



Genome - Wide Linkage Mapping

Teacher instructions and answers

Introduction

This activity is based on the work of Dr. Christine Seidman et al that was published in *Circulation*, 1998, vol 97, pgs 2043-2048.

Students should be familiar with the following concepts before beginning this activity. This activity could be used to review the topics.

Patterns of inheritance – dominant, recessive, and sex-linked

Meiosis including crossing over

PCR – polymerase chain reaction

Gel electrophoresis

Step 1 - Constructing a Pedigree

If your students would like more information and examples of how to construct a pedigree, see accompanying handout.

Step 2 - Patterns of Inheritance of Human Disorders

Family	Percent affected	Sex of affected individuals	Pattern of inheritance
A	8/16	6 male, 2 female	dominant
B	2/20	1 male, 1 female	recessive
C	2/12	2 male, 0 female	sex-linked
ASD	10/19	7 male, 3 female	dominant

Now return to your family with ASD. Which pattern of inheritance does ASD exhibit? Record your results in the table.

dominant

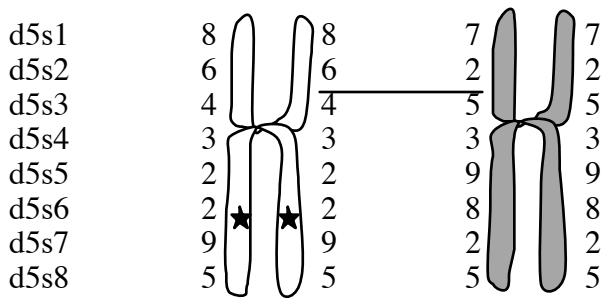
Next you will be looking for the location of the mutation leading to ASD in the human genome. Considering the type of inheritance ASD exhibits, will you look on the X chromosome or one of the other chromosomes?

Not the X chromosome

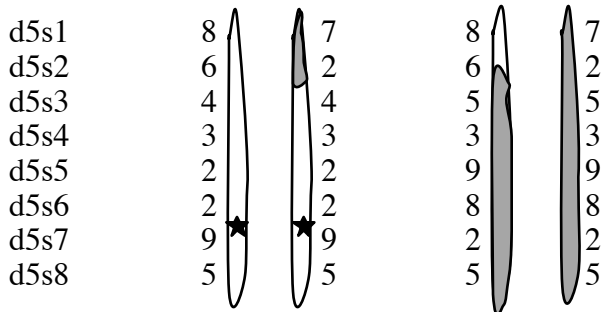
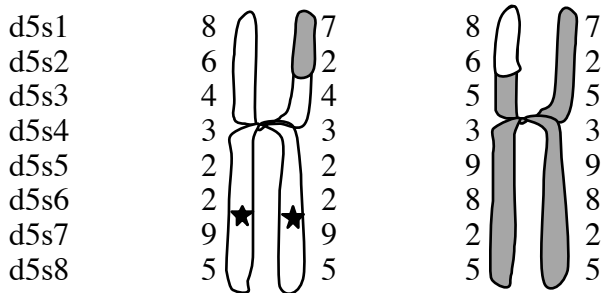
Again considering the type of inheritance ASD exhibits, will you be looking for one or two copies of the mutation in affected individuals?

One copy

Part IV – Segregation of STS Markers During Meiosis



Chromosomes have replicated and homologous chromosomes are aligned.



Questions

What marker(s) and repeat numbers are most closely linked to the mutation?

d5s6 – 2 repeats, d5s7 – 9 repeats

Next, compare the 4 products. Which markers and repeat numbers are inherited with the mutation on both chromosomes that contain the mutation

in this example?

d5s3 – 4 repeats, d5s4 – 3 repeats, d5s5 – 2 repeats

d5s6 – 2 repeats, d5s7 – 9 repeats, d5s8 – 5 repeats

in the example on the previous page?

d5s6 – 2 repeats, d5s7 – 9 repeats, d5s8 – 5 repeats

Note – the closer the mutation is to the marker, the more commonly it is inherited with the marker.

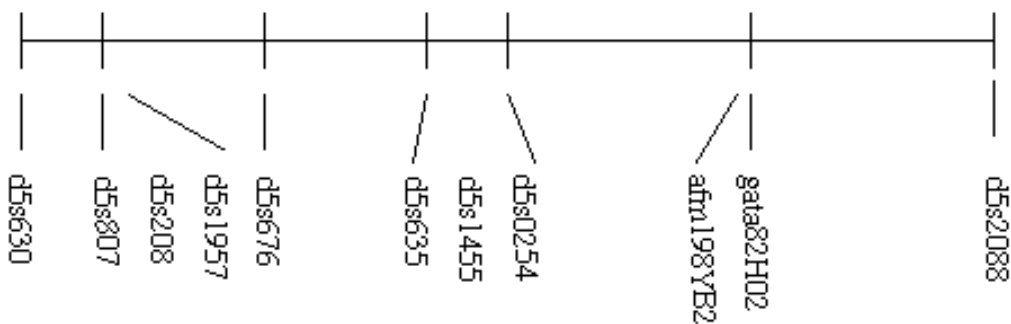
Part V – Determining the Number of Repeats by PCR and Gel Electrophoresis

- Record your data with the other class data (see attached data sheet).

A completed data sheet is attached

Part VI – Analyze the Data and Determine Linkage

Below is a map of the region of chromosome 5 you are examining. Note that the STS markers you have used are noted on the map. These are also the same markers found on the data table.



What series of markers and repeat numbers is inherited together with the mutation?

The mutation is between d5s635 and d5s807

d5s676, d5s1957, d5s208 are always inherited with the defect, however the mutation could be just outside these markers, hence the statement that the mutation is between d5s635 and d5s807.

Teacher prep for gel electrophoresis

- If your students want to know more about gel electrophoresis use the accompanying handout entitled “gel electrophoresis.”
- For general information on how to make, pour and run the gels, as well as general information on stain ordering and preparation see the accompanying handout entitled “Gel electrophoresis – teacher instructions”
- For specific sample prep for the samples used in this activity – see below
- Also, the pages that show what numbers of repeats are possible for each of the markers to be tested are included in this handout. These pages should be distributed to the students depending on which marker they run.
- The last two pages of the handout are marker sheets for your students to use as a key or standard of what numbers of repeats are possible and where they would run on a gel. They will need this sheet to understand the results of their gels.

Sample prep

The samples concern the following individuals and markers

	II-1	II-2	III-1	III-2	III-3	III-4	III-5
d5s635	1 5	1 5	1 1	1 1	5 5	5 5	5 5
d5s676	1 5	4 5	4 5	4 5	5 1	1 5	5 5

	II-3	II-4	II-5	III-6	III-7	III-8	II-6
d5s635	1 5	1 1	4 1	1 1	4 1	1 1	1 5
d5s676	5 1	5 5	5 5	5 5	5 5	5 5	5 5

Rack 1 – II-1 through III-5, d5s635

Rack 2 – II-3 through II-6. d5s635

Rack 3 - II-1 through III-5, d5s676

Rack 4 – II-3 through II-6. d5s676

Rack 1

Tube label Family member Marker tested	Number of repeats	Xylene Cyanol	Bromophenol blue	Orange G
Control d5s635	1, 4, or 5	Yes	Yes	Yes
II-1 d5s635	1 5	Yes	No	Yes
II-2 d5s635	1 5	Yes	No	Yes
III-1 d5s635	1 1	No	No	Yes
III-2 d5s635	1 1	No	No	Yes
III-3 d5s635	5 5	Yes	No	No
III-4 d5s635	5 5	Yes	No	No
III-5 d5s635	5 5	Yes	No	No

Rack 2

Tube label Family member Marker tested	Number of repeats	Xylene Cyanol	Bromophenol blue	Orange G
Control d5s635	1, 4, or 5	Yes	Yes	Yes
II-3 d5s635	1 5	Yes	No	Yes
II-4 d5s635	1 1	No	No	Yes
II-5 d5s635	4 1	No	Yes	Yes
III-6 d5s635	1 1	No	No	Yes
III-7 d5s635	4 1	No	Yes	Yes
III-8 d5s635	1 1	No	No	Yes
II-6 d5s635	1 5	Yes	No	Yes

Rack 3

Tube label Family member Marker tested	Number of repeats	Xylene Cyanol	Bromophenol blue	Orange G
Control d5s676	1, 4, or 5	Yes	Yes	Yes
II-1 d5s676	1 5	Yes	No	Yes
II-2 d5s676	4 5	Yes	Yes	No
III-1 d5s676	4 5	Yes	Yes	No
III-2 d5s676	4 5	Yes	Yes	No
III-3 d5s676	5 1	Yes	No	Yes
III-4 d5s676	1 5	Yes	No	Yes
III-5 d5s676	5 5	Yes	No	No

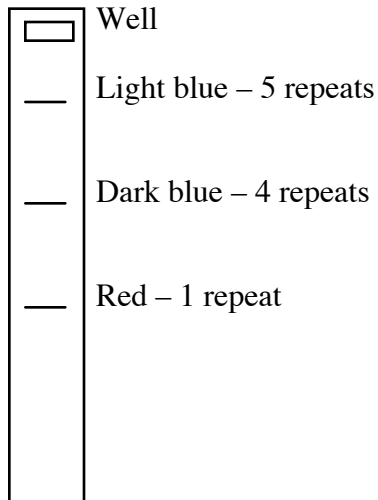
Rack 4

Tube label Family member Marker tested	Number of repeats	Xylene Cyanol	Bromophenol blue	Orange G
Control d5s676	1, 4, or 5	Yes	Yes	Yes
II-3 d5s676	5 1	Yes	No	Yes
II-4 d5s676	5 5	Yes	No	No
II-5 d5s676	5 5	Yes	No	No
III-6 d5s676	5 5	Yes	No	No
III-7 d5s676	5 5	Yes	No	No
III-8 d5s676	5 5	Yes	No	No
II-6 d5s676	5 5	Yes	No	No

STS Marker d5s635 – Chromosome 5

Three different numbers of repeats are found in the members of this extended family
1 repeat, 4 repeats and 5 repeats

A gel of the size standard would look as follows



STS Marker d5s676 – Chromosome 5

Three different numbers of repeats are found in the members of this extended family
1 repeat, 4 repeats and 5 repeats

A gel of the size standard would look as follows

