

# DICE & SLICE

## DAY ONE

You will receive two plates of the nematode worm *C.elegans*. One contains the wild type with the **dpy-13 (+)** gene. The other contains the mutant with the **dpy-13 (-)** gene.

Identify the difference between the **phenotype** of the worm with the **dpy-13 (+)** gene and the **phenotype** of the worm with the **dpy-13 (-)** gene. Write this in the boxes on the right.

Wild type dpy-13 (+)	Mutant dpy-13 (-)

### Identifying L4s

You need to identify the **L4 larval stage**. These are smaller than the adult and are characterized by a **light patch** about half way along the body. This is where the eggs will be released in the adult worm.

If you can't find any L4s, then transfer skinny worms instead.

### The media

You will receive 3 plates containing nematode medium on which *E. coli* (OP50) are growing. One of these plates is labeled L4440/IPTG. This one has a special *E. coli* that contains the L4440 plasmid and an inducer (IPTG) in the nematode medium. The other two have normal *E. coli* (OP50) and no inducer.

**Label these three plates:**  
**dpy-13 (+) /OP50 (L4440)**  
**dpy-13 (+) /OP50**  
**dpy-13 (-) /OP50**

**Put your initials and the date on all three plates.**

Transfer five L4 stage wild type **dpy-13 (+)** worms in to the OP50 (L4440) plate and five to a regular OP 50 plate.

Transfer five L4 stage mutant **dpy-13 (-)** worms to a regular OP50 plate.

You must sterilize the worm pick **each time you transfer a different worm type or transfer the same worm type into a different plate**. If you don't sterilize your pick, **your experiment will fail!**

This should give you the plates with the following combinations of worms and mediums:



*C. elegans* L4 larva, showing the characteristic light patch on the lower side of the worm, a little to the left of the middle of the worm. The head of the worm is to the right. An egg is also visible in the bottom left corner of the picture.

W O R M S	Wild type dpy-13 (+)	Wild type dpy-13 (+)	Mutant dpy-13 (-)
M E D I U M	OP50 with L4440 plasmid	OP50	OP 50

## DAY TWO

The first generation, which were the worms you put in the plates on the first day have been removed. You should now have a mixture of two generations: the adult **second generation**

What happens between Day One and Day Two?

(which reached maturity approximately 50 hours after being laid as eggs) and **L1s** (the first larval stage of **the third generation**, which are the offspring of the second generation).

You will receive three PCR tubes containing **Proteinase K + buffer**.

Label these tubes:

**R** for the **dpy-13 (+)** /OP50 (L4440)  
**W** for the wild type **dpy-13 (+)** /OP50  
**M** for the mutant **dpy-13 (-)** /OP50

For the following step, you must **sterilize the worm pick** between **R, W** and **M**. If you don't, **your experiment will fail!**

Remove the adult **dpy-13 (+)** / OP50 worms, **dpy-13 (+)** /OP50 (L4440), worms and **dpy-13 (-)** /OP50 worms from their plates and put them in the three tubes **R, W, M** containing **Proteinase K + buffer**.

1. Spin these in a centrifuge for 5 seconds.
2. Put them on dry ice for 10 minutes
3. Heat them to 65 deg.C in a water bath for 1 hour.

After they have been in the water bath for 1 hour, the worm digests will be heated for 15 minutes to 95 deg. C to denature the **Proteinase K enzyme**, and the product will be put in the freezer overnight.

[Preparing worms for PCR](#)

## DAY THREE

### PCR

The worm digests are put into the PCR machine (thermal cycler).

At the end of the final PCR cycle, the amplified product is put in the freezer overnight.

Digitally photograph the worms. Characterise and compare the worm types.

[DNA amplification by PCR](#)

## DAY FOUR

### DNA Electrophoresis

Pipette the **PCR product** from the three tubes into three electrophoresis gel wells.

For the following step, you must sterilize the worm pick **each time you transfer a different worm type or transfer the same worm type into a different plate**. If you don't sterilize your pick, **your experiment will fail!**

Label three new plates: **dpy-13 (+) RNAi /OP 50, dpy-13 (+) /OP 50, dpy-13 (-) /OP 50**. All these plates have regular OP 50.

The *E. coli* with the L4440 plasmid made dsRNA. This dsRNA was taken up by *C. elegans*.

*C. elegans* treats this dsRNA as if it were a virus infection and starts to dice & slice this and all matching RNAs. This includes the **mRNA** transcribed from the worms own **dpy-13 (+)** gene.

The wild type gene **dpy-13 (+)** is therefore 'silenced'.

This is the phenomenon of RNA interference, or **RNAi**

<b>W O R M S</b>	Wild type dpy-13 (+)	Wild type dpy-13 (+)	Mutant dpy-13 (-)
<b>M E D I U M</b>	OP50 with L4440 plasmid	OP50	OP 50
<b>T Y P E</b>			

**Predicted outcome (what you expect to see) after DNA electrophoresis**

Transfer five L4 worms from each of the three plates **dpy-13 (+) /OP 50 (L4440)**, **dpy-13 (+) /OP 50**, **dpy-13 (-) /OP 50**, to the three new plates.

[DNA electrophoresis](#)

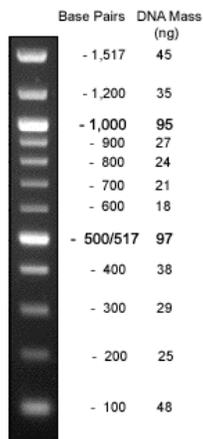


## DAY FIVE

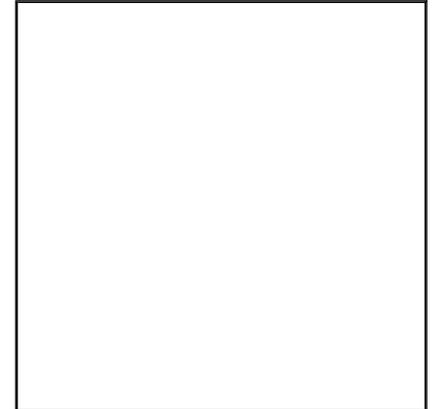
For the following step, you must sterilize the worm pick **each time you transfer a different worm type or transfer the same worm type into a different plate**. If you don't sterilize your pick, **your experiment will fail!**

Remove and discard all the adult worms from the new plates.

Analyse the data from the electrophoresis gels, using the 'ladder' to figure out the lengths of the DNA fragments.



**Observed outcome (what you actually saw) after DNA electrophoresis**



## DAY SIX

Observe and digitally photograph the new adult worms that have appeared on the three OP 50 plates since Day Five, and compare them with the photographs you took of the adult worms on Day Three.

Explain the relationship between the electrophoresis results and the worm phenotypes under the different feeding conditions.

# BLAST

Doing a BLAST search to find out the gene sequence for the dsRNA.

[BLAST = Basic Local Alignment Search Tool]

1. go to [www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)
2. select **BLAST** from the menu bar across the top of the page.
3. in the Nucleotide box, find

**Nucleotide-nucleotide BLAST (blastn )**

4. in the **Search** window, type in the following two sequences. Double check you have the correct sequences.

These are the sites the primers recognise, not the actual base sequence of the primers.

**AGTCGTCTTCTCCGTTATCG**  
**GAGCAACGCATAAGGCAAAG**

5. Go to **Options** and change *All organisms* to *Caenorhabditis elegans*.

6. Click on **BLAST!** When a new window opens, click on **Format!** to get your results. This may take a short while, but the results appear automatically.

The information is sent to the **National Center for Biotechnology** (NCBI) in Bethesda, Maryland. BLAST then tries to match these two sequences to the millions of sequences that it has stored in its DNA database.

The search engine tries to match the sequence you typed in in several ways. It tries both the **forwards and backwards versions of the sequence**. It also tries to find a match using the sequence **complementary to the sequence** you typed in, in both its forwards and backwards versions.

For example if you type in AAAGGG, the search engine would look for AAAGGG, but also for the backwards sequence GGGAAA, and also for the corresponding complementary sequences TTCCC and CCCTTT.

If any of these match it records a 'hit'.

## How to interpret the results

**BLAST** tells you if there are any 'hits'. That is, whether it has found matches for the two primer recognition site sequences (or their complements, either forwards or backwards).

When it finds a 'hit' it presents this in the following way.

```
Query  1  AAAGGG 6  ← The sequence you are looking for
      | | | | |
Sbjct  59  AAAGGG 65 ← What the search engine found
```

Here the letters are the same in the two rows, so this is a perfect match. The numbers at the beginning and end of the top row identify the first and last base you typed in. The number of the first base is 1. You typed in six bases, so the last number is 6.

The numbers on the bottom row identify the position of the hit in the genome, relative to a known starting point or origin.

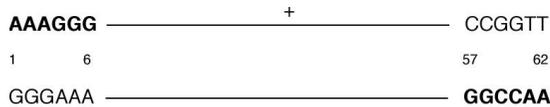
To see the complete nucleotide sequence (including the primer recognition sites and the bit in between, click on the blue underlined link beginning with **gi** at the left end of the hit.

This gives the complete **nucleotide base sequence**. The base sequence shown is the + strand. It also gives the amino acid sequence (using the one amino acid = one letter code) that would be the result of translating this base sequence.

Print out the base sequence and locate the two primer recognition sites on it. Remember, the second primer recognition site is actually on the - strand, so to locate it on the + strand, it must first be converted to its complement and reversed.

For example, If the second primer recognises **AACCGG**, then the search engine will find a

match with **CCGGTT** (the backwards complementary sequence).



The hit recorded on the screen however, is not shown as the backwards complementary sequence, but as the original forwards sequence. Because it is written AAGGCC on the screen and not GGCCAA, it begins with the higher number (e.g. 62) and finishes with the lower number (e.g. 57).

You also want to know if a matching sequence is likely to occur by chance. The likelihood of any match being due to chance decreases as the sequence gets longer. When long sequences are involved, hits are much less likely to be due to chance. The number at the right end of the hit is the **E-value**. The lower the **E-value**, the more likely you are to have a real hit.

Do two hits for two primer recognition sites always mean the bit between them is the gene-of-interest?

This is likely if they are close together, and unlikely if they are very far apart, or on different chromosomes! You can judge how far apart they are by calculating the distance between them.

The distance between the beginning of the first primer recognition site and the end of the second primer recognition site is calculated by subtracting the smallest number from the largest (e.g. 62 - 1), and adding 1.

[A sequence of ten numbers spans 1 to 10. But 10 - 1 = 9. So the total length is 9 + 1 = 10]

Calculate gene sizes in bp. for the two shorter versions of the wild type gene, one based on DNA and one based on mRNA.

What is the difference in length between the DNA version and the mRNA? Look at the amino acid sequence. Note that it is identical for both versions.

Explain this in terms of what happens to mRNA in eukaryotes? Identify the missing bits in the mRNA version. What name would you give to these missing bits?

### Looking for homologs in the human genome

1. Click on the link beginning with **gi** to get details of the gene you identified in the BLAST search. Near the top left of the page find the **Display** menu. Change the **Display** option to **FASTA**. This gives you the complete sequence which you can cut and paste into the BLAST search window.

2. Copy the nucleotide sequence for the DPY-13 (-) mutant gene.

3. Go back to **BLAST**, and in a new window paste the complete sequence.

4. Go to Options and change *All organisms* to *Homo sapiens*.

5. Click on **BLAST!** When the new window opens, click on **Format!** to get your results. This may take a short while, but the results appear automatically.

Length of dpy-13 (+) gene DNA version	Length of dpy-13 (+) gene mRNA version

6. Go to the second hit on the 'results of Blast' page and click on the blue underlined link

**gi** |14719826|ref|NM\_000088.2| for *Homo sapiens collagen, Type 1, alph*

7. Find a **named disease (syndrome)** associated with this base sequence.

8. *Google* this name to find out more about the disease. Describe its symptoms and figure out why it shares its symptoms with Dumpy 13 .

### To locate this gene on the human genome map

Copy the reference number in the **gi** string.

Go to [www.ncbi.nlm.nih.gov/mapview](http://www.ncbi.nlm.nih.gov/mapview)

Choose *Homo sapiens* (human) Build 36

Paste the reference number into Search for (delete the | at the end, if this is carried over)

Click on **Find**.  
This will identify the chromosome involved.  
Note its number.

Click on the number beneath the chromosome to see the actual locus of the gene on the chromosome.

Find the red line which identifies the locus of the gene. Click on the blue rectangle next to the red line in the Genes\_seq column to open the zoom window.

Click on Show 10M for maximum zoom.

Click on Show Sequence to see the complete sequence in place.

Chromosome number:

