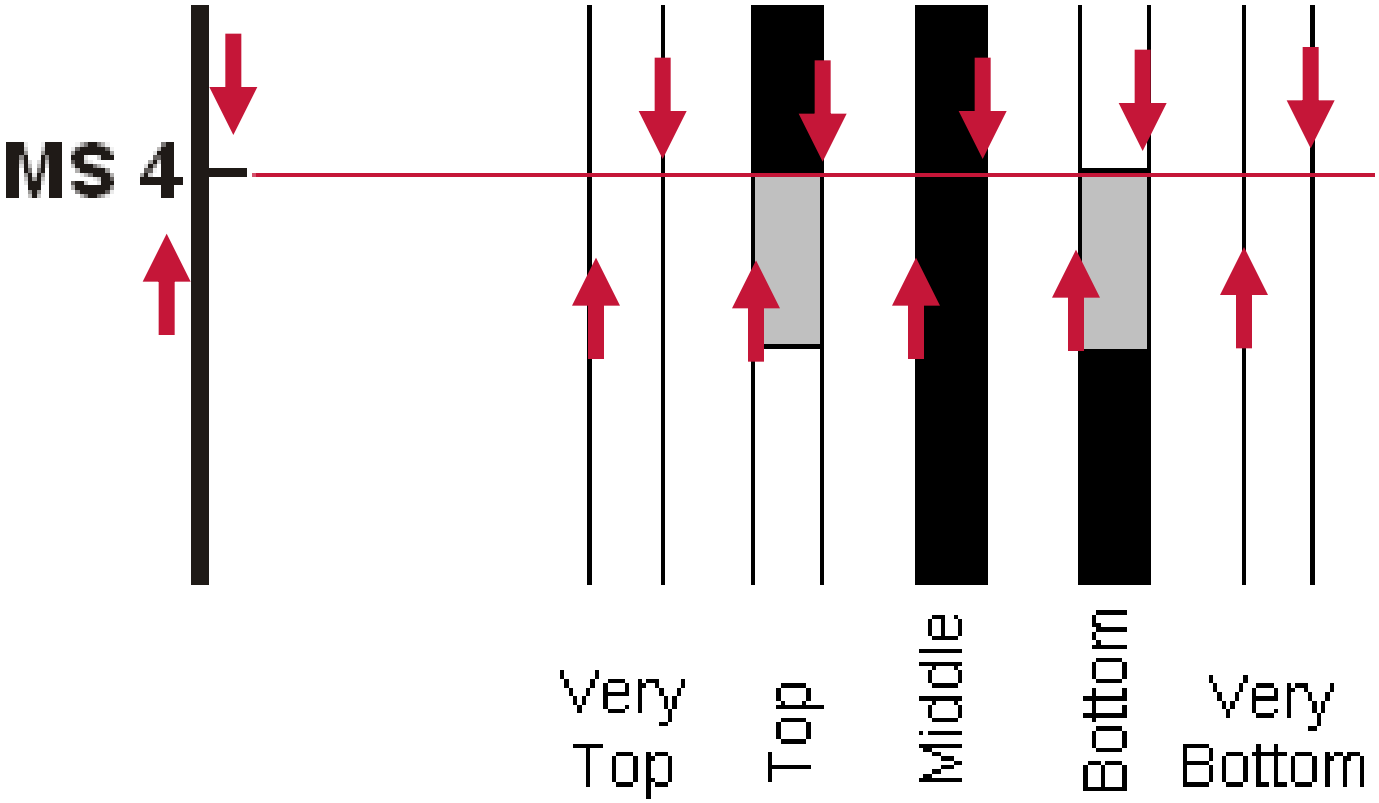
Using microsatellite analysis to identify different BXSB congenic mice

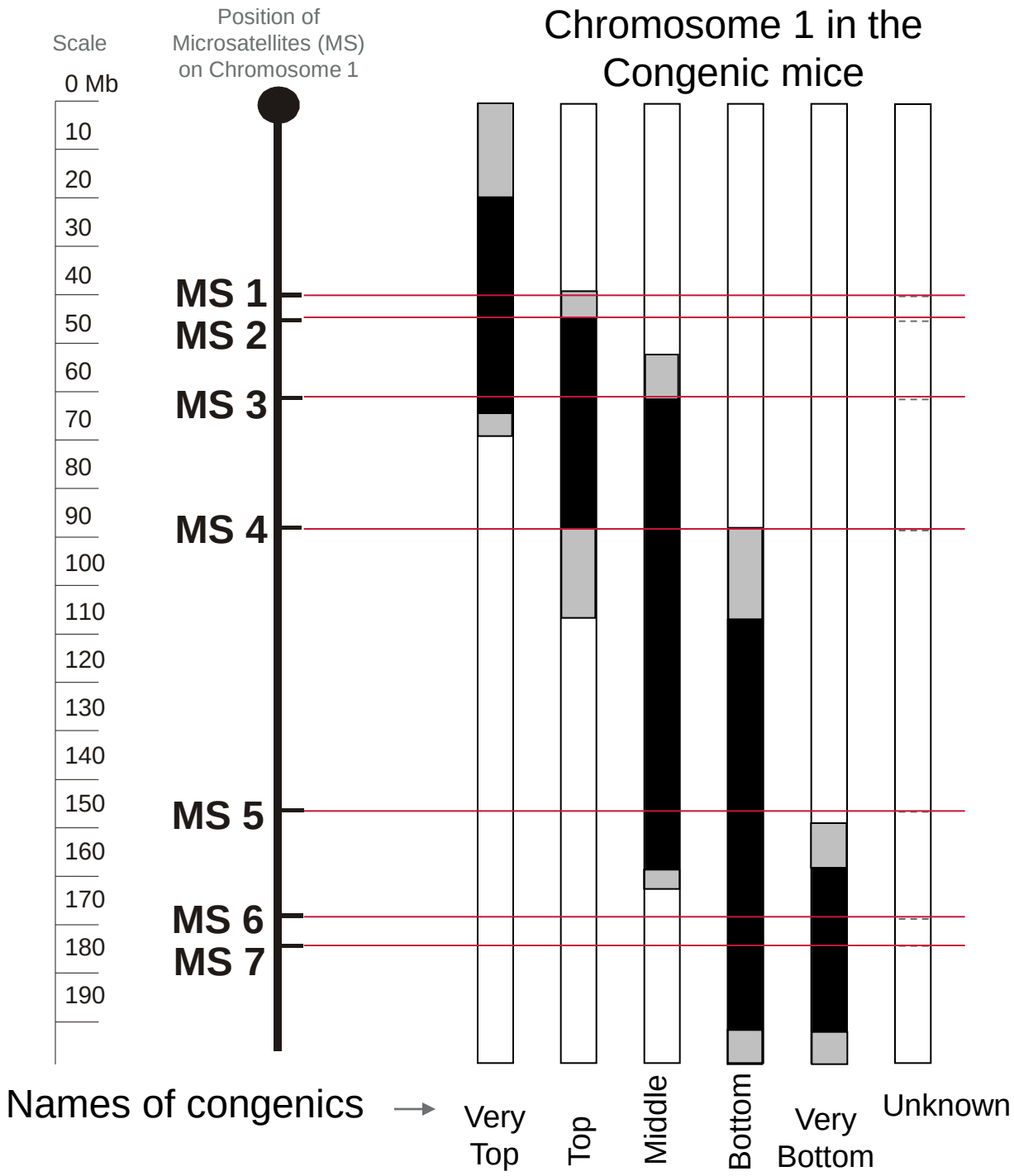


BXSB Congenic Mouse




Chromosome 1 in the Congenic mice
Scale
Position of Microsatellites (MS) on Chromosome 1
0 Mb
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
MS 1
MS 2
MS 3
MS 4
MS 5
MS 6
MS 7
BXSB-derived
B10-derived
Derivation unconfirmed
Names of congenics
Very Top
Top
Middle
Bottom
Very Bottom


MS 4
Very Top
Top
Middle
Bottom
Very Bottom



Key:

- BXSB-derived
- B10-derived
- Derivation unconfirmed

MS	Size in B10	Size in BXSB
MS 1	140 bp	185 bp
MS 2	150 bp	135 bp
MS 3	137 bp	125 bp
MS 4	146 bp	128 bp
MS 5	112 bp	122 bp
MS 6	160 bp	184 bp
MS 7	150 bp	124 bp

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The Practical: Congenics and microsatellites. 5 min challenge!

What size would the PCR products be in the following experiments?

1. If you were given:

- Primers that amplify MS 1
- DNA from mouse strain Very Top

2. If you were given:

- Primers that amplify MS4
- DNA from mouse strain Very bottom

3. If you were given:

- Primers that amplify MS 7
- DNA from an F1 mouse (BXSB x B10)

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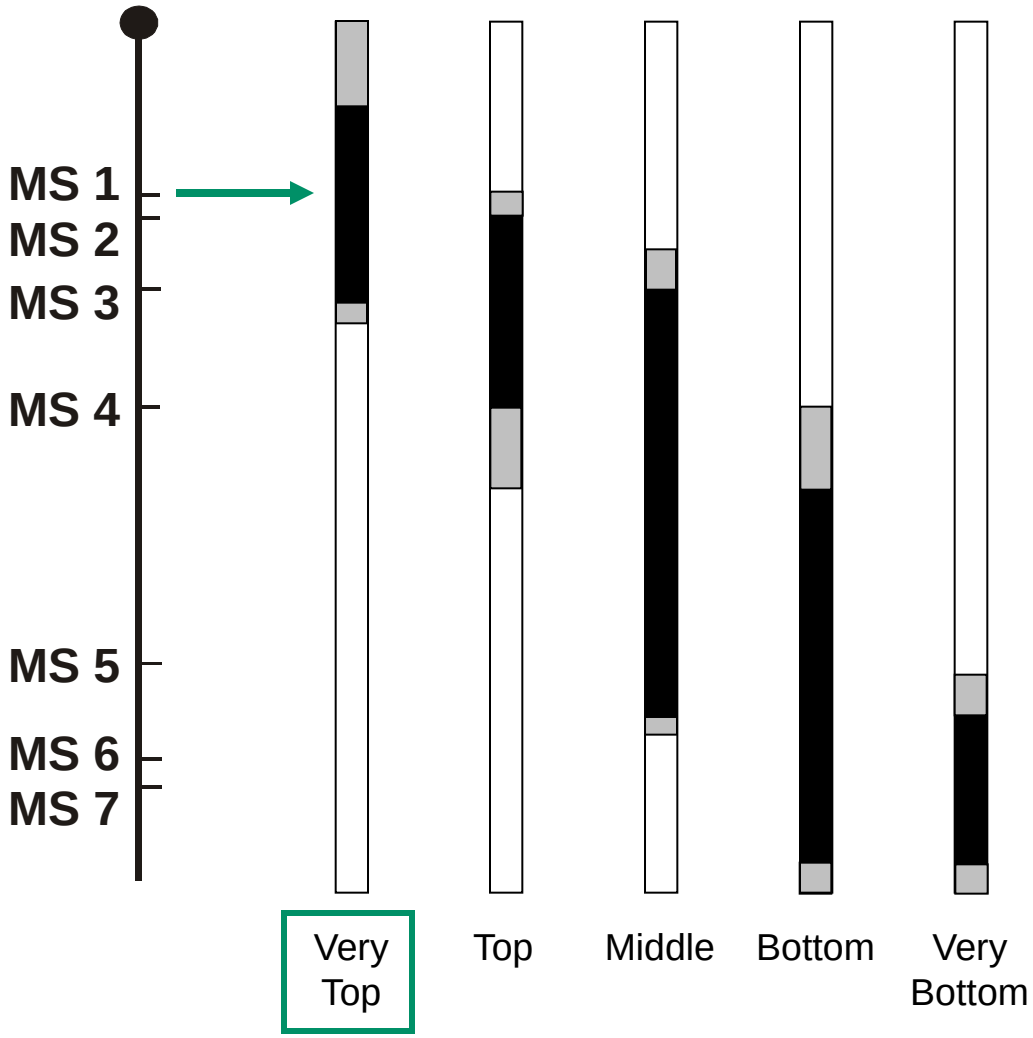
1. If you were given:
   - Primers that amplify MS 1
   - DNA from Very Top



MS	Size in B10	Size in BXSB
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MS 4	146 bp	128 bp
MS 5	112 bp	122 bp
MS 6	160 bp	184 bp
MS 7	150 bp	124 bp

Key:
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- B10-derived
- Derivation unconfirmed

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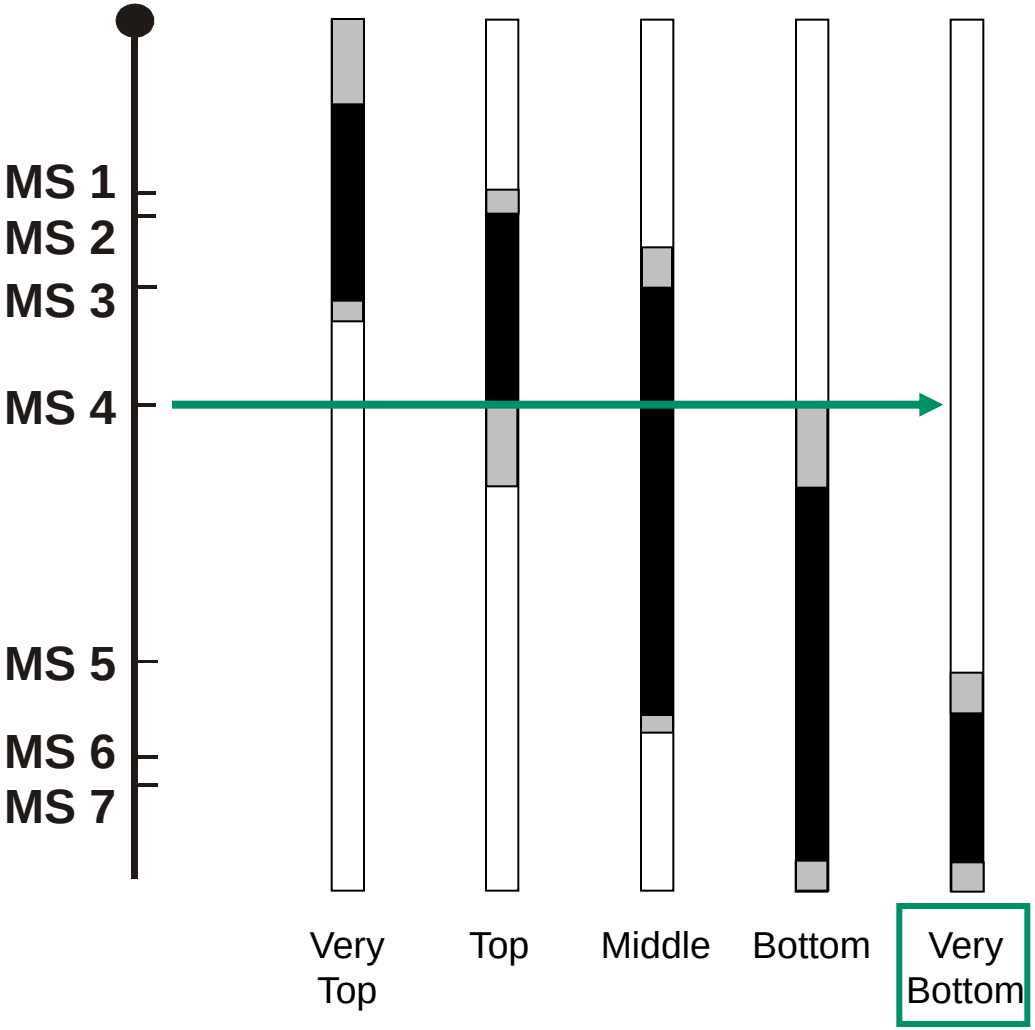
1. If you were given:
   - ➢ Primers that amplify MS4
   - ➢ DNA from Very bottom



MS	Size in B10 ↓	Size in BXSB ↓
MS 1	140 bp	185 bp
MS 2	150 bp	135 bp
MS 3	137 bp	125 bp
MS 4	146 bp	128 bp
MS 5	112 bp	122 bp
MS 6	160 bp	184 bp
MS 7	150 bp	124 bp

Key:
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- Derivation unconfirmed

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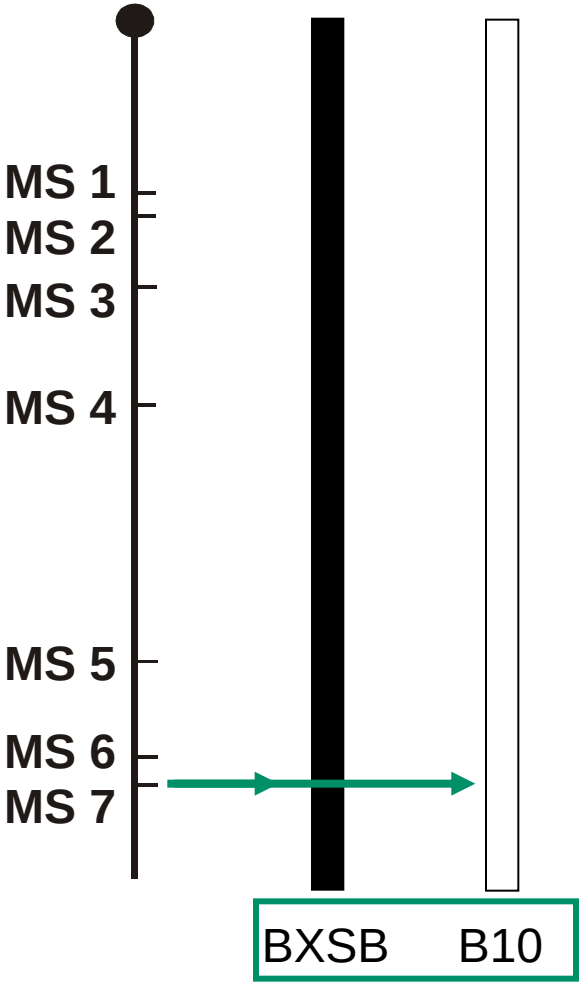
1. If you were given:
   - ➢ Primers that amplify MS7
   - ➢ DNA from F1 (BXSB x B10)



MS	Size in B10 ↓	Size in BXSB ↓
MS 1	140 bp	185 bp
MS 2	150 bp	135 bp
MS 3	137 bp	125 bp
MS 4	146 bp	128 bp
MS 5	112 bp	122 bp
MS 6	160 bp	184 bp
MS 7	150 bp	124 bp

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Aims and Objectives

- Explain the concept of PCR
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The Practical: Congenics and microsatellites. 5 min challenge!

For this practical, you will be provided with two DNA samples that were taken from the mice during the generation and characterization of the congenics. Consequently, the samples may be from any of the following:

- The parental control strain BXSB or B10
- F1 breeding crosses
- 1 of the 5 congenics

Your challenge (should you choose to accept it!) is to identify the DNA you have been given.

How?

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The Practical: Congenics and microsatellites. 5 min challenge!

What do you need to do and what reagents do you need?

Primers for the Microsatellites

Controls – DNA from parental strains B10 and BXSB

PCR reagents

Agarose gel electrophoresis

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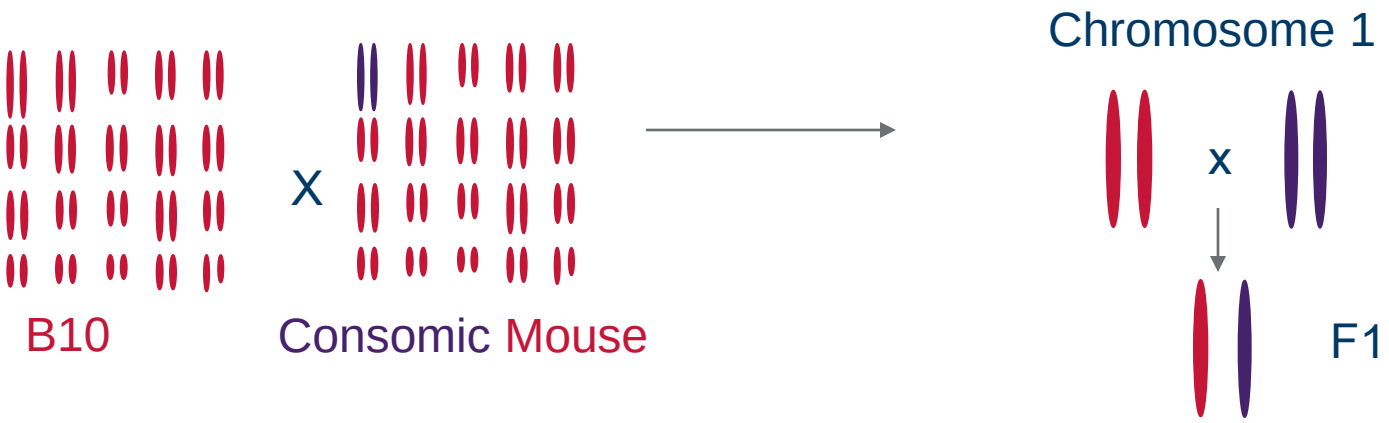
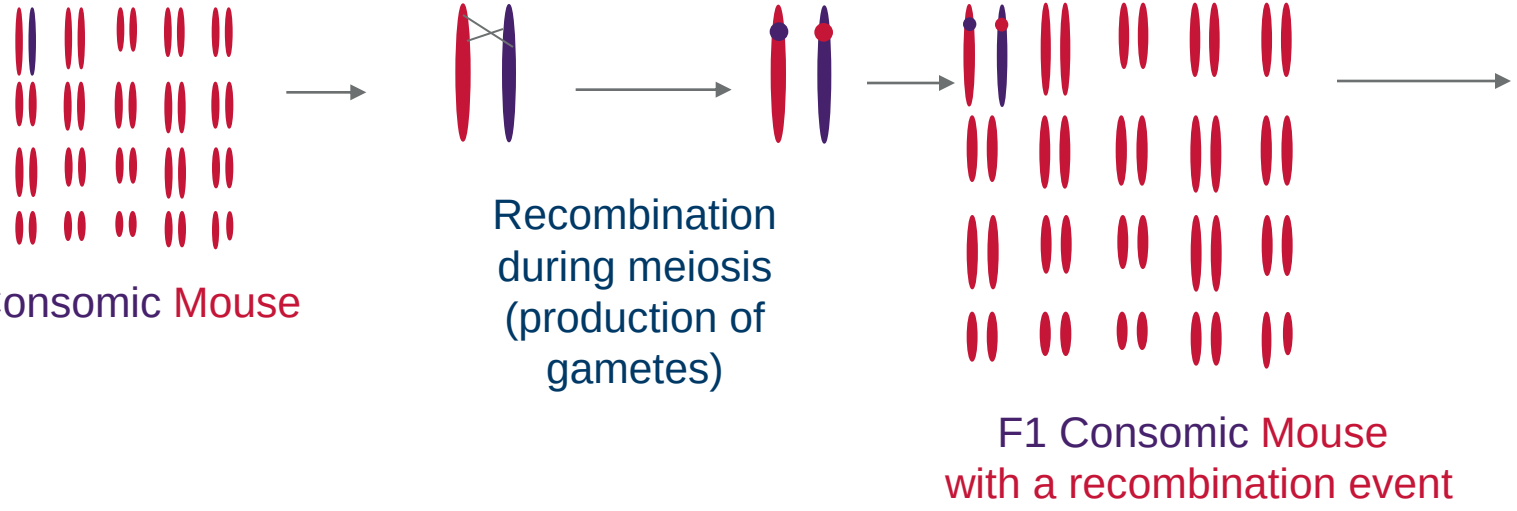
How do you make a congenic? More details…





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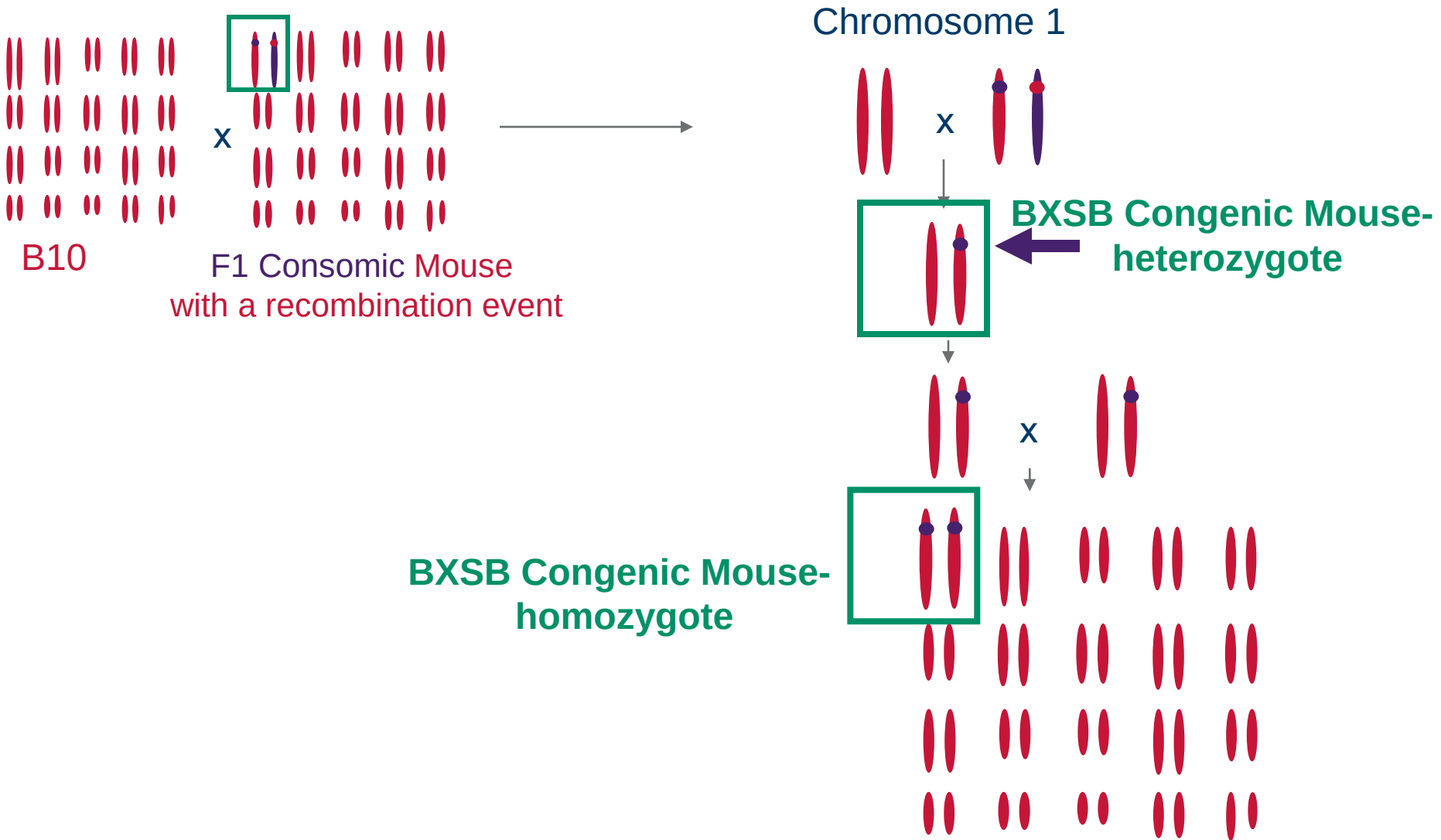
The Practical: Congenic (genetically modified) mice



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The Practical: Congenic (genetically modified) mice

BXSB Congenic Mouse-
homozygote