Development of a Universal System to Close Microbial Genomes

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Objective

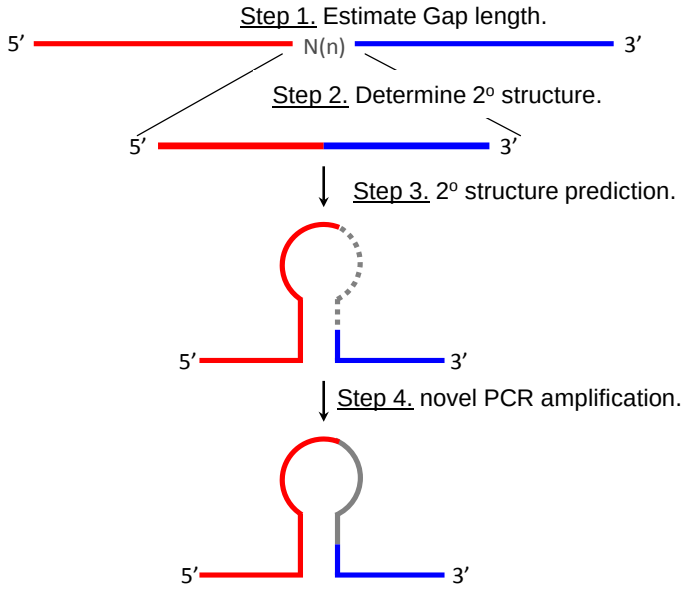
- Develop a universal method to close “non-contiguous finished” genomes.

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.



Step 5. Resolution of gap sequence with sequencing.

Survey of Additional Non-contiguous Finished Genomes

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

Hurt, 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).

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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.

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