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The attachment of a nuclear localization signal and a peroxisome targeting signal to mCherry and the effect on the localization of mCherry in a MDA-MB-231 cell line

While target peptides themselves have been studied widely, dual targeting and prioritization of signals in cellular trafficking is relatively unstudied. The goal of this experiment was to determine the localization of a mCherry protein that contained both a nuclear localization signal (NLSSV40) and a peroxisome transit signal (PTS1) in a MDA-MB-231 cell line.

Oligonucleotides were designed for each target peptide that contained the sequence for the target peptide and a restriction enzyme site in order to confirm the clones. They were allowed to anneal, leaving overhangs that corresponded with restriction site overhangs in the multiple cloning site and in the mCherry protein coding sequence. The annealed oligonucleotides were added to the digested plasmids and ligated. Two very similar plasmids were used for this – the PTS1 was added to the plasmid with the multiple cloning site (MCS) on the carboxy terminus of the protein and NLSSV40 was added to the plasmid with the MCS on the amino terminus.

As of right now, both the plasmid containing NLSSV40 and the one containing PTS1 have been successfully cloned. The next step in this research is to confirm the efficacy of the individual target peptides by transfecting the MDA-MB-231 cell line with the plasmids and staining with DAPI and a peroxisome labeling kit in order to visualize the localization of mCherry in the cells. Then, the plasmids containing the NLSSV40 and PTS1 target peptides will be combined into one, and the protein will be expressed in a MDA-MB-231 cell line. This will be done by digesting both plasmids with two single cutter restriction enzymes and ligating the fragments that contain the target peptides together. This research can help improve the understanding of eukaryotic cellular processes overall, more specifically with the targeting of proteins to different organelles.

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