Biology Hour\_\_\_\_\_ Name\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
Wexler/Fennelly
Extraction of DNA from Wheat Germ
Date:

**Instructions:**

1. Place 1 gram of raw wheat germ in a 50ml centrifuge tube
2. Add 20 ml of TE and mix by inversion for 2 minutes.

*Note: TE contains Tris buffer and EDTA. The EDTA binds divalent cations (Mg2+ and Zn2+), preventing nucleases from attacking the DNA when the cells are lysed.*
3. Add 1 ml of dishwashing detergent and mix gently by inversion for 3 minutes. Try not to create foam.
Detergents dissolve the cell membrane and nuclear membrane, releasing the DNA.
4. Centrifuge for 2 minutes to pellet the lysed wheat germ solids.
5. Carefully pipet the DNA-containing supernatant into a clean 50mL centrifuge tube.

**Questions: What is the consistency of the supernatant when you pipet it? Why is it like that? (hint – it is due to one aspect of the structure of DNA).**

1. Gently layer two volumes of 95% ethanol.
2. Observe the precipitation of the DNA. What do you see?
3. Gather the DNA with a hook and transfer it to a 15mL tube containing 3 mL of TE buffer.
4. Allow the DNA to dissolve without shaking (gentle rotation of the tube is OK).
5. Add 10ul DNA to 10ul Gel Red on a square of parafilm.
6. Observe fluorescence of the drop – this indicates that DNA is present.
7. Mix 20µl of your DNA prep with 5 ul of 5X sample solution.
8. Load into a well of an agarose minigel containing Gel Red. The electrophoresis buffer also contains Gel Red.
9. After electrophoresis, observe the gel under UV light.

**Questions:** **What do you see?**

**What does this mean in terms of the size of the DNA and its degree of intactness?**