Inoculation of *Komagataeibacter rhaeticus*

Introduction

This protocol inoculates *Komagataeibacter rhaeticus iGEM* on Hestrin Schramm media for further growth. The growth period of *Komagataeibacter rhaeticus iGEM* is approximately 6-10 days.

Reagents

- 250 mL Hestrin Schramm (HS) media
- 5 mL of 5 mL *K. rhaeticus* liquid culture
- 5 mL of 10 mL *K. rhaeticus* liquid culture
- Sample of a *K. rhaeticus* plated colony

Equipment

- 3 250 mL Erlenmeyer flasks
- Pipetboy
- Aluminum foil

Procedure

1. Split the 250 mL HS media evenly between 3 separate 250mL Erlenmeyer flasks (about 85mL).
2. Acquire 10 mL *K. rhaeticus* liquid culture and 5 mL *K. rhaeticus* liquid culture and prepare them for transfer by pipetting them up and down to mix.
3. Add 5 mL of the 10 mL *K. rhaeticus* liquid culture to one flask, 5 mL of the 5 mL *K. rhaeticus* liquid culture to the second flask, and the sample of a plated bacterial cellulose colony to the third flask.
4. Cover flasks with aluminum foil and incubate at 30°C without shaking.