Ruegeria pomeroyi RB-TnSeq Transponson Mutant Library Screen Experimental Data February 2021 (C-CoMP Marine Bacterial Transporters project)

Website: https://www.bco-dmo.org/dataset/893256
Data Type: experimental
Version: 1
Version Date: 2023-04-05

Project
» Function and Importance of Marine Bacterial Transporters of Plankton Exometabolites (C-CoMP Marine Bacterial Transporters)

Program
» Center for Chemical Currencies of a Microbial Planet (C-CoMP)

Contributors	Affiliation	Role
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Abstract
A barcoded transposon mutant library (RB-TnSeq) of Ruegeria pomeroyi DSS-3 was screened on 23 substrates, each as a sole carbon source. In February of 2021 the RB-TnSeq library was grown in the laboratory in minimal medium with a substrate of interest, cultures were sequentially transfered four times to increase selection. Mutants with phenotypes that decreased fitness on the substrate of interest grow at a lower rate than mutants with no loss of fitness. Sequencing the barcodes of mutants after the screen shows which mutants are enriched or depleted on each substrate, indicating the fitness advantage provided by each gene when grown on each substrate. This can indicate genes that are involved in the uptake and metabolism of each substrate.

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Coverage

Temporal Extent: 2021-02 - 2021-02

Data Processing Description

Processing Notes from Researcher:

Sequence data were processed according to Wetmore et al (2015). Following quality control, an average of 35,090 unique barcodes mapped to insertions that fell within the interior 10 to 90% of *R. pomeroyi* coding sequences. In total, 55 million reads were mapped to insertions in 3,570 genes (out of 4,469 protein-encoding genes in the *R. pomeroyi* genome) with a median of 404,513 mapped reads per sample. Reads mapping to different insertion sites within the same coding sequence were pooled.

BCO-DMO Processing Notes:

- Removed spaces and special characters from column header names (replaced with underscores "_")

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Data Files

File	
rbtnseq_bcodmo_2.csv	(Comma Separated Values (.csv), 1,014.10 KB) MD5:de395169ec8a3d2f6ffab393359813ba
Primary data file for 893256. File processed with laminar pipeline "893256_v1_RB-TnSeq_Data" at path 893256/1/data/rbtnseq_bcodmo_2.csv	

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Related Publications

Schroer, W. F., Kepner, H. E., Uchimiya, M., Mejia, C., Rodriguez, L. T., Reisch, C. R., & Moran, M. A. (2023). Function and Importance of Marine Bacterial Transporters of Plankton Exometabolites. https://doi.org/10.1101/2023.01.19.524783
Results

Schroer, W. F., Kepner, H. E., Uchimiya, M., Mejia, C., Rodriguez, L. T., Reisch, C. R., & Moran, M. A. (2023). Functional annotation and importance of marine bacterial transporters of plankton exometabolites. ISME Communications, 3(1). https://doi.org/10.1038/s43705-023-00244-6
Results

Wetmore, K. M., Price, M. N., Waters, R. J., Lamson, J. S., He, J., Hoover, C. A., Blow, M. J., Bristow, J., Butland, G., Arkin, A. P., & Deutschbauer, A. (2015). Rapid Quantification of Mutant Fitness in Diverse Bacteria by Sequencing Randomly Bar-Coded Transposons. MBio, 6(3). https://doi.org/10.1128/mbio.00306-15 https://doi.org/https://doi.org/10.1128/mBio.00306-15
Methods

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Parameters

Parameter	Description	Units
Locus ID	Locus ID of a gene in the Ruegeria pomeroyi DSS-3 genome.	unitless
T0_1	Time 0 replicate 1	hours

T0_2	Time 0 replicate 2	hours
T0_3	Time 0 replicate 3	hours
T0_4	Time 0 replicate 4	hours
Cysteate_1	Replicate 1 sample grown on Cysteate.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Cysteate_2	Replicate 2 sample grown on Cysteate.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Cysteate_3	Replicate 3 sample grown on Cysteate.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Cysteate_4	Replicate 4 sample grown on Cysteate.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
DMSP_1	Replicate 1 sample grown on DMSP.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
DMSP_2	Replicate 2 sample grown on DMSP.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
DMSP_3	Replicate 3 sample grown on DMSP.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
DMSP_4	Replicate 4 sample grown on DMSP.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
DHPS_1	Replicate 1 sample grown on DHPS.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
DHPS_2	Replicate 2 sample grown on DHPS.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
DHPS_3	Replicate 3 sample grown on DHPS.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
DHPS_4	Replicate 4 sample grown on DHPS.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Leucine_1	Replicate 1 sample grown on Leucine.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Leucine_2	Replicate 2 sample grown on Leucine.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Leucine_3	Replicate 3 sample grown on Leucine.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Leucine_4	Replicate 4 sample grown on Leucine.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Arginine_1	Replicate 1 sample grown on Arginine.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Arginine_2	Replicate 2 sample grown on Arginine.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Arginine_3	Replicate 3 sample grown on Arginine.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Arginine_4	Replicate 4 sample grown on Arginine.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Ectoine_1	Replicate 1 sample grown on Ectoine.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Ectoine_2	Replicate 2 sample grown on Ectoine.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Ectoine_3	Replicate 3 sample grown on Ectoine.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.

Ectoine_4	Replicate 4 sample grown on Ectoine.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
GlcNAc_1	Replicate 1 sample grown on GlcNAc.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
GlcNAc_2	Replicate 2 sample grown on GlcNAc.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
GlcNAc_3	Replicate 3 sample grown on GlcNAc.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
GlcNAc_4	Replicate 4 sample grown on GlcNAc.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Glucose_1	Replicate 1 sample grown on Glucose.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Glucose_2	Replicate 2 sample grown on Glucose.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Glucose_3	Replicate 3 sample grown on Glucose.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Glucose_4	Replicate 4 sample grown on Glucose.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Xylose_1	Replicate 1 sample grown on Xylose.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Xylose_2	Replicate 2 sample grown on Xylose.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Xylose_3	Replicate 3 sample grown on Xylose.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Xylose_4	Replicate 4 sample grown on Xylose.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Glycolate_1	Replicate 1 sample grown on Glycolate.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Glycolate_2	Replicate 2 sample grown on Glycolate.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Glycolate_3	Replicate 3 sample grown on Glycolate.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Glycolate_4	Replicate 4 sample grown on Glycolate.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Phenylacetate_1	Replicate 1 sample grown on Phenylacetate.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Phenylacetate_2	Replicate 2 sample grown on Phenylacetate.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Phenylacetate_3	Replicate 3 sample grown on Phenylacetate.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Phenylacetate_4	Replicate 4 sample grown on Phenylacetate.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Malate_1	Replicate 1 sample grown on Malate.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Malate_2	Replicate 2 sample grown on Malate.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Malate_3	Replicate 3 sample grown on Malate.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Malate_4	Replicate 4 sample grown on Malate.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.

3-Hydoxybutyrate_1	Replicate 1 sample grown on 3-Hydroxybutyrate.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
3-Hydoxybutyrate_2	Replicate 2 sample grown on 3-Hydroxybutyrate.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
3-Hydoxybutyrate_3	Replicate 3 sample grown on 3-Hydroxybutyrate.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
3-Hydoxybutyrate_4	Replicate 4 sample grown on 3-Hydroxybutyrate.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Acetate_1	Replicate 1 sample grown on Acetate.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Acetate_2	Replicate 2 sample grown on Acetate.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Acetate_3	Replicate 3 sample grown on Acetate.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Acetate_4	Replicate 4 sample grown on Acetate.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Spermidine_1	Replicate 1 sample grown on Spermidine.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Spermidine_2	Replicate 2 sample grown on Spermidine.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Spermidine_3	Replicate 3 sample grown on Spermidine.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Spermidine_4	Replicate 4 sample grown on Spermidine.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Threonine_1	Replicate 1 sample grown on Threonine.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Threonine_2	Replicate 2 sample grown on Threonine.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Threonine_3	Replicate 3 sample grown on Threonine.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Threonine_4	Replicate 4 sample grown on Threonine.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Tyramine_1	Replicate 1 sample grown on Tyramine.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Tyramine_2	Replicate 2 sample grown on Tyramine.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Tyramine_3	Replicate 3 sample grown on Tyramine.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Tyramine_4	Replicate 4 sample grown on Tyramine.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Gluconate_1	Replicate 1 sample grown on Gluconate.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Gluconate_2	Replicate 2 sample grown on Gluconate.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Gluconate_3	Replicate 3 sample grown on Gluconate.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Gluconate_4	Replicate 4 sample grown on Gluconate.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.

Serine_1	Replicate 1 sample grown on Serine.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Serine_2	Replicate 2 sample grown on Serine.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Serine_3	Replicate 3 sample grown on Serine.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Serine_4	Replicate 4 sample grown on Serine.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Glycine_1	Replicate 1 sample grown on Glycine.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Glycine_2	Replicate 2 sample grown on Glycine.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Glycine_3	Replicate 3 sample grown on Glycine.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Glycine_4	Replicate 4 sample grown on Glycine.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Ribose_1	Replicate 1 sample grown on Ribose.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Ribose_2	Replicate 2 sample grown on Ribose.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Ribose_3	Replicate 3 sample grown on Ribose.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Ribose_4	Replicate 4 sample grown on Ribose.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Proline_1	Replicate 1 sample grown on Proline.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Proline_2	Replicate 2 sample grown on Proline.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Proline_3	Replicate 3 sample grown on Proline.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
Proline_4	Replicate 4 sample grown on Proline.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
IAM_1	Replicate 1 sample grown on IAM.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
IAM_2	Replicate 2 sample grown on IAM.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
IAM_3	Replicate 3 sample grown on IAM.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.
IAM_4	Replicate 4 sample grown on IAM.	Number of sequence counts (barcodes) that mapped to each gene in the treatment.

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Instruments

Dataset-specific Instrument Name	NextSeq SE150 (Illumina, San Diego, CA, USA)
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	An aliquot of 8 ng of product from each sample was pooled, purified using HiPrep beads (MagBio, Gaithersburg, MD, USA), and sequenced on a NextSeq SE150 Mid Output flow cell (SE150) at the Georgia Genomics and Bioinformatics Core Facility (Athens, Georgia, USA).
Generic Instrument Description	General term for a laboratory instrument used for deciphering the order of bases in a strand of DNA. Sanger sequencers detect fluorescence from different dyes that are used to identify the A, C, G, and T extension reactions. Contemporary or Pyrosequencer methods are based on detecting the activity of DNA polymerase (a DNA synthesizing enzyme) with another chemoluminescent enzyme. Essentially, the method allows sequencing of a single strand of DNA by synthesizing the complementary strand along it, one base pair at a time, and detecting which base was actually added at each step.

Dataset-specific Instrument Name	Synergy H1 plate reader (BioTek, Winooski, VT, USA)
Generic Instrument Name	plate reader
Dataset-specific Description	After growth with shaking at 25oC for 72 h in a Synergy H1 plate reader (BioTek, Winooski, VT, USA) , cultures were serially transferred into fresh media four times and then transferred to 1.5 ml tubes, pelleted by centrifugation at 8,000 x g for 3 min, and stored at -80oC until further processing.
Generic Instrument Description	Plate readers (also known as microplate readers) are laboratory instruments designed to detect biological, chemical or physical events of samples in microtiter plates. They are widely used in research, drug discovery, bioassay validation, quality control and manufacturing processes in the pharmaceutical and biotechnological industry and academic organizations. Sample reactions can be assayed in 6-1536 well format microtiter plates. The most common microplate format used in academic research laboratories or clinical diagnostic laboratories is 96-well (8 by 12 matrix) with a typical reaction volume between 100 and 200 uL per well. Higher density microplates (384- or 1536-well microplates) are typically used for screening applications, when throughput (number of samples per day processed) and assay cost per sample become critical parameters, with a typical assay volume between 5 and 50 µL per well. Common detection modes for microplate assays are absorbance, fluorescence intensity, luminescence, time-resolved fluorescence, and fluorescence polarization. From: http://en.wikipedia.org/wiki/Plate_reader, 2014-09-0-23.

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Project Information

Function and Importance of Marine Bacterial Transporters of Plankton Exometabolites (C-CoMP Marine Bacterial Transporters)

Project:

Observing expression of marine bacterial transporter systems with transcriptomic or proteomic tools can provide valuable information about the metabolomic environment. However, these ‘omics approaches are limited by the low rate of transporter gene annotation. Here, a barcoded, arrayed, mutant library of the marine bacterium *Ruegeria pomeroyi* DSS-3 is employed in high throughput screens to identify the target substrates

of 13 transporter systems. A set of 156 isolated putative transporter mutants were screened for growth on minimal medium with 63 substrates, each as a sole carbon source. Mutants that demonstrated a growth defect on a specific substrate were selected for secondary, higher resolution, growth screening. Mutants that continued to demonstrate growth defect relative to the pooled-mutant library (pooled-BarSeq, used as an analog for wildtype) were screened for their ability to drawdown the target substate. Gene annotations were made when mutants of the given transporter demonstrated both growth and drawdown defects on the target substrate.

In addition to the isolated mutant screens, the pooled barcoded transposon mutant library (pooled-BarSeq) was grown on minimal medium with selected substrates, each as sole carbon source, such that the relative enrichment or depletion of each mutant could demonstrate its fitness cost associated with the loss of each disrupted gene when grown on each substrate. The results of pooled-BarSeq screens had mixed consistency with the isolated mutant screens, demonstrating the value of isolated mutants for transporter annotation.

Program:

The Center for Chemical Currencies of a Microbial Planet (C-CoMP) integrates research, education and knowledge transfer activities to develop a mechanistic understanding of surface ocean carbon flux within the context of a changing ocean and through increased participation in ocean sciences. C-CoMP supports science teams that merge biology, chemistry, modeling, and informatics to close long-standing knowledge gaps in the identities and dynamics of organic molecules that serve as the currencies of elemental transfer between the ocean and atmosphere.

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Program Information

Center for Chemical Currencies of a Microbial Planet (C-CoMP)

Website: https://ccomp-stc.org/

Coverage: North Atlantic, BATS, global/other

Functions carried out by microscopic inhabitants of the surface ocean affect every aspect of life on our planet, regardless of distance from the coast. Ocean phytoplankton are responsible for half of the photosynthesis on Earth, the first step in a complex system that annually withdraws 50 billion metric tons of carbon from the atmosphere to sustain their growth. Of this, 25 billion metric tons participate in a rapid cycle in which biologically reactive material is released into seawater and converted back into carbon dioxide by marine bacteria within hours to days. The chemical-microbe network at the heart of this fast cycle remains poorly constrained; consequently, its primary currencies and controls remain elusive; its sensitivities to changing ocean conditions are unknown; and its responses to future climate scenarios are not predictable. The Center for Chemical Currencies of a Microbial Planet (C-CoMP) integrates research, education and knowledge transfer activities to develop a mechanistic understanding of surface ocean carbon flux within the context of a changing ocean and through increased participation in ocean sciences. C-CoMP supports science teams that merge biology, chemistry, modeling, and informatics to close long-standing knowledge gaps in the identities and dynamics of organic molecules that serve as the currencies of elemental transfer between the ocean and atmosphere. C-CoMP fosters education, outreach, and knowledge transfer activities that engage students of all ages, broaden participation in the next generation of ocean scientists, and extend novel open-science approaches into complementary academic and industrial communities. The Center framework is critical to this mission, uniquely facilitating an open exchange of experimental and computational science, methodological and conceptual challenges, and collaborations that establish integrated science and education partnerships. With expanded participation in ocean science research and ocean literacy across the US society, the next generation of ocean scientists will better reflect the diverse US population.

Climate-carbon feedbacks on the marine carbon reservoir are major uncertainties for future climate projections, and the trajectory and rate of ocean changes depend directly on microbial responses to temperature increases, ocean acidification, and other perturbations driven by climate change. C-CoMP research closes an urgent knowledge gap in the mechanisms driving carbon flow between ocean and atmosphere, with global implications for predictive climate models. The Center supports interdisciplinary

science teams following open and reproducible science practices to address: (1) the chemical currencies of surface ocean carbon flux; (2) the structure and regulation of the chemical-microbe network that mediates this flux; and (3) sensitivity of the network and its feedbacks on climate. C-CoMP leverages emerging tools and technologies to tackle critical challenges in these themes, in synergy with existing ocean programs and consistent with NSF’s Big Ideas. C-CoMP education and outreach activities seek to overcome barriers to ocean literacy and diversify participation in ocean research. The Center is developing (1) initiatives to expand ocean literacy in K-12 and the broader public, (2) ocean sciences undergraduate curricula and research opportunities that provide multiple entry points into research experiences, (3) post-baccalaureate programs to transition undergraduates into graduate education and careers in ocean science, and (4) interdisciplinary graduate student and postdoctoral programs that prepare the next generation of ocean scientists. The C-CoMP team includes education faculty who evaluate the impacts of education and outreach activities and export successful STEM initiatives to the education community. C-CoMP is revolutionizing the technologies for studying chemical transformations in microbial systems to build understanding of the outsized impact of microbes on elemental cycles. Open science, cross-disciplinary collaborations, community engagement, and inclusive practices foster strategic advances in critical science problems and STEM initiatives. C-CoMP science, education, and knowledge-transfer themes are efficiently addressed through a sustained network of scientists addressing critical research challenges while broadening the workforce that will tackle multi-disciplinary problems with academic, industrial and policy partners.

This award reflects NSF's statutory mission and has been deemed worthy of support through evaluation using the Foundation's intellectual merit and broader impacts review criteria.

The Program's Data Management Plan (DMP) is available as a PDF document.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-2019589
Simons Foundation (Simons)	542391

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