

Development of a Universal System to Close Microbial Genomes

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DOE/Office of Science/Biological & Environmental Research

Objective

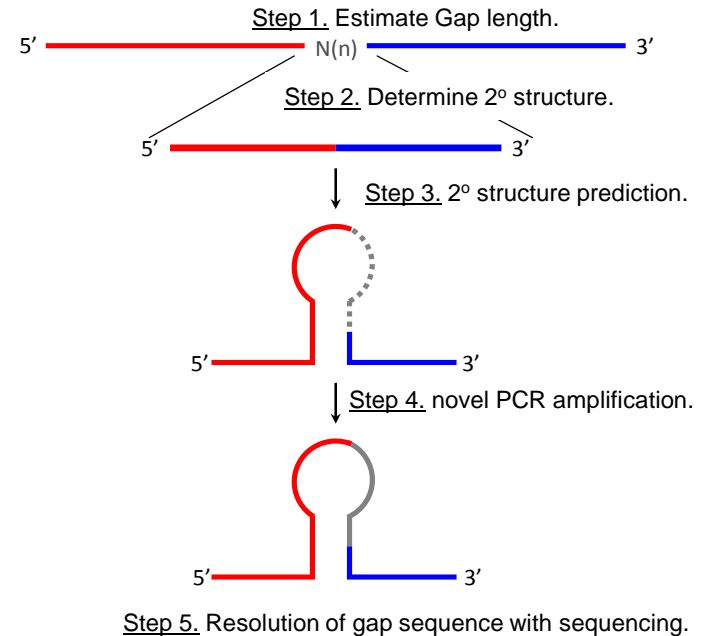
- Develop a universal method to close “non-contiguous finished” genomes.

New Science

- Novel method is demonstrated for resolving, amplifying and sequencing gaps in genomic regions.
- Our novel method closed 7 gaps in 2 bacterial genomes within 2 months, after unsuccessful efforts with conventional methods over 3 years.

Significance

- “Non-contiguous finished” has been used to describe genomes considered finished but still have gaps. Unfinished genomes are increasing with exponential increase of sequencing speed and capacity. A universal method developed here will help close these gaps in any microbial genomes.



Survey of Additional Non-contiguous Finished Genomes

Non-contiguous Finished Genome	Total Number of Gaps	Positive for 2° Structure ^c
<i>Brenneria</i> sp. EniD312	9	5
<i>Burkholderia cepacia</i> Bu72	3	3
<i>Clostridium</i> sp. DL-VIII	1	0
<i>Desulfovibrio africanus</i>	1	1
<i>Desulfovibrio desulfuricans</i> ND132	6	5
<i>Desulfovibrio</i> sp. FW1012B	2	2
<i>Frankia</i> sp. EUN1f	1	1
<i>leptonema illini</i> DSM 21528	17	5
<i>Methanofollis liminatans</i> DSM 4140	6	3
<i>Methylocystis</i> sp. ATCC 49242	1	1
<i>Shewanella baltica</i> OS183	4	2
<i>Thermotogales bacterium</i> mesG1.Ag.4.2	2	2

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Advancement in high throughput DNA sequencing technologies has supported a rapid proliferation of microbial genome sequencing projects, providing the genetic blueprint for in-depth studies. While sequencing speed and capacity has increased exponentially during the last decade, so too has the relative proportion of genomes with sequencing gaps. “Non-contiguous finished” has been used to describe genomes considered finished but that still have gaps. Oftentimes, difficult to sequence regions in microbial genomes are ruled “intractable” resulting in a growing number of genomes with sequence gaps deposited in databases. A procedure was developed to sequence such problematic regions in the “non-contiguous finished” *Desulfovibrio desulfuricans* ND132 genome (6 intractable gaps) and the *Desulfovibrio africanus* genome (1 intractable gap). The polynucleotides surrounding each gap formed GC rich secondary structures making the regions refractory to amplification and sequencing. Strand-displacing DNA polymerases used in concert with a novel ramped PCR extension cycle supported amplification and closure of all gap regions in both genomes. The developed procedures support accurate gene annotation, and provide a step-wise method that reduces the effort required for genome finishing.

Hurt Jr., R.A., S.D. Brown, M. Podar, A.V. Palumbo and D.A. Elias. 2012. Sequencing intractable DNA to close microbial genomes. PLoS ONE 7:e41295 (doi:10.1371/journal.pone.0041295).