Sample: Replicate-A-15min-Flip-B

Status	**Public on XXX**
Title	**Exposed to Streptococcus gordonii competence signaling peptide for 15 minutes**
Sample Type	**RNA**

Channel IA

Source Name	**Streptococcus gordonii culture exposed to 100 ng/ml competence signaling peptide (CSP) for 15 minutes.**
Organism(s)	**Streptococcus gordonii challis CH1**
Characteristics	**-**

Treatment protocol
Total RNA from cell cultures was extracted with hot acid phenol and purified on Qiagen RNeasy columns.

Growth protocol
Cells were grown at 36^0C in Todd Hewitt broth with 8mM HCl to OD_{600} of 0.4. Synthetic competence signaling peptide (CSP) was added to cell cultures to achieve a concentration of 100 ng/ml. After 15 minutes, a 10 ml aliquot of cells was removed and mixed with hot acid phenol to extract RNA.

Extracted molecule
RNA

Extraction protocol
Ten milliliters of cells were mixed with hot acid phenol (95^0C), incubated for 10 minutes at 95^0C, chilled on ice. A total of 0.2 volumes of chloroform was added, mixed well and centrifuged to separate the aqueous and organic layers. The aqueous layer (containing the RNA) was re-extracted with 1 volume of acid phenol-chloroform and centrifuged. The supernatant was adjusted to 0.3 M sodium acetate, mixed with one volume of isopropanol and centrifuged to precipitate the RNA. The RNA pellet was resuspended in DEPC treated water containing 150 U of RNase inhibitor (RNaseOUT, Invitrogen, Carlsbad, CA) per ml and purified on a Qiagen RNeasy midi-column (Qiagen, Valencia, CA) according to manufacturer's recommendations. After elution from the Qiagen RNeasy column, 50 U of RNase inhibitor was added to the RNA prior to storage at -80^0C.

Label
Cy3

Label protocol
Streptococcus gordonii RNA was first converted to amino-allyl cDNA (aa-cDNA) by reverse transcription with Superscript III reverse transcriptase (Invitrogen) in a deoxynucleotide triphosphate mixture containing amino-allyl dUTP (Ambion, Austin, TX). The RNA template was removed by alkaline hydrolysis and aa-cDNA recovered by purification on Qiagen QIAquick PCR columns (Qiagen). The cDNA was dried in a SpeedVac^{TM}, labeled by coupling to either Cy3 or Cy5 NHS-Cy dye (GE Healthcare Bio-Sciences Corp., Piscataway, NJ) and purified on Qiagen QIAquick PCR columns (Qiagen). The quality of the probes and their specific activity were confirmed by a spectrophotometric scan from 200 to 700 nm. Typical yields from this synthesis were 3

ug of cDNA with 150-200 pmol of dye molecule incorporated per microgram of cDNA produced. Only targets with > 200 pmol of dye incorporation per sample and a ratio of less than 50 nucleotides/dye molecules were used for hybridizations. Arabidopsis control oligonucleotides for array quality were printed onto each slide.

Channel IB

Source Name	**Streptococcus gordonii culture untreated**
Organism(s)	**Streptococcus gordonii challis CH1**
Characteristics	**-**

Treatment protocol
Total RNA from cell cultures was extracted with hot acid phenol and purified on Qiagen RNeasy columns.

Growth protocol
Cells were grown at 36^0C in Todd Hewitt broth with 8mM HCl to OD_{600} of 0.4. The cells were grown for an additional 15 min. at 36^0C as the control for cells incubated with competence signaling peptide (CSP). A 10 ml aliquot of cells was removed and mixed with hot acid phenol to extract RNA.

Extracted molecule
RNA

Extraction protocol
Ten milliliters of cells were mixed with hot acid phenol (95^0C), incubated for 10 minutes at 95^0C, chilled on ice. A total of 0.2 volumes of chloroform was added, mixed well and centrifuged to separate the aqueous and organic layers. The aqueous layer (containing the RNA) was re-extracted with 1 volume of acid phenol-chloroform and centrifuged. The supernatant was adjusted to 0.3 M sodium acetate, mixed with one volume of isopropanol and centrifuged to precipitate the RNA. The RNA pellet was resuspended in DEPC treated water containing 150 U of RNase inhibitor (RNaseOUT, Invitrogen, Carlsbad, CA) per ml and purified on a Qiagen RNeasy midi-column (Qiagen, Valencia, CA) according to manufacturer's recommendations. After elution from the Qiagen RNeasy column, 50 U of RNase inhibitor was added to the RNA prior to storage at -80^0C.

Label
Cy5

Label protocol
Streptococcus gordonii RNA was first converted to amino-allyl cDNA (aa-cDNA) by reverse transcription with Superscript III reverse transcriptase (Invitrogen) in a deoxynucleotide triphosphate mixture containing amino-allyl dUTP (Ambion, Austin, TX). The RNA template was removed by alkaline hydrolysis and aa-cDNA recovered by purification on Qiagen QIAquick PCR columns (Qiagen). The cDNA was dried in a SpeedVac^{TM}, labeled by coupling to either Cy3 or Cy5 NHS-Cy dye (GE Healthcare Bio-Sciences Corp., Piscataway, NJ) and purified on Qiagen QIAquick PCR columns (Qiagen). The quality of the probes and their specific activity were confirmed by a spectrophotometric scan from 200 to 700 nm. Typical yields from this synthesis were 3 ug of cDNA with 150-200 pmol of dye molecule incorporated per microgram of cDNA produced. Only targets with > 200 pmol of dye incorporation per sample and a ratio of

less than 50 nucleotides/dye molecules were used for hybridizations. Arabidopsis control oligonucleotides for array quality were printed onto each slide.

Hybridization protocol
Before hybridization, slides were pre-treated in a 5X SSC, 0.1% SDS, and 1% BSA solution for 1 hr at 42ºC. Slides were then washed twice in H_20 followed by one wash in isopropanol and dried. Fifty ul of hybridization solution (50% formamide, 5X SSC, 0.1% SDS) filtered through a Nalgene 0.45uM filter and containing 0.5mg of salmon sperm DNA was used to resuspend each dried probe. Resuspended probes were denatured at 95ºC, chilled on ice to 4ºC and added between slides and lifterslips (Erie Scientific, Portsmouth, NH) and sealed in hybridization chambers (Corning Life Sciences, Lowell, MA) containing 40ul of H_20 to maintain humidity. Slides were incubated in the dark in a 42ºC water bath overnight. Slides were washed once in 1X SSC/0.2% SDS for 5 min at ~45ºC, once in 1X SSC/0.1% SDS for 5 min at room temperature, and three times in 0.1X SSC for 5 min each at room temperature. Slides were dried and scanned immediately or the same day after storage in the dark.

Scan protocol
Microarrays were scanned using GenePix Pro v5.0 with a Axon 4000B GenePix scanner (Molecular Devices, Sunnyvale, CA). The intensities of the two dyes at each spot were quantified using TIGR spotfinder (http://www.tigr.org).

Description
Biological replicate 2 of 2. Bacterial culture exposed to 100 ng/ml competence signaling peptide (CSP) for 15 minutes.

Data processing
The TIGR TM4 Microarray Software Suite (http://www.tigr.org) was used for all analyses, including analysis of TIF files using TIGR Spotfinder and normalization using Microarray Data Analysis System (MIDAS). Normalization included LocFit, a low intensity filter of 10,000, and in-slide replicate analysis which calculated geometric means for in-slide replicates. Geometric means were then calculated for flip dye pairs using flip dye pair consistency checking in MIDAS. Statistical analyses were conducted using Multiexperiment Viewer (MEV).

Submission date	
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Platform ID	**XXXX**
Series	**Genome-wide Transcriptional Changes in *Streptococcus gordonii* in Response to Competence Signaling Peptide**

Normalized data table header descriptions
ORF: identifier for ORFs in Streptococcus gordonii Challis CH1 genome
LOCI: identifier for gene loci in Streptococcus gordonii Challis CH1 genome
DESCRIPTION: functional name of Streptococcus gordonii Challis CH1 locus
GENBANK ID: genbank accession ID
GENE SYMBOL: -
VALUE: Log(base2) of Channel 2/Channel 1 Normalized Ratio

Raw data table header descriptions
UID: unique identifier for this spot
IA: intensity value in channel A (cy3)
IB: intensity value in channel B (cy5)
R: row (slide row)
C: column (slide column)
MR: meta-row (block row)
MC: meta-column (block column)
SR: sub-row
SC: sub-column
SA: actual sport area (in pixels)
SF: saturation factor
QCscore: cumulative quality control score
QCA: quality control score in channel A
QCB: quality control score in channel B
BkgA: background value in channel A
BkgB: background value in channel B
ORF: identifier for ORFs in Streptococcus gordonii Challis CH1 genome

Data table