Connect-A-Contig

Rope version

Abstract

Students align pieces of rope "DNA" based on the distance between "markers" to generate a DNA consensus sequence. The activity helps students see how fragments of tagged DNA can be used to make a physical map of a genome.

Learning Objectives

- To sequence or map long stretches of DNA, researchers first cut the DNA into shorter fragments, then piece them back together.
- Overlapping sequences or features on short segments of DNA can be used to assemble much longer contiguous DNA sequences, or "contigs."

Estimated time

- Set-up: 2–3 hours (one time only)
- Classroom implementation: 20-30 minutes

Materials

For all three contig sets (total cost is approximately $30):

- 50 feet (15 meters) of soft, flexible rope (we used ¼-inch braided nylon rope)
- Heavy-duty scissors
- Masking tape or clear tape (for sealing the rope ends)
- (Optional) Lighter or other source of flame
- Measuring tape or meter stick
- Colored tape (such as electrical or lab tape) in 7 different colors

Set-up Instructions

1. Cut rope into pieces according to the lengths below each photo. Tape or burn the ends to prevent unraveling. Keep each contig set separate.

   Tip: If you're using tape to seal the ends, first wrap the clear or masking tape tightly around the rope, then cut the rope through the middle of the taped area.

2. Create a consensus sequence. Mark the longest rope (marked "C" in the photos) with colored tape, approximating the colors and spacing shown.

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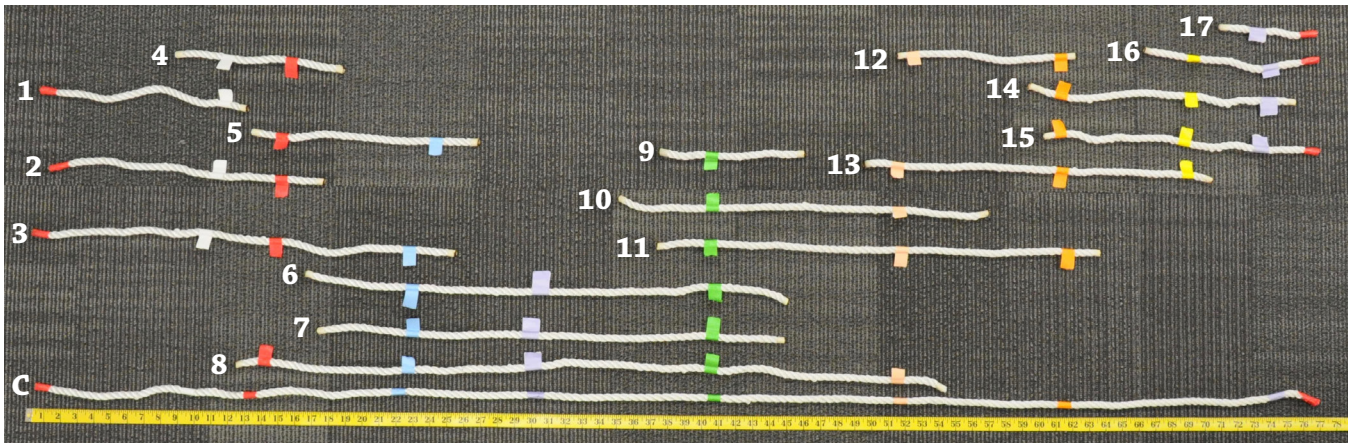
3. Arrange the smaller pieces of rope along the consensus sequence as shown in the photo. Mark the smaller pieces with tape to precisely match the color and spacing of the consensus sequence.

Tip: You don't need to exactly duplicate the lengths of the fragments shown in the photos. However, be sure that the fragments overlap, the coverage is consistent, and the spacing and colors of the markers on the fragments precisely match those on the consensus.

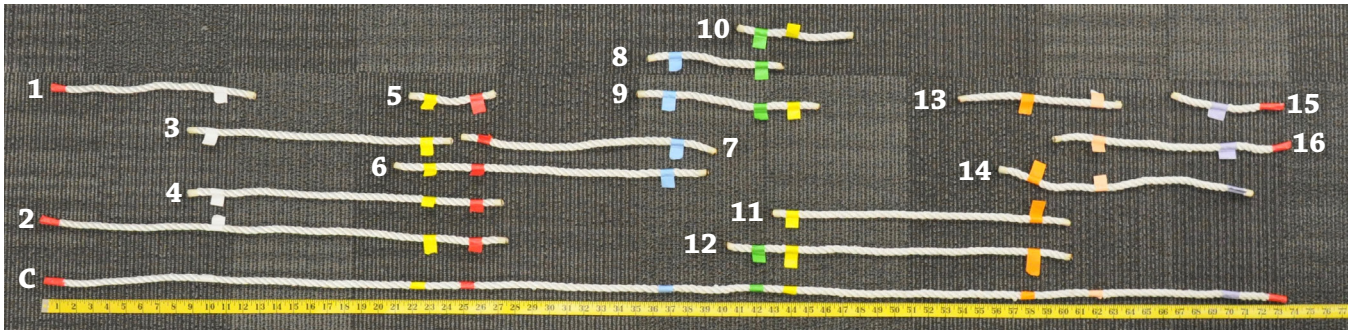


Lengths for Contig Set 1 — inches (cm)

Consensus: 77 (196)

Fragments:

1. 13 (33)
2. 16 (41)
3. 25 (64)
4. 10 (25)
5. 13 (33)
6. 29 (74)
7. 28 (71)
8. 42 (107)
9. 9 (23)
10. 22 (56)
11. 26 (66)
12. 11 (28)
13. 20 (51)
14. 16 (41)
15. 16 (41)
16. 11 (28)
17. 6 (15)



Lengths for Contig Set 2 — inches (cm)

Consensus: 74 (188)

Fragments:

1. 12 (31)
2. 28 (71)
3. 16 (41)
4. 19 (48)
5. 5 (13)
6. 19 (48)
7. 15 (38)
8. 8 (20)
9. 11 (28)
10. 7 (18)
11. 18 (46)
12. 21 (53)
13. 10 (25)
14. 15 (38)
15. 7 (18)
16. 14 (36)

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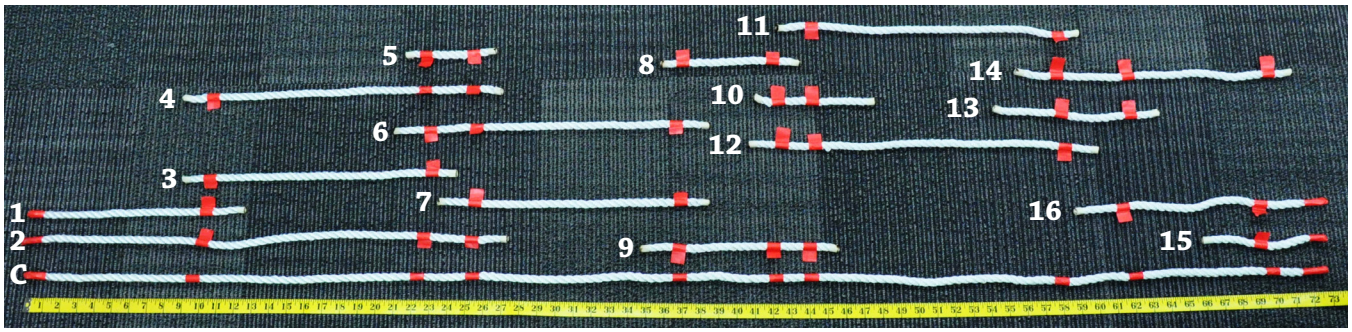

Contig Set 3

The lengths of the fragments and the spacing of the markers are the same as for Set 2, but Set 3 has just one color of marker tape.

Classroom Implementation

The rope contig sets can be used as a whole-classroom demonstration, or they can be given to students to solve in small groups or individually. You may wish to first demonstrate this rope-based activity and then have students complete the paper version of Connect-A-Contig and/or the online contig-building activity.

1. Introduce the activity by reviewing relevant content from the Genome Mapping web page: http://learn.genetics.utah.edu/content/cotton/genome/

   Be sure to cover the following key ideas:

   - Whole chromosomes are made of single DNA molecules that are many millions of base pairs long. With current technology, the longest pieces of DNA we can "read" are much shorter than that—up to several thousand base pairs long.
   - In order to map a whole chromosome (or other very long stretches of DNA), researchers first collect many copies of it, then they break the copies randomly into smaller fragments. They read the fragments, and then they use computer software to put the pieces together into a contig (short for "contiguous") to get a full-length consensus sequence.

2. Distribute contig sets. Explain that the students will act like the software programs that researchers use to assemble genomes. They will need to match up the spacing of the markers (colored tape) on the overlapping shorter lengths of DNA to build a "contig."

   For an easier exercise, include the consensus sequence in each contig set. For an extra challenge, leave it out.

3. If students have trouble, consider offering the following hints:

   - Begin by straightening each rope and laying them horizontally.
   - Choose a longer rope, then find markers on other ropes that overlap with it.
   - If you don't see where a rope fits, try flipping it around 180 degrees.

4. Students should condense their contig solution into a "consensus sequence." Display the solution to each contig set and ask students to compare their answers to it.

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Discuss

Which pieces of DNA are the most informative? Why? *(Answer: the longer ones, because they contain information about the spacing of more markers.)*

If you have a reference sequence (i.e., a consensus), it's easier to align other tagged sequences to it than to build a reference sequence from scratch. *(Using only the fragments is like sequencing a genome for the first time. Using a consensus sequence is like comparing the genomic sequence of a new individual to one that has been sequenced previously—the sequences will have some differences, but they will be largely identical (about 99.9% for people).*

Explore the concept of "depth of coverage" (the number of fragments that cover a particular span of the contig). Where is the greatest depth of coverage? Where is the least depth of coverage?

What do the tape tags on the rope represent? *(Answer: a specific DNA sequence. In the case of optical mapping, which this exercise approximates, they represent restriction enzyme sites—specific, short DNA sequences where a restriction enzyme will nick the DNA and add a fluorescent tag.)*

Was it easier to assemble fragments that had multiple types of markers vs. just one type? Why? *(Answer: with just one type of marker, you have only the spacing of the fragments to guide you. With multiple colors, you can use both the color and the spacing.)*

Assembling contigs out of DNA sequences (strings of As, Cs, Gs, and Ts) follows the same principle: instead of using markers, you line up fragments by overlapping DNA sequences.

Variations

To explore more concepts, you can build additional contig sets:

- Fragments consisting of long pieces of rope—24 inches (60 cm) or longer—will be easier to assemble.
- Fragments consisting of short pieces of rope—10 inches (25 cm) or shorter—will be harder to assemble. For equal depth of coverage to the above, you will need many more fragments.
- Include an "outlier" fragment, where the markers do not line up properly with the consensus. Sometimes, non-adjacent DNA fragments will join together. If you have enough depth of coverage, a fragment like this will stand out as an obvious outlier, but if depth of coverage is poor, it can cause a problem with the consensus sequence.

Credits

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