**Tips for staining for pErk**

For pErk staining, the following tips should be followed to get optimal staining:

1. Unless the experimental setup prohibits it, stain ovarioles from flies that have eclosed no more than three days prior to dissection.
2. It is important to feed the flies fresh, very wet yeast for at least two days prior to dissection. Do not keep a large amount of flies in each bottle (20-40 flies per bottle – or 10-20 flies per vial).
3. Feed flies fresh wet yeast 4-6 hours before dissecting*. 🡨 This is probably the most important step!!!*
4. Place dissected, teased-apart ovaries into fixing solution as soon as possible (do not leave sitting in Grace’s Medium).
5. It does not make a difference whether dissected on ice, but I do use chilled Grace’s Medium.