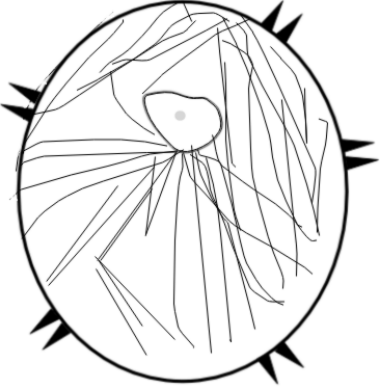
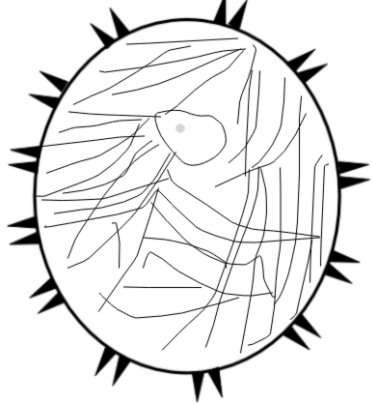
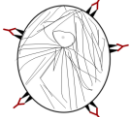
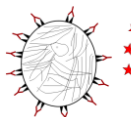
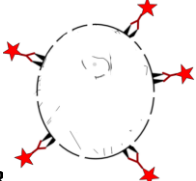
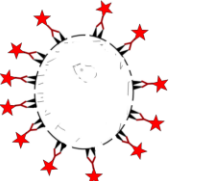






Modeling Cell Staining: Using fluorescent stain to detect overexpressed mER TEACHER ANSWER KEY
Shoreline Biotech Experience Cancer Biology Kit

Compare results of Staining

Breast Cancer Cell expressing normal levels of mER [Low levels mER]	Breast Cancer Cell over-expressing mER [High Levels mER]
	
<p>Staining protocol:</p> <p>A. Stain the cell membrane with an antibody (Ab) that is specific for mER. This will have a red fluorescent tag.</p> <p>NOTE: Excess Ab is washed away during this step</p>	<p>How to model:</p> <p>A. Draw 'Y' shaped Ab molecules binding to all mER proteins found on each cell. Be sure to draw "arms" of the 'Y' to the antibody. Draw a red star on the other end of the 'Y' Ab symbol to represent the red fluorescent tag.</p> <div style="display: flex; justify-content: space-around; align-items: center;">   </div> <p>Low mER High mER</p>
<p>B. Permeabilize the cell (using a mild detergent solution) to allow the stain that binds to actin and the stain that binds to DNA to enter the cell.</p> <p>Cells are again washed after this step.</p>	<p>B. Use white out or similar to represent holes the form in both the cell membrane and the nuclear membrane.</p> <div style="display: flex; justify-content: space-around; align-items: center;">   </div> <p>Low mER high mER</p>
<p>C. Add the actin stain, which is labeled with a green fluorescent protein.</p> <p>Excess stain will be washed away.</p>	<p>C. Stain the actin filaments green throughout the cytoplasm of the cell.</p> <div style="display: flex; justify-content: space-around; align-items: center;">   </div> <p>Low mER high mER</p>
<p>D. Add the nucleic acid stain, which fluoresces blue.</p>	<p>D. Color the nucleus with blue pencil.</p> <div style="display: flex; justify-content: space-around; align-items: center;">   </div> <p>Low mER high mER</p>

E. Staining is complete.	
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Analysis

- 1) Compare the two cells.

The cell that is over-expressing mER has a lot of red stain on the cell membrane (binding to mER), which can be visualized with a fluorescence microscope. The cell expressing normal levels of mER has so little fluorescently tagged anti-mER antibody binding to it that you cannot detect it with a fluorescence microscope. You can see the actin and nuclei in both types of cells.

- 2) When observing actual slides, what do you think a cell with very low levels of mER would look like?
You would expect to see green-stained actin filaments and blue stained nuclei, but very little red fluorescence.

- 3) What does it likely mean if cells are stained red.
That cell expresses a large quantity of Estrogen Receptor on its membrane. High levels of membrane-bound Estrogen Receptor (mER) can be a cause of breast cancer.

- 4) Which cell parts are clearly visible on both types of cells?
Actin (cytoskeleton) and nuclei

- 5) How do you distinguish a breast cancer cell that expresses 'normal' levels of mER from a breast cancer cell that over-expresses mER using a fluorescent microscope?
Breast cancer cells expressing low (normal) levels of mER will not appear red in the fluorescent image since there is so little red 'signal' to detect. The cause of the breast cancer in these patients is something other than over-expression of mER.
Breast cancer cells that over-express mER will have patches of bright red staining in the fluorescent image.

- 6) How could you modify this fluorescent staining protocol to look for the over-expression of other receptors that might lead to the development of other types of cancer?
There are many other types of receptors that are involved in signaling the cell to grow and divide. Over-expression of some of these receptors has been shown to be a cause of other types of cancer. Staining cells with a fluorescently tagged antibody specific for one of these other membrane-expressed receptors would help identify patients whose cancer could be caused by over-expression of that receptor.
Example: Over-expression of Epidermal Growth Factor Receptor (EGFR) on the cell membrane correlates with poor prognosis in ovarian, cervical, bladder cancers and Non-Small Cell Lung cancer (NSCLC).