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What a long trip it's been: Trying to create a CSN-5 and GLH-1 mutant

It is known in *Caenorhabditis elegans* that non-membrane-bound cytoplasmic complexes called P granules are specific to the germline lineage. P granules contain four GLH proteins, germline RNA helicases, which are critical to the development of the germline lineage and the fertility of the nematode. The CSN-5, KGB-1, ZYX-1, and PAN-1 proteins all interact with the GLH proteins as found with a yeast two-hybrid screen, with CSN-5 and KGB-1 both critical for meiotic germline development. The Bennett lab has not been able to isolate a mutant csn-5, nor have they been able to create a knockout glh-1 mutant strain that is null. This summer we have been attempting to create both a new glh-1and a csn-5 mutant strain. In looking for the csn-5 and glh-1 deletion strains, I have been using a library technique that has been successfully used in the Bennett lab for several years. C. elegans wild type worms are grown to adults on plates and their eggs are harvested. Approximately 500,000-600,000 of these eggs are hatched and mutagenized by exposure to the chemical trimethylpsoralen and UV light. After 24 hours, 750,000-1 million progeny of the mutagenized worms are collected and distributed among 960 plates. Once adults, DNA is isolated from some of the worms on each plate and tested by PCR looking for a deletion band smaller than the wild type. However, this summer the PCR and gel electrophoresis have not quite gone as planned. Many problems arose with primers, solutions, and with the DNA. Much time has been spent trouble shooting. Recently gels have been working and three potential deletions were found and tested. Upon confirmation, it was discovered that none of the three are the deletions we were looking for.