

POTASSIUM SENSOR MODULE: LAB NOTEBOOK WEEKLY SUMMARY

Week 1 (1/6/2015-5/6/2015)

1. Summer training about the basic techniques
2. Project plans discussion

Week 2 (8/6/2015-12/6/2015)

1. Ligation of pSB1C3-*P_{kdpF}*-WT RBS
2. Ligated *P_{kdpF}*-WT RBS into pSB1C3-BBa_I13401

Week 3 (15/6/2015-19/6/2015)

1. Sent a request to get TK2240 strain
2. Ordered the WT *P_{kdpF}* without WT RBS and *P_{kdpF}* [-15, T>G] oligos
3. Preliminary confirmed the construct pSB1C3-WT *P_{kdpF}*-WT RBS-BBa_I13401
4. Sent the construct pSB1C3-WT *P_{kdpF}*-WT RBS-BBa_I13401 for sequencing

Week 4 (22/6/2015-26/6/2015)

1. The TK2240 strain has arrived. Made the glycerol stock
2. Ligated WT *P_{kdpF}* into pSB1C3-BBa_E0240
3. Ligated *P_{kdpF}* [-15, T>G] into pSB1C3-BBa_E0240
4. Confirmed the construct pSB1C3-WT *P_{kdpF}*-WT RBS-BBa_I13401 by sequencing.
5. Did functional assay for pSB1C3-WT *P_{kdpF}*-WT RBS-BBa_I13401

Week 5 (29/6/2015-3/7/2015)

1. Confirmed the constructs pSB1C3-WT *P_{kdpF}*-BBa_E0240 by restriction check and sequencing result
2. Confirmed the constructs pSB1C3-*P_{kdpF}* [-15, T>G]-BBa_E0240 by restriction check and sequencing.
3. Migrated WT *P_{kdpF}*-BBa_E0240 into pSB3K3
4. Migrated *P_{kdpF}* [-15, T>G]-BBa_E0240 into pSB3K3
5. Did the functional assay for the pSB3K3-WT *P_{kdpF}*-BBa_E0240
6. Did the functional assay for the pSB3K3-*P_{kdpF}* [-15, T>G]-BBa_E0240
7. Made competent cells for TK2240 strains

Week 6 (6/7/2015-10/7/2015)

1. Failed to do ligation of P_{kdpF} [-15, T>A] into pSB1C3-BBa_E0240
2. Ligated P_{kdpF} [-15, T>C] into pSB1C3-BBa_E0240
3. Confirmed the construct pSB1C3- P_{kdpF} [-15, T>C]-BBa_E0240 by colony PCR and restriction check
4. Sent the construct pSB1C3- P_{kdpF} [-15, T>C]-BBa_E0240 for sequencing.

Week 7 (13/7/2015-17/7/2015)

1. Ligated P_{kdpF} [-15, T>A] into pSB1C3-BBa_E0240
2. Migrated P_{kdpF} [-15, T>A]-BBa_E0240 into pSB3K3
3. Migrated P_{kdpF} [-15, T>C]-BBa_E0240 into pSB3K3
4. Did the preliminary measurement to measure the activity of pSB3K3- P_{kdpF} [-15, T>G]-BBa_E0240 in both TK2240 and DH10B strain

Week 8 (20/7/2015-24/7/2015)

1. Prepared the constructs of mutant promoters to be sent for submission
2. Did preliminary RPU measurement for pSB3K3- P_{kdpF} [-15, T>G]-BBa_E0240 using FACS method
3. Did relative fluorescence measurement for all mutant constructs of pSB3K3- P_{kdpF} -BBa_E0240 in DH10B with 3 time points every 30 minutes

Week 9 (27/7/2015-31/7/2015)

1. Did two sets of RPU measurement for pSB3K3- P_{kdpF} [-15, T>G]-BBa_E0240 using FACS method
2. Did one set of relative fluorescence measurement for all mutant constructs of pSB3K3- P_{kdpF} -BBa_E0240 in DH10B and also pSB3K3- P_{kdpF} [-15, T>G]-BBa_E0240 in TK2240
3. Failed to make pSB1C3- P_{kdpF} [-15, T>G] construct

Week 10 (3/8/2015-7/8/2015)

1. Failed one RPU measurement for pSB3K3- P_{kdpF} [-15, T>G]-BBa_E0240 using FACS method
2. Did two set of relative fluorescence measurement for all constructs of pSB3K3- P_{kdpF} [-15, T>G]-BBa_E0240 in both DH10B and TK2240

Week 11 (10/8/2015- 14/8/2015)

1. Did one sets of RPU measurement for pSB3K3- P_{kdpF} [-15, T>G]-BBa_E0240 using FACS method, data sets are completed

2. Failed first GFP synthesis rate measurement for all mutant constructs of pSB3K3- P_{kdpF} - BBa_E0240 in DH10B and also pSB3K3- P_{kdpF} [-15, T>G]-BBa_E0240 in TK2240

Week 12 (17/8/2015- 23/8/2015)

1. Did two sets of GFP synthesis rate measurement for all mutant constructs of pSB3K3- P_{kdpF} -BBa_E0240 in DH10B and also pSB3K3- P_{kdpF} [-15, T>G]-BBa_E0240 in TK2240
2. Ordered primers to ligate P_{dcus} -BBa_E0420 into pSB3K3- P_{kdpF} -BBa_E0240 together with Gibson Assembly

Week 13 (24/8/2015-30/8/2015)

1. Did two trials for Gibson Assembly, failed the both batches of Gibson Assembly, proved by restriction check