**Safety First**

- Vectors with PR for screening (BBa_K572100) and expression (BBa_K572000)
- Normal
- Prototrophic expression bacteria
- Can rescue the cell
- Entire genome is cloned to provide any desired advantage

**Antibiotic Resistance Marker**

- PR
- High growth rate
- Resistant to Kanamycin

**Proteorhodopsin Marker**

- PR even if horizontally transferred does not have any harmful effects

- PR based selection can be prolonged using endogenous Retinal production
- PR based selection can be prolonged using endogenous retinal production
- Environment friendly

**Toxic and unsafe**

- Retinal – not cytotoxic
- Antibiotics degrade after 16 hours
- PR released into the environment

**Parts Design**

- BBa_K572000: Carbon Stress Response Indicator
- BBa_K572200: Protein expression
- BBa_K572201: Protein expression

**Experimental Results**

- Growth rate is seen to decrease with decreasing substrate concentration
- Protein expression increases at low substrate concentrations

**Conclusions**

- Carbon stress expression indicator (BBa_K572200) characterized and works as expected
- Proteorhodopsin coding gene (BBa_K572005) modified to Parts Registry Standard 10, characterized and works as expected
- CP1 cassette from K572009 modified to Parts Registry standard 10 and submitted
- Improved and characterized existing part PCK promoter (BBa_K118101) activity
- Created software tool for generating Site Directed Mutagenesis primers for silent mutation of Parts Registry Standard restriction enzyme sites from coding sequence
- Designed a computational model and validated that PR confers metabolic advantage to cells under carbon stress
- Designed plasmids with PR selectivity marker

**Way Forward**

- Proteorhodopsin in B.subtilis and Agrobacterium sp for
  - Hyaluronic acid Production
  - Curcumin production
- Anaerobic phased solid digestor for PR expressing bacteria
- Characterize Blue Light Absorbing PR

**References**