

Protocol for inducing *cep290* mutant cells to grow flagella (courtesy Branch Craige):

Chlamydomonas cep290 mutant cells are palmelloid and must be induced to grow flagella (Craige et al., 2010). The most straightforward way to accomplish this is to hatch the cells with autolysin. (The autolysin must be high quality for this to work well – one protocol for making autolysin is at <https://www.chlamycollection.org/methods/preparation-of-gamete-autolysin-improved-protocol/>). We have had better results when the cells are grown in M medium; Like some other strains, this mutant does not flagellate very well if cultured in TAP medium. Here is the protocol:

1. Culture cells in M medium (Witman, 1986) until the culture is fairly dense but not stationary. We have had best results when aeration was used for the first 3 days of culturing, then aeration was stopped for another 2-3 days.
2. Pellet the cells by centrifugation.
3. Wash the cells in 10 mM HEPES pH 7.4; pellet the cells and repeat the wash 2 X.
4. Resuspend the cell pellet in autolysin. The volume of autolysin needs to be at least 20 X the volume of the cell pellet. For example, if the cell pellet is 0.5 ml, you should use at least 10 ml autolysin, preferably more (20-30 ml).
5. Monitor the cells for hatching and growth of flagella. If successful, the cells will be freed from the mother cell wall within 5-10 min, and ~50% of the cells will show signs of motility within 30-60 min. If the cells are still palmelloid after 10 min, pellet the cells and resuspend in a new volume of fresh autolysin and/or try using a larger volume of fresh autolysin.
6. After ~ 1-2 hrs, ~50% of the cells should be motile. Approximately half of the cells will never grow flagella. We usually harvested the flagella ~3 hrs after resuspending cells in autolysin.

The most important things are that the autolysin be of high-quality, and that sufficient amounts of it be used to resuspend the cells.

References:

Craige, B., C.-C. Tsao, D. R. Diener, Y. Hou, K.-F. Lechtreck, J. L. Rosenbaum, and G. B. Witman. 2010. CEP290 tethers flagellar transition zone microtubules to the membrane and regulates flagellar protein content. *J. Cell Biol.* 190: 927-940. (PMCID: PMC2935561).

Witman, G. B. 1986. Isolation of *Chlamydomonas* flagella and flagellar axonemes. *Meth. Enzymol.* 134: 280-290.