

# LTDE BGE Experiment

*Mario E. Muscarella, Jay T. Lennon*

*10 May, 2016*

## Overview

Is growth efficiency an evolvable trait? Here, we explore patterns in bacterial growth efficiency using a set of isolates from the long-term starvation experiment. Each isolate has an ancestor and at least three derived strains that were isolated after three years of starvation in PBS. We are using <sup>14</sup>C succinate as the sole carbon source for these experiments.

## Experiment 1: BGE Evolution Experiment

### Data Import

```
# Import Data
# KBS0707.data <- read.csv("../data/BGE/20160216_BRBP.csv", header = T)
# Not using this run because it doesn't appear that the cultures grew enough
KBS0707.data <- read.csv("../data/BGE/20160228_BRBP.csv", header = T)
KBS0802.data <- read.csv("../data/BGE/20160214_BRBP.csv", header = T)
```

### Data Processing

```
# Raw Data For Intra Vial Test
voucher707 <- KBS0707.data[grepl("Voucher", KBS0707.data$Tube), ]
voucher802 <- KBS0802.data[grepl("Voucher", KBS0802.data$Tube), ]
control707 <- KBS0707.data[grepl("Control", KBS0707.data$Tube), ]
control802 <- KBS0802.data[grepl("Control", KBS0802.data$Tube), ]

# Merge Datasets
BGE.data.full <- rbind(KBS0707.data, KBS0802.data)

# Remove Control and Voucher
BGE.data <- BGE.data.full[~which(BGE.data.full$Tube == "Voucher" |
                                BGE.data.full$Tube == "Control"), ]

BGE.data$CPM.c <- NA

for (i in 1:dim(BGE.data)[1]){
  if (BGE.data$Sample[i] == "Cells"){
    BGE.data$CPM.c[i] <- BGE.data$CPM[i] * (6150/1500)
  } else {
    if (BGE.data$Sample[i] == "Trap"){
      BGE.data$CPM.c[i] <- BGE.data$CPM[i] * (500/100)
    } else {
      if (BGE.data$Sample[i] == "Sup"){
```

```

    BGE.data$CPM.c[i] <- BGE.data$CPM[i] * (6150/100)
  }
  else {
    BGE.data$CPM.c[i] <- NA
  }}
}

BGE.data.m <- melt(BGE.data)

## Using Tube, Sample, Organism as id variables

BGE.data.p <- cast(BGE.data.m, Tube + Organism + Sample ~ variable,
                  subset=variable=="CPM.c", fun.aggregate = c(mean, CV))

BGE.data.p$CPM.p <- NA
for (i in 1:dim(BGE.data.p)[1]){
  if (grep("707", BGE.data.p$Organism[i])){
    BGE.data.p$CPM.p <- BGE.data.p$CPM.c_mean / voucher707$CPM
  } else {
    if (grep("802", BGE.data.p$Organism[i])){
      BGE.data.p$CPM.p <- BGE.data.p$CPM.c_mean / voucher802$CPM
    } else{
      BGE.data.p$CPM.p <- NA
    }}
}

mass.l <- melt(as.data.frame(BGE.data.p))

## Using Tube, Organism, Sample as id variables

# Mass Balance
mass <- cast(mass.l, Tube + Organism ~ variable, subset=variable == "CPM.p",
            fun.aggregate = sum)
mean(mass$CPM.p)

# Data For Plots
BGE.707 <- droplevels(BGE.data.p[grep("707", BGE.data.p$Organism), ])
BGE.802 <- droplevels(BGE.data.p[grep("802", BGE.data.p$Organism), ])
levels(BGE.802$Tube)

```

## 802 Plot

```

BGE.802.w <- reshape(BGE.802, idvar = "Tube", direction="wide",
                    v.names = "CPM.p", timevar = "Sample",
                    drop = c("CPM.c_mean", "CPM.c_CV"))

BGE.802.w$BGE <- BGE.802.w$CPM.p.Cells / (BGE.802.w$CPM.p.Cells +
                                           BGE.802.w$CPM.p.Trap)
BGE.802.m <- melt(data.frame(BGE.802.w[,c(1,2,6)]))

## Using Tube, Organism as id variables

```

```

BGE.802.c <- cast(BGE.802.m, Organism ~ variable, fun.aggregate=c(mean, se))

# Plot
png(filename="../figures/BGE802.png",
      width = 1000, height = 800, res = 96*2)

par(mar=c(3,5.5,2.5,1))
bge_plot <- plot(BGE.802.c$BGE_mean, main = "LTDE: KBS0802 BGE",
                 pch = 15, cex = 1.5,
                 ylab = "", yaxt = "n", xaxt = "n", xlab = "",
                 xlim = c(0.5, 4.5), ylim = c(0.26, 0.33))

arrows(x0 = c(1:4), y0 = BGE.802.c$BGE_mean, y1 = BGE.802.c$BGE_mean -
       BGE.802.c$BGE_se, angle = 90,
       length=0.1, lwd = 2)
arrows(x0 = c(1:4), y0 = BGE.802.c$BGE_mean, y1 = BGE.802.c$BGE_mean +
       BGE.802.c$BGE_se, angle = 90,
       length=0.1, lwd = 2)

axis(side = 1, labels = c("Ancestor", "A-1", "B-1", "C-1"),
      at = c(1:4), lwd.ticks = 2, cex = 2)
axis(side = 2, labels = T, at = c(0.26, 0.28, 0.30, 0.32), las = 1,
      lwd.ticks = 2)
axis(side=1, lwd.ticks = 2, tck = -0.02, labels=F, cex.axis=1, at = c(1:4))
axis(side=3, lwd.ticks = 2, tck = -0.02, labels=F, cex.axis=1, at = c(1:4))
axis(side=1, lwd.ticks = 2, tck = 0.01, labels=F, cex.axis=1, at = c(1:4))
axis(side=3, lwd.ticks = 2, tck = 0.01, labels=F, cex.axis=1, at = c(1:4))
axis(side = 2, lwd.ticks = 2, tck = -0.02, labels=F, cex.axis=1,
      at = c(0.26, 0.28, 0.30, 0.32))
axis(side = 4, lwd.ticks = 2, tck = -0.02, labels=F, cex.axis=1,
      at = c(0.26, 0.28, 0.30, 0.32))
axis(side = 2, lwd.ticks = 2, tck = 0.01, labels=F, cex.axis=1,
      at = c(0.26, 0.28, 0.30, 0.32))
axis(side = 4, lwd.ticks = 2, tck = 0.01, labels=F, cex.axis=1,
      at = c(0.26, 0.28, 0.30, 0.32))

box(lwd = 2)

mtext("BGE", side = 2 , line =3.5, cex = 1.5)

dev.off() # this writes plot to folder

```

```

## pdf
## 2

```

```

graphics.off() # shuts down open devices

```

## 707 Plot

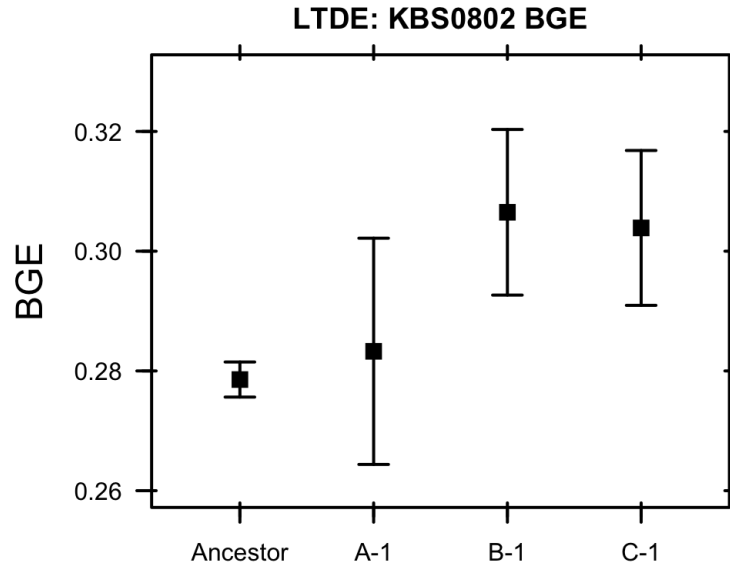


Figure 1: 14C-Succinate Recovery and BGE

```
BGE.707.w <- reshape(BGE.707, idvar = "Tube", direction="wide",
                      v.names = "CPM.p", timevar = "Sample",
                      drop = c("CPM.c_mean", "CPM.c_CV"))

BGE.707.w$BGE <- BGE.707.w$CPM.p.Cells / (BGE.707.w$CPM.p.Cells +
                                         BGE.707.w$CPM.p.Trap)
BGE.707.m <- melt(data.frame(BGE.707.w[,c(1,2,6)]))

## Using Tube, Organism as id variables

BGE.707.c <- cast(BGE.707.m, Organism ~ variable,
                  fun.aggregate=c(mean, se))

# Plot
png(filename="../figures/BGE707.png",
     width = 1000, height = 800, res = 96*2)

par(mar=c(3,5.5,2.5,1))
bge_plot <- plot(BGE.707.c$BGE_mean, main = "LTDE: KBS0707 BGE",
                 pch = 15, cex = 1.5, ylab = "", yaxt = "n",
                 xaxt = "n", xlab = "",
                 xlim = c(0.5, 4.5), ylim = c(0.22, 0.28))

arrows(x0 = c(1:4), y0 = BGE.707.c$BGE_mean, y1 = BGE.707.c$BGE_mean -
      BGE.707.c$BGE_se, angle = 90,
      length=0.1, lwd = 2)
arrows(x0 = c(1:4), y0 = BGE.707.c$BGE_mean, y1 = BGE.707.c$BGE_mean +
      BGE.707.c$BGE_se, angle = 90,
      length=0.1, lwd = 2)
```

```

axis(side = 1, labels = c("Ancestor", "A-1", "B-1", "C-1"),
     at = c(1:4), lwd.ticks = 2, cex = 2)
axis(side = 2, labels = T, at = c(0.22, 0.24, 0.26, 0.28),
     las = 1, lwd.ticks = 2)
axis(side=1, lwd.ticks = 2, tck = -0.02, labels=F, cex.axis=1, at = c(1:4))
axis(side=3, lwd.ticks = 2, tck = -0.02, labels=F, cex.axis=1, at = c(1:4))
axis(side=1, lwd.ticks = 2, tck = 0.01, labels=F, cex.axis=1, at = c(1:4))
axis(side=3, lwd.ticks = 2, tck = 0.01, labels=F, cex.axis=1, at = c(1:4))
axis(side = 2, lwd.ticks = 2, tck = -0.02, labels=F, cex.axis=1,
     at = c(0.22, 0.24, 0.26, 0.28))
axis(side = 4, lwd.ticks = 2, tck = -0.02, labels=F, cex.axis=1,
     at = c(0.22, 0.24, 0.26, 0.28))
axis(side = 2, lwd.ticks = 2, tck = 0.01, labels=F, cex.axis=1,
     at = c(0.22, 0.24, 0.26, 0.28))
axis(side = 4, lwd.ticks = 2, tck = 0.01, labels=F, cex.axis=1,
     at = c(0.22, 0.24, 0.26, 0.28))
box(lwd = 2)

mtext("BGE", side = 2 , line =3.5, cex = 1.5)

dev.off() # this writes plot to folder

```

```

## pdf
## 2

```

```

graphics.off() # shuts down open devices

```

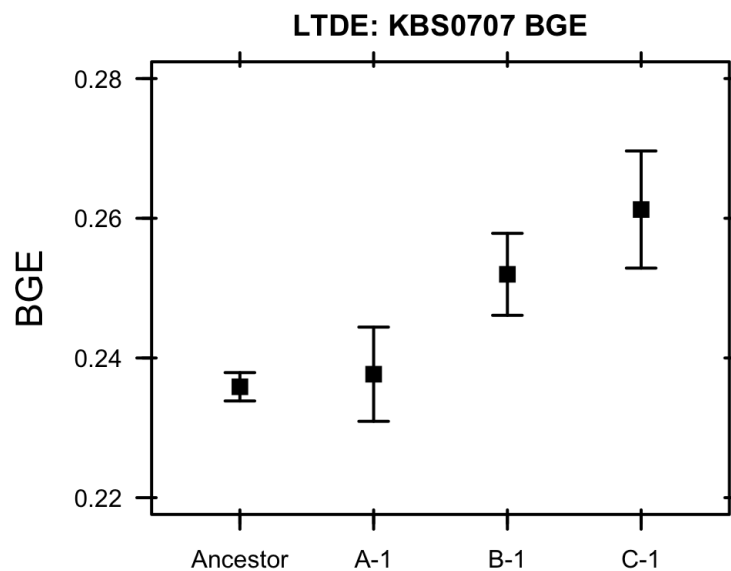


Figure 2: 14C-Succinate Recovery and BGE

## Experiment 2: <sup>14</sup>C Succinate BGE Using Michaelis-Menten Experiment

### Data Import

```
KBS0802.data <- read.csv("../data/BGE/20160306_BRBP.csv", header = T)
KBS0707.data <- read.csv("../data/BGE/20160430_BRBP.csv", header = T)
```

### Data Processing

```
# Raw Data For Intra Vial Test
voucher802 <- KBS0802.data[grep("Voucher", KBS0802.data$Tube), ]
control802 <- KBS0802.data[grep("Control", KBS0802.data$Tube), ]
voucher707 <- KBS0707.data[grep("Voucher", KBS0707.data$Tube), ]
control707 <- KBS0707.data[grep("Control", KBS0707.data$Tube), ]
blank802 <- KBS0802.data[grep("Blank", KBS0802.data$Organism), ]
kill802 <- KBS0802.data[grep("KC", KBS0802.data$Organism), ]
blank707 <- KBS0707.data[grep("Blank", KBS0707.data$Organism), ]
kill707 <- KBS0707.data[grep("KC", KBS0707.data$Organism), ]

# Merge Datasets
BGE.data.full <- rbind(KBS0802.data, KBS0707.data)

# Remove Control and Voucher
BGE.data <- BGE.data.full[-which(BGE.data.full$Tube == "Voucher" |
                                BGE.data.full$Tube == "Control" |
                                BGE.data.full$Organism == "Blank" |
                                BGE.data.full$Organism == "KC"), ]

BGE.data$CPM.c <- NA

# Turn Conc into Factor
BGE.data$Conc <- as.factor(BGE.data$Conc)

# Create Vector of Final Vol
BGE.data$Vol <- NA
for (i in 1:dim(BGE.data)[1]){
  if (BGE.data$Organism[i] == "KBS0802"){
    BGE.data$Vol[i] = 6150
  } else {
    if (BGE.data$Organism[i] == "KBS0707") {
      BGE.data$Vol[i] = 6200
    }
  }
}

# Correct CPM data for entire contents of fraction
for (i in 1:dim(BGE.data)[1]){
  if (BGE.data$Sample[i] == "Cells"){
    BGE.data$CPM.c[i] <- BGE.data$CPM[i] * (BGE.data$Vol[i]/1500)
  } else {
```

```

if (BGE.data$Sample[i] == "Trap"){
  BGE.data$CPM.c[i] <- BGE.data$CPM[i] * (500/100)
} else {
  if (BGE.data$Sample[i] == "Sup"){
    BGE.data$CPM.c[i] <- BGE.data$CPM[i] * (BGE.data$Vol[i]/100)
  }
  else {
    BGE.data$CPM.c[i] <- NA
  }}
}

# Re-organize data
BGE.data.m <- melt(BGE.data)

```

## Using Tube, Sample, Organism, Conc as id variables

```

BGE.data.p <- cast(BGE.data.m, Tube + Organism + Conc +
  Sample ~ variable, subset=variable=="CPM.c",
  fun.aggregate = mean)

# Calculate Percentages given Voucher
BGE.data.p$CPM.p <- NA
for (i in 1:dim(BGE.data.p)[1]){
  if (BGE.data.p$Organism[i] == "KBS0802"){
    BGE.data.p$CPM.p[i] <- BGE.data.p$CPM.c[i] / voucher802$CPM
  } else {
    if (BGE.data.p$Organism[i] == "KBS0707"){
      BGE.data.p$CPM.p[i] <- BGE.data.p$CPM.c[i] / voucher707$CPM
    } else {}
  }
}

# Mass Balance
mass.l <- melt(as.data.frame(BGE.data.p))

```

## Using Tube, Organism, Conc, Sample as id variables

```

mass <- cast(mass.l, Tube + Organism + Conc ~ variable,
  subset=variable == "CPM.p", fun.aggregate = sum)
mean(mass$CPM.p)

# Drop Levels (needed?)
BGE.802 <- droplevels(BGE.data.p[grepl("802", BGE.data.p$Organism), ])
BGE.707 <- droplevels(BGE.data.p[grepl("707", BGE.data.p$Organism), ])

# Turn percentages into concentrations given experimental concentration
BGE.802.w <- reshape(BGE.802, idvar = "Tube", direction="wide",
  v.names = "CPM.p", timevar = "Sample",
  drop = c("CPM.c"))
BGE.802.w$CPM.p.Resp <- 1 - (BGE.802.w$CPM.p.Cells + BGE.802.w$CPM.p.Sup)
BGE.802.w$Conc <- as.numeric(as.character(BGE.802.w$Conc))
BGE.802.w$Conc.Cells <- BGE.802.w$CPM.p.Cells * BGE.802.w$Conc

```

```

BGE.802.w$Conc.Sup <- BGE.802.w$CPM.p.Sup * BGE.802.w$Conc
BGE.802.w$Conc.Trap <- BGE.802.w$CPM.p.Trap * BGE.802.w$Conc
BGE.802.w$Conc.Resp <- BGE.802.w$CPM.p.Resp * BGE.802.w$Conc

BGE.707.w <- reshape(BGE.707, idvar = "Tube", direction="wide",
                      v.names = "CPM.p", timevar = "Sample",
                      drop = c("CPM.c"))
BGE.707.w$CPM.p.Resp <- 1 - (BGE.707.w$CPM.p.Cells + BGE.707.w$CPM.p.Sup)
BGE.707.w$Conc <- as.numeric(as.character(BGE.707.w$Conc))
BGE.707.w$Conc.Cells <- BGE.707.w$CPM.p.Cells * BGE.707.w$Conc
BGE.707.w$Conc.Sup <- BGE.707.w$CPM.p.Sup * BGE.707.w$Conc
BGE.707.w$Conc.Trap <- BGE.707.w$CPM.p.Trap * BGE.707.w$Conc
BGE.707.w$Conc.Resp <- BGE.707.w$CPM.p.Resp * BGE.707.w$Conc

# Calculate BGE
BGE.802.w$BGE <- BGE.802.w$CPM.p.Cells / (BGE.802.w$CPM.p.Cells +
                                           BGE.802.w$CPM.p.Resp)
BGE.707.w$BGE <- BGE.707.w$CPM.p.Cells / (BGE.707.w$CPM.p.Cells +
                                           BGE.707.w$CPM.p.Resp)

# Recovery
BGE.802.w$Recovery <- rowSums(BGE.802.w[,4:6])
BGE.707.w$Recovery <- rowSums(BGE.707.w[,4:6])

```

## Michaelis Menten Plot KBS802

```

# Plot
png(filename="../figures/MM802-14C.png",
     width = 1000, height = 1600, res = 96*2)

par(mfrow = c(1,1), mar = c(0, 6, 0, 0) + 0.5, oma = c(5, 0, 4, 1) + 0.5)
layout(rbind(1, 2, 3), height = c(3, 3, 3))

prod_plot <- plot(Conc.Cells ~ jitter(Conc), data = BGE.802.w,
                  pch = 22, cex = 3, col = "black", bg = "gray", lwd = 2.5,
                  xaxt = 'n', yaxt = 'n', xlab = '', ylab = '',
                  ylim = c(0.0, 0.15))
axis(side=2, lwd.ticks = 2, tck=-0.04, labels=T, las = 1, cex.axis = 1.5)
axis(side=2, lwd.ticks = 2, tck= 0.02, labels=F)
axis(side=4, lwd.ticks = 2, tck=-0.02, labels=F)
axis(side=4, lwd.ticks = 2, tck= 0.02, labels=F)
axis(side = 1, lwd.ticks = 2, cex.axis = 1, las = 1, tck = -0.04,
     at = c(0, 2.5, 5, 7.5, 10), labels = F)
axis(side = 1, lwd.ticks = 2, cex.axis = 1, las = 1, tck = 0.02,
     at = c(0, 2.5, 5, 7.5, 10), labels = F)
axis(side = 3, lwd.ticks = 2, cex.axis = 1, las = 1, tck = -0.02,
     at = c(0, 2.5, 5, 7.5, 10), labels = F)
axis(side = 3, lwd.ticks = 2, cex.axis = 1, las = 1, tck = 0.02,
     at = c(0, 2.5, 5, 7.5, 10), labels = F)
mtext("BP", side = 2, line = 4, cex = 1.5)
mtext("KBS0802", side = 3, line = 1.5, outer = F, cex = 1.5)

```



```

# mtext("(mM Carbon)", side = 2, line = 3, cex = 0.8)
box(lwd = 3)
resp_plot <- plot(Conc.Resp ~ jitter(Conc), data = BGE.802.w,
  pch = 22, cex = 3, col = "black", bg = "gray", lwd = 2.5,
  xaxt = 'n', yaxt = 'n', xlab = '', ylab = '',
  ylim = c(0.1, 0.9))
axis(side=2, lwd.ticks = 2, tck=-0.04, labels=T, las = 1, cex.axis = 1.5)
axis(side=2, lwd.ticks = 2, tck= 0.02, labels=F)
axis(side=4, lwd.ticks = 2, tck=-0.02, labels=F)
axis(side=4, lwd.ticks = 2, tck= 0.02, labels=F)
axis(side = 1, lwd.ticks = 2, cex.axis = 1, las = 1, tck = -0.04,
  at = c(0, 2.5, 5, 7.5, 10), labels = F)
axis(side = 1, lwd.ticks = 2, cex.axis = 1, las = 1, tck = 0.02,
  at = c(0, 2.5, 5, 7.5, 10), labels = F)
axis(side = 3, lwd.ticks = 2, cex.axis = 1, las = 1, tck = -0.02,
  at = c(0, 2.5, 5, 7.5, 10), labels = F)
axis(side = 3, lwd.ticks = 2, cex.axis = 1, las = 1, tck = 0.02,
  at = c(0, 2.5, 5, 7.5, 10), labels = F)
mtext("BR", side = 2, line = 4, cex = 1.5)
# mtext("(mM Carbon)", side = 2, line = 3, cex = 0.8)
box(lwd = 3)
bge_plot <- plot(BGE ~ Conc, data = BGE.802.w,
  pch = 22, cex = 3, col = "black", bg = "gray", lwd = 2.5,
  xaxt = 'n', yaxt = 'n', xlab = '', ylab = '',
  ylim = c(0.05, 0.22))
axis(side=2, lwd.ticks = 2, tck=-0.04, labels=T, las = 1, cex.axis = 1.5)
axis(side=2, lwd.ticks = 2, tck= 0.02, labels=F)
axis(side=4, lwd.ticks = 2, tck=-0.02, labels=F)
axis(side=4, lwd.ticks = 2, tck= 0.02, labels=F)
axis(side = 1, lwd.ticks = 2, cex.axis = 1.5, las = 1, tck = -0.04,
  at = c(0, 2.5, 5, 7.5, 10), labels = T)
axis(side = 1, lwd.ticks = 2, cex.axis = 1, las = 1, tck = 0.02,
  at = c(0, 2.5, 5, 7.5, 10), labels = F)
axis(side = 3, lwd.ticks = 2, cex.axis = 1, las = 1, tck = -0.02,
  at = c(0, 2.5, 5, 10), labels = F)
axis(side = 3, lwd.ticks = 2, cex.axis = 1, las = 1, tck = 0.02,
  at = c(0, 2.5, 5, 7.5, 10), labels = F)
mtext("BGE", side = 2, line = 4, cex = 1.5)
box(lwd = 3)
mtext("Succinate (mM)", side = 1, line = 3.5, outer=F, cex = 1.5)

dev.off() # this writes plot to folder
graphics.off() # shuts down open devices
#```

#```{r fig.width=4, fig.height=4,echo=FALSE,fig.cap="BGE Under Michaelis Menten Dynamics"}
img <- readPNG("../figures/MM802-14C.png")
grid.raster(img)

```

## Michaelis Menten Plot KBS707

```
# Plot
png(filename="../figures/MM707-14C.png",
     width = 1000, height = 1600, res = 96*2)

par(mfrow = c(1,1), mar = c(0, 6, 0, 0) + 0.5, oma = c(5, 0, 4, 1) + 0.5)
layout(rbind(1, 2, 3), height = c(3, 3, 3))

prod_plot <- plot(Conc.Cells ~ jitter(Conc), data = BGE.707.w,
                 pch = 22, cex = 3, col = "black", bg = "gray", lwd = 2.5,
                 xaxt = 'n', yaxt = 'n', xlab = '', ylab = '',
                 ylim = c(0.01, 0.09), xlim = c(0, 10))
axis(side=2, lwd.ticks = 2, tck=-0.04, labels=T, las = 1, cex.axis = 1.5)
axis(side=2, lwd.ticks = 2, tck= 0.02, labels=F)
axis(side=4, lwd.ticks = 2, tck=-0.02, labels=F)
axis(side=4, lwd.ticks = 2, tck= 0.02, labels=F)
axis(side = 1, lwd.ticks = 2, cex.axis = 1, las = 1, tck = -0.02,
     at = c(0, 2.5, 5, 7.5, 10), labels = F)
axis(side = 1, lwd.ticks = 2, cex.axis = 1, las = 1, tck = 0.02,
     at = c(0, 2.5, 5, 7.5, 10), labels = F)
axis(side = 3, lwd.ticks = 2, cex.axis = 1, las = 1, tck = -0.02,
     at = c(0, 2.5, 5, 7.5, 10), labels = F)
axis(side = 3, lwd.ticks = 2, cex.axis = 1, las = 1, tck = 0.02,
     at = c(0, 2.5, 5, 7.5, 10), labels = F)
mtext("BP", side = 2, line = 4, cex = 1.5)
mtext("KBS0707", side = 3, line = 1.5, outer = F, cex = 1.5)
# mtext("(mM Carbon)", side = 2, line = 3, cex = 0.8)
box(lwd = 3)

resp_plot <- plot(Conc.Resp ~ jitter(Conc), data = BGE.707.w,
                 pch = 22, cex = 3, col = "black", bg = "gray", lwd = 2.5,
                 xaxt = 'n', yaxt = 'n', xlab = '', ylab = '',
                 ylim = c(0, 1.5), xlim = c(0, 10))
axis(side=2, lwd.ticks = 2, tck=-0.04, labels=T, las = 1, cex.axis = 1.5)
axis(side=2, lwd.ticks = 2, tck= 0.02, labels=F)
axis(side=4, lwd.ticks = 2, tck=-0.02, labels=F)
axis(side=4, lwd.ticks = 2, tck= 0.02, labels=F)
axis(side = 1, lwd.ticks = 2, cex.axis = 1, las = 1, tck = -0.02,
     at = c(0, 2.5, 5, 7.5, 10), labels = F)
axis(side = 1, lwd.ticks = 2, cex.axis = 1, las = 1, tck = 0.02,
     at = c(0, 2.5, 5, 7.5, 10), labels = F)
axis(side = 3, lwd.ticks = 2, cex.axis = 1, las = 1, tck = -0.02,
     at = c(0, 2.5, 5, 7.5, 10), labels = F)
axis(side = 3, lwd.ticks = 2, cex.axis = 1, las = 1, tck = 0.02,
     at = c(0, 2.5, 5, 7.5, 10), labels = F)
mtext("BR", side = 2, line = 4, cex = 1.5)
# mtext("(mM Carbon)", side = 2, line = 3, cex = 0.8)
box(lwd = 3)

bge_plot <- plot(BGE ~ jitter(Conc), data = BGE.707.w,
                 pch = 22, cex = 3, col = "black", bg = "gray", lwd = 2.5,
                 xaxt = 'n', yaxt = 'n', xlab = '', ylab = '',
                 ylim = c(0.02, 0.18), xlim = c(0, 10))
axis(side=2, lwd.ticks = 2, tck=-0.04, labels=T, las = 1, cex.axis = 1.5)
```

```

axis(side=2, lwd.ticks = 2, tck= 0.02, labels=F)
axis(side=4, lwd.ticks = 2, tck=-0.02, labels=F)
axis(side=4, lwd.ticks = 2, tck= 0.02, labels=F)
axis(side = 1, lwd.ticks = 2 , cex.axis = 1.5, las = 1, tck = -0.04,
      at = c(0, 2.5, 5, 7.5, 10), labels = T)
axis(side = 1, lwd.ticks = 2 , cex.axis = 1, las = 1, tck = 0.02,
      at = c(0, 2.5, 5, 7.5, 10), labels = F)
axis(side = 3, lwd.ticks = 2 , cex.axis = 1, las = 1, tck = -0.02,
      at = c(0, 2.5, 5, 10), labels = F)
axis(side = 3, lwd.ticks = 2 , cex.axis = 1, las = 1, tck = 0.02,
      at = c(0, 2.5, 5, 7.5, 10), labels = F)
mtext("BGE", side = 2 , line = 4, cex = 1.5)
box(lwd = 3)
mtext("Succinate (mM)", side = 1, line = 3.5, outer=F, cex = 1.5)

dev.off() # this writes plot to folder
graphics.off() # shuts down open devices

```

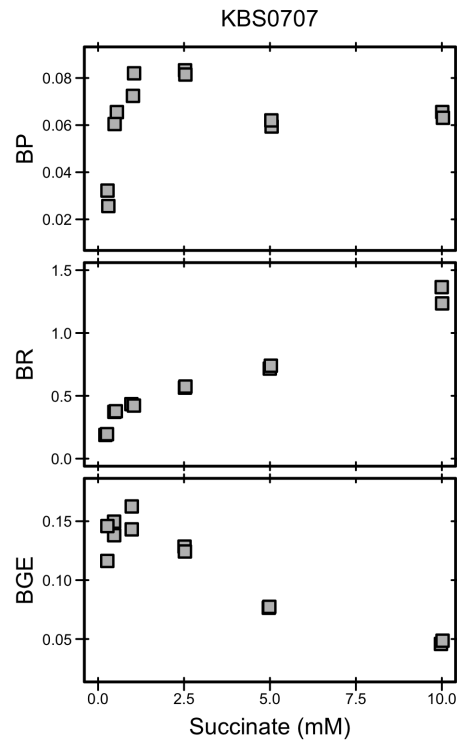


Figure 3: BGE Under Michaelis Menten Dynamics