

### Activity Overview:

The focus of the activity is to provide a clear understanding of the steps involved in Sanger Sequencing method. The key concept that students should be able to grasp after the activity is the process in which the four types of chain terminating dideoxynucleosides, including adenine, cytosine, guanine and thymine, emit different fluorescent dyes when terminated. The paper cutting exercise with different colors for the four nucleotides exhibit this process.

### Instructions:

1. Print out the activity worksheet for students
2. Explain the concept behind Sanger Sequencing
3. Ask the students to find the sequence that will be read

### For Teachers:

#### Sanger Sequencing Activity Sheet

### Answer:

5 T A C C A C T C G T T C C C G C T C C T C G A C A A G T 3

**For Students:**  
**Sanger Sequencing Activity Sheet**

**References**

Nucleotides:

A T G C

Dideoxynucleoside Terminators with Fluorescent Dyes:

A T G C

Primer Sequence:

C C G G G G

DNA template, primer, polymerase, NTPs, and **adenine** dNTPs added into Reaction Vessel 1.  
DNA template, primer, Polymerase, NTPs, and **thymine** dNTPs added into Reaction Vessel 2.  
DNA template, primer, Polymerase, NTPs, and **guanine** dNTPs added into Reaction Vessel 3.  
DNA template, primer, Polymerase, NTPs, and **cytosine** dNTPs added into Reaction Vessel 4.

Below are some of the resulting fragments inside the four reaction vessels:

3 I C A C C G G G G 5

3 G G C G A G G A G C T G T T C A C C G G G G 5

3 I G T T C A C C G G G G 5

3 A G C T G T T C A C C G G G G 5

3 I T C A C C G G G G 5

3 A T G G T G A G C A A G G G C G A G G A G C T G T T C A C C G G G G 5

3 A C C G G G G 5

3 G C T G T T C A C C G G G G 5

3 G A G C T G T T C A C C G G G G 5

3 C A C C G G G G 5

3 G C G A G G A G C T G T T C A C C G G G G 5

3 I G A G C A A G G G C G A G G A G C T G T T C A C C G G G G 5

3 C T G T T C A C C G G G G 5

3 A G C T G T T C A C C G G G G 5

Below is the result from polyacrylamide gel electrophoresis plate:

Reaction Vessel			
1 A	2 T	3 G	4 C
	_____		
_____			_____
			_____
_____			_____
	_____		_____
		_____	
	_____		
	_____		
			_____
			_____
			_____
		_____	
	_____		_____
			_____
	_____		_____
			_____
_____		_____	

Write down the template strand, which **does not** include the primer, that should be read.

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