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Random barcode transposon-site sequencing of *Rhodopseudomonas palustris* CGA009

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Data Note

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Abstract

Rhodopseudomonas palustris is one of the most metabolically versatile organisms ever described and is of interest for a variety of biotechnology applications. Here we describe a random barcoded transposon (Tn) insertion library in the strain CGA009 along with gene fitness analyses from 208 experiments spanning 54 growth conditions.

Announcement

Rhodopseudomonas palustris CGA009 is a model purple phototrophic bacterium of interest for its metabolic versatility and biotechnological potential (1, 2). Here we describe a CGA009 random barcoded Tn insertion library and its application in 208 sequencing experiments (RB-TnSeq) to identify genes that are important for fitness in 54 conditions (Table S1).

The CGA009 RB-TnSeq library was constructed by conjugation with *Escherichia coli* AMD289 (strain WM3064 with barcoded Tn5 library pHLL249_NN1). pHLL249_NN1, was constructed by assembling part vectors pHLL212, pHLL214, PJW9, pJW13, and pJW19 into pHLL249, and then adding millions of barcodes, all using Golden Gate assembly with BbsI (3). AMD289 was grown in LB with carbenicillin (50 µg/mL) and 2,6-diaminopimelic acid (DAP; 300 µM) to early stationary phase. CGA009 was grown in and in CAAc (Table S2) to mid-exponential phase. Strains were harvested, washed with CAAc plus DAP, mixed 1:2 respectively, incubated on a filter on CAAc agar with DAP for 20 h, then resuspended in CAAc with 25% glycerol and stored at -80ºC as described (4). CGA009 Tn mutants were selected on CAAc agar with kanamycin (Km, 100 µg/ml) at 30ºC with light for 7 days in a GasPak EZ Anaerobe Pouch System (Becton Dickenson). Colonies were scraped, pooled in CAAc with Km and 25% glycerol, and stored at -80 ºC. This mutant library was later inoculated into 50 ml anoxic CAAc with Km (100% N_2) in a 70-ml serum vial to 0.2 OD_{660}. After cultivation at 30°C with light to ~1.0 OD_{660}, the library was mixed 1:1 with 50% glycerol then stored at -80°C. Five libraries created by this method were then thawed, combined, and 1 ml aliquots were stored at -80°C.

Tn sites were mapped and linked to DNA barcodes using a two-step PCR approach that amplifies Tn insertion junctions and adds Illumina adapters (5). The final mutant library (RPal_CGA009_ML8) was mapped using two independently prepared sequencing libraries with paired end sequencing (2 x 150; Illumina NovaSeq; Novogene; 26,635,532 and 47,634,263 paired reads across both libraries). We confidently mapped 261,878 uniquely barcoded insertion mutants with at least 10 supporting reads for each, meeting quality criteria (6).

Competitive fitness assays were performed by culturing a 1-ml mutant library aliquot in 50-ml anoxic CAAc as above. The culture (2.0 – 3.0 OD_{660}) was pelleted, washed in 50 ml PM (Table S2), and resuspended in PM to 3.0 OD_{660}. Some washed library (equivalent to 1.0 OD_{660} in 1 ml) was harvested for gDNA extraction to determine zero-timepoint frequencies. Assays (3 or 4 replicates) were inoculated

with 0.15 ml of washed library, grown for 4-5 doublings (Table S1), and then harvested for gDNA extraction.

Barcode sequencing (7) was performed with both P1 and P2 oligos indexed to minimize impacts from misassigned indexes in Illumina NovaSeq runs (8). Strain and gene fitness scores and a t-like statistic describing gene fitness score confidence was calculated as described (6).

Declarations

Data availability

RB-TnSeq software is available at https://bitbucket.org/berkeleylab/feba/src/master/.

All fitness data is available at https://morgannprice.org/FEBA/RPal_CGA009. Data can be explored at the Fitness Browser (fit.genomics.lbl.gov).

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Supplementary Files

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