**Manual assembly of *Mycobacterium chimaera* strain 852 genome**

*De novo* genome assembly of *M. chimaera* 852 was performed using Flye v2.8.3 assembler (see main text). However the assembler failed in the reconstruction of a complete (i.e single, circular) chromosome generating 4 circular contigs (95.6, 26.9, 21.1 and 15.9 Kb in length) and 5 linear contigs (3.1 Mb, 2.8 Mb, 289 Kb, 24 Kb and 19 Kb in length). The output (**.gfa** file) was inspected using Bandage (v0.8.1), highlighting the presence of multiple links/connection among contigs, failing in circularization (see **Figure S1**). Using the blast algorithm, the two longest contigs (3.1 and 2.8 Mb) were determined to belong to *Mycobacterium chimaera* chromosome, while the smaller contigs shared high similarities with a *M. chimaera* plasmid available in GenBank database ([NZ\_LT703506.1](https://www.ncbi.nlm.nih.gov/nuccore/NZ_LT703506.1)).

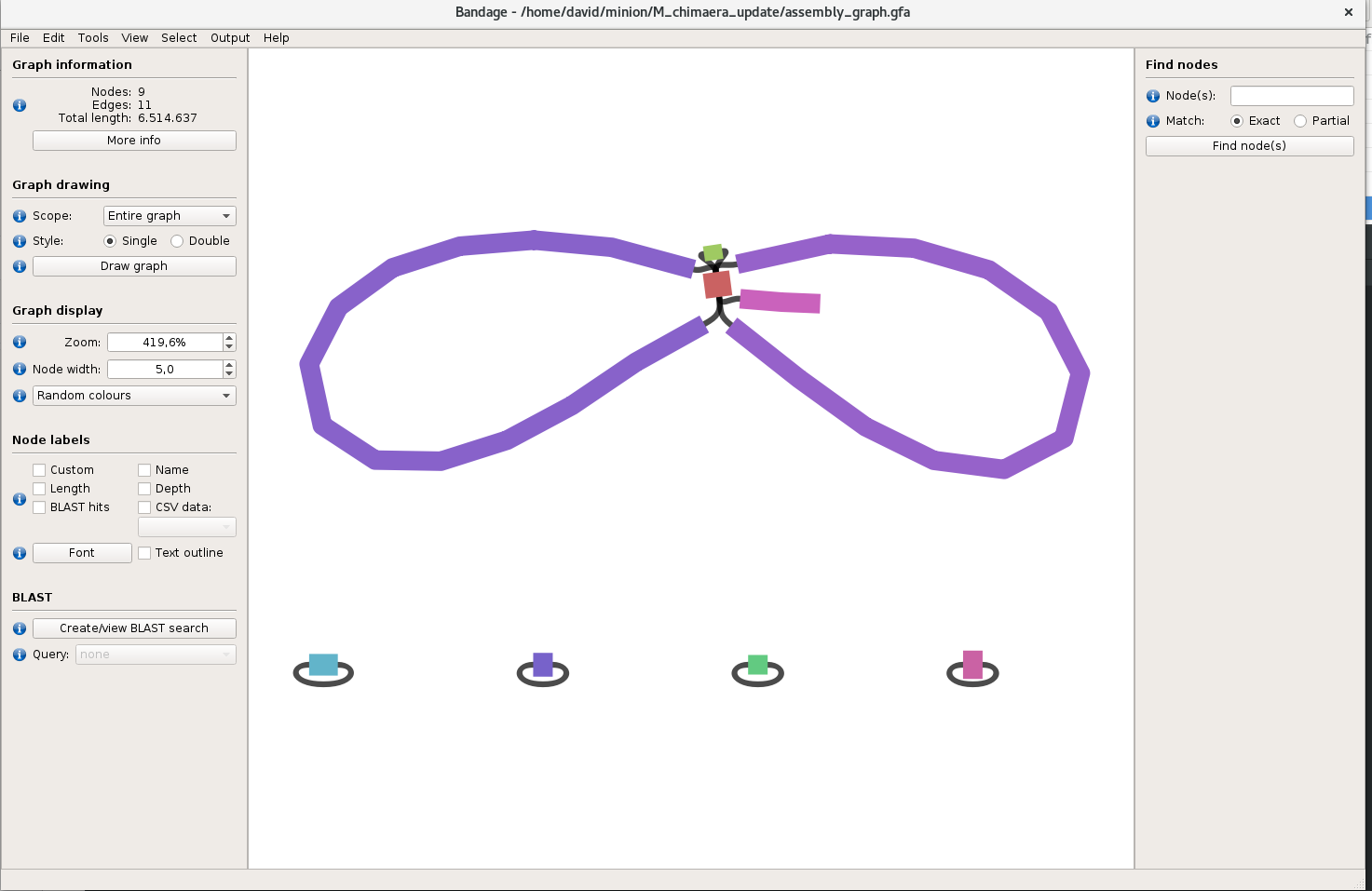
A manual curation was necessary to reconstruct both the chromosome and plasmid. The procedure was performed as follows.

Chromosomal contigs, previously identified using blast, were aligned against the *M. chimaera* 850 chromosome using the progressiveMauve aligner (Mauve v2.4.0) and manually reordered. The same procedure was performed for contigs belonging to the putative plasmid.

Nanopore reads were then aligned against the manually assembled contigs using minimap2 v 2.20. Aligned reads were visualized in Tablet v1.17 inspecting the coverage and assessing possible coverage gaps.

The draft assembled genome was polished using Medaka v1.4.1 and Pilon v1.2.4 tools, and its quality was evaluated using Ideel.

**Figure1S**:



The output of the *de novo* genome assembly process was visually represented using the tool Bandage.

The small circular contigs (bottom figure) are plasmids further listed as Plasmid2 (95.6 Kb), Plasmid3 (26.9 Kb), Plasmid4 (21.1 Kb) and Plasmid5 (15.9 Kb) in Table1 (main text). The long purple contigs (3.1 and 2.8 Mb in length) were identified as part of the *M. chimaera* chromosome by blast analysis. Pink, Orange and Green labelled contigs were instead linked to a putative plasmid homologous to [NZ\_LT703506.1](https://www.ncbi.nlm.nih.gov/nuccore/NZ_LT703506.1) and further named as Plasmid1 (322 Kb) (Table1).