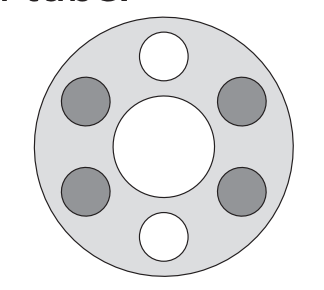
**Lab 3: Building the pARA-R Plasmid: Ligation**

1. Place the K+ and A+ tubes from Lab 2 into a float rack and into the 80°C water bath for 20 minutes. Alternatively place the tubes directly into the 80°C heat block. CAUTION: 80°C is very hot, use caution!

2. Label the LIG tube with your group and period ID.

3. After 20 minutes, remove the K+ and A+ tubes. Quickly spin them down to pool all reagents.



4. Using a new tip for each reagent, add the following directly to the solution at the bottom of the LIG tube:

4.0µL of A+

4.0µL of K+

3.0µL of 5xB

2.0µL of dH20

5. After adding the last reagent, gently pump the solution up and down to mix with the pipette. Cap the tube.

6. Quickly spin the LIG tube down to pool all reagents.

7. Place your LIG, K+ and A+ tubes in the microfuge racks designated by your teacher. The LIG tube needs to incubate at room temperature for at least 20 minutes. Your A+ and K+ tubes will be returned to the freezer for Lab 4.