6.3: Two-dimensional gels to analyze the number and position of replication origins

For the most common replicative structure, in which both strands are replicated simultaneously to form replication “eyes”, replication origins can be mapped on the basis of the shapes of origin-containing fragments. If a population of replicating DNA molecules is cleaved by restriction endonucleases, the resulting fragments will have distinctive shapes depending on whether or not they contain a replication origin (Figure 6.5). A restriction fragment encompassing the origin will form a replication bubble, whereas other DNA fragments without origins will have a replication fork moving through them and thus will have a Y-shape.

**Figure 6.5.** Replicating molecules have different shapes generated by replication bubbles and forks. The simple Y results from a single replication fork moving through the DNA fragment, whereas the double Y results from two forks moving in opposite directions through the DNA fragment. Movement of a replication fork from an origin generates a bubble. If the origin is centrally located in the DNA fragment, all or almost all of the replication intermediates will contain a bubble (illustrated in the second panel). Under some circumstances, such as when an origin is close to one end or when fork movement is unidirectional, one fork can reach one end before the other. In this case, early replicate structures have a bubble and later replicated structures have a Y (illustrated in the fourth panel).

In 1987, Brewer and Fangman introduced the use of two-dimensional agarose gels (2-D gels) to distinguish these shapes and thereby map origins of replication. The key experimental advance was to design electrophoretic conditions
that would resolve the nonlinear, replicating DNA molecules from the linear, nonreplicating molecules. This was accomplished by using two-dimensional agarose gels. The first dimension is a conventional separation by size. In the second dimension (run perpendicular to the first) the molecules are separated mainly on the basis of their shape. Nonlinear DNA molecules move anomalously on agarose gels when compared to linear DNA. This anomalous migration is accentuated by increasing the voltage and concentration of agarose, so that deviation from a linear rod-shape gives a much slower mobility. In practice, the first dimension is run in 0.5% agarose at 1 v/cm, and the second dimension is run in 1.0% agarose at 8 v/cm, and with ethidium bromide.

Replicating DNA molecules are isolated (e.g. from rapidly growing cells in culture), cleaved with restriction endonucleases and separated on a two-dimensional agarose gel. In the gel, all the fragments of the chromosome are present, but particular fragments from a digest can be visualized by Southern blot-hybridization, using the particular fragment as a hybridization probe.

If the hybridization probe is a DNA fragment containing an origin, it will reveal a series of DNA fragments containing bubbles of different sizes. As illustrated in Figure 6.6A, molecules that have just initiated replication are smaller and will move fast in the first dimension. They will also have a small replication bubble, and hence they will move fast in the second dimension. However, those with more extensive replication will have a larger bubble. These molecules are larger, and thus move more slowly in the first dimension, but importantly, the larger bubbles will move even more slowly in the second dimension, since they have the greater deviation from linearity. This generates a characteristic "bubble arc" on the two-dimensional gel. The mobility expected for linear molecules increasing in size from one unit to two is shown as a dashed line in Figure 6.6, so that the deviations from linearity can be seen more clearly.

![Figure 6.6.A](https://bio.libretexts.org/Bookshelves/Genetics/Book%3A_Working_with_Molecular_Genetics_(Hardison)/Unit_II%3A_Replica…)

**Figure 6.6.A.** Bubble arcs on 2-D gels. A DNA fragment containing a replication origin will have one or two replication forks moving through it, generating bubbles of increasing size. When such a fragment is used as a hybridization probe, the population of origin-containing fragments will be detected as bubble arcs on two-dimensional gels.

A DNA fragment without an origin will be copied by a replication fork moving through it. Hence it will generate a series of "Y" shapes on the two-dimensional gels. A Y with a fork in the middle of the fragment gives a very slow mobility in the second dimension, because of its large deviation from linearity. In contrast, a small Y at one end or a Y almost at the end of the fragment moves essentially like a linear rod. Hence the "Y arc" on the two-dimensional gels starts on the diagonal expected for simple linear molecules (unit length), moves through an arc with an apex at 1.5 unit lengths (the slowest mobility in the second dimension) an returns to the diagonal at 2 unit lengths.
Similar logic applies to fragments with replication forks coming in from both sides ("double Y arc"), which shows the approximate position of a terminus (Figure 6.6.C, third panel). Also, fragments in which the origin is off-center (an asymmetric arc, which is a combination between a "bubble arc" and a "Y arc") allow one to map the position of the origin precisely (Figure 6.6.C, fourth panel). It can be calculated from the site where the "bubble arc" shifts to a "Y arc".

**Figure 6.6.B.** Y-arcs on two-dimensional gels of replicating molecules.

**Question 6.4.** A restriction map is shown for a portion of a chromosome below, along with the patterns on two-dimensional gels for the replication intermediates formed by each fragment. Where are the origins and termini? Can you deduce the direction of replication fork movement?

**Restriction fragments:**

**Question 6.5.** How can you calculate the position of an origin within a DNA fragment from an asymmetric fork/bubble pattern on a 2-D gel of replicating DNA molecules?
Contributors

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