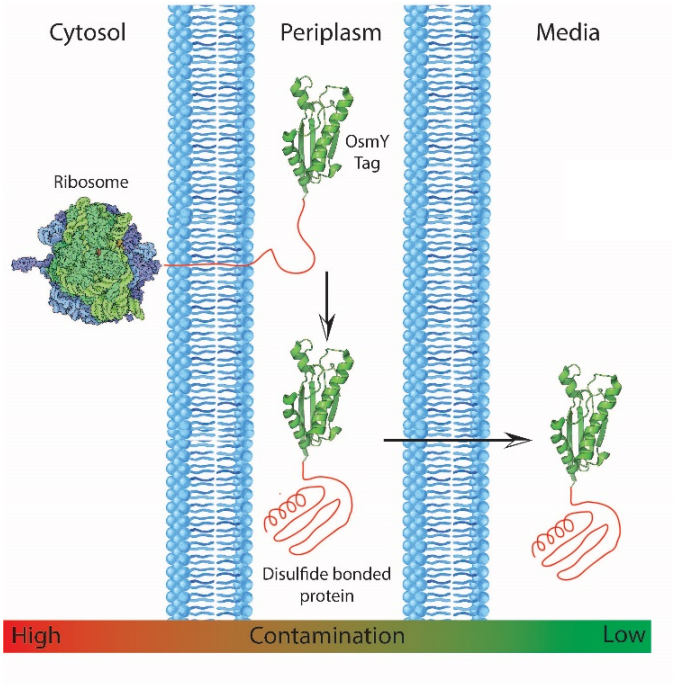
Our Solution

Bacteria Aimed at Removing and Terminating Intestinal Invaders

Gliadin Degrader

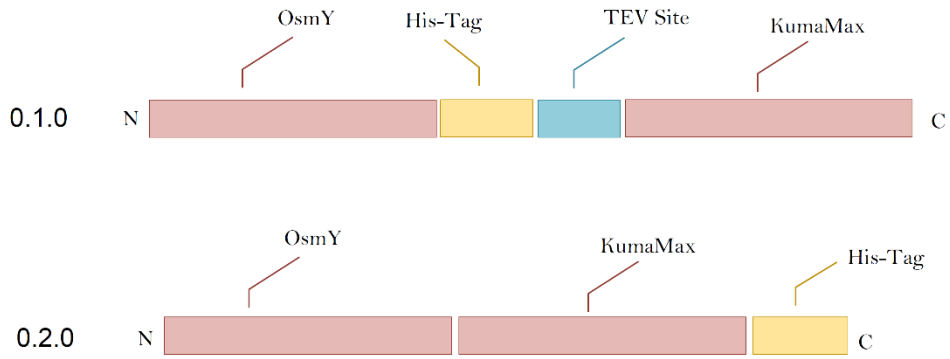
The first thing to do was determine an enzyme that would 'break down' gliadin and one of the first ones we came across was something called KumaMax which was developed by WashU's iGEM team in 2011. We decided to use this enzyme because it was shown to be quite effective but now the next step was to improve it for our use. Searching for a way to secrete this enzyme was the first battle as we had to retain biological activity after it is secreted. We determined that a protein called OsmY, which has been shown in past iGEM projects, could be used to transport a fusion construct through both the inner and outer membrane. This method relies on a ABC transport system and that system is what recognizes the signal peptide that OsmY possesses.

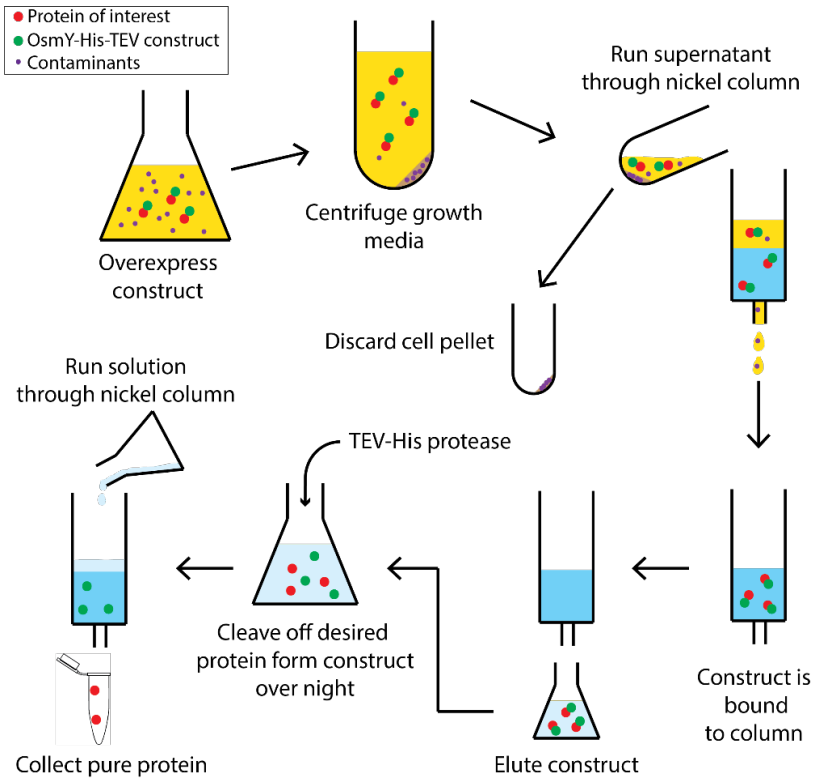


Credit: iGEM_UMich_2014

We finalized two designs that are very similar on paper but serve different purposes. 0.1.0 would be used to test the effectiveness of OsmY as a transporter and give us the ability to test the activity of the enzyme. It would be tested by running a Nickel column that binds to the his-tag and then the construct would be eluted. You would then cleave the construct with TEV protease and it would cut at the designed TEV Site. Running the solution through a nickel column again would allow the pure enzyme to pass straight through since there isn't a his-tag attach to it, only the OsmY. 0.2.0 is simpler in that we plan to just produce the protein and see how much could be autocatalytically cleaved as OsmY has been shown cases depending on the fusion protein. Purification would be done with a nickel column and a SDS-PAGE would be done to determine the extent of auto-cleaving.

CDS Breakdown





0.1.0 Testing Protocol
Credit: GEM_UMich_2014