

Sample: Replicate-A-40min-Flip-B

Status	Public on XXX
Title	Exposed to <i>Streptococcus gordonii</i> competence signaling peptide for 40 minutes
Sample Type	RNA

Channel IA

Source Name	<i>Streptococcus gordonii</i> culture exposed to 100 ng/ml competence signaling peptide (CSP) for 40 minutes.
--------------------	--

Organism(s)	<i>Streptococcus gordonii</i> 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°C in Todd Hewitt broth with 8mM HCl to OD₆₀₀ of 0.4. Synthetic competence peptide (CSP) was added to cell cultures to achieve a concentration of 100 ng/ml. After 40 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°C), incubated for 10 minutes at 95°C, 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°C.

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 SpeedVacTM, 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°C in Todd Hewitt broth with 8mM HCl to OD₆₀₀ of 0.4. The cells were grown for an additional 40 min. at 36°C 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°C), incubated for 10 minutes at 95°C, 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°C.

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 SpeedVacTM, 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 42°C. Slides were then washed twice in H₂O 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 95°C, chilled on ice to 4°C and added between slides and lifterslips (Erie Scientific, Portsmouth, NH) and sealed in hybridization chambers (Corning Life Sciences, Lowell, MA) containing 40ul of H₂O to maintain humidity. Slides were incubated in the dark in a 42°C water bath overnight. Slides were washed once in 1X SSC/0.2% SDS for 5 min at ~45°C, 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 40 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

Contact name	Steven R. Gill
E-mail	srgill@buffalo.edu
Phone	(716) 881-8956
Organization Name	SUNY-Buffalo
Department	Infectious Disease and Genomics
Street address	701 Ellicott Street
City	Buffalo
State/province	NY
ZIP/Postal code	14203
Country	USA

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: $\text{Log}(\text{base}2)$ 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 spot 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