**Creation of iBB151**

Creation of the B. burgdorferi metabolic model was done with the aid of the RAVEN package, which has been published elsewhere (<https://doi.org/10.1371/journal.pcbi.1006541>). Raven 2.5.3 and MATLAB R2020A were used throughout.

RAVEN scripts, documentation, and tutorials are available at <https://github.com/SysBioChalmers/RAVEN>.

* Protein coding sequences were derived from the complete *B. burgdorferi* B31 genome (Genbank assembly GCF\_000008695.2).
* Metacyc model was generated using getMetaCycModelForOrganism
* KEGG model was pulled directly from KEGG using getKEGGModelForOrganism and the organism reference *bbu*.

RAVEN 2.5.3 includes Hidden Markov Models trained to either prokaryotic or eukaryotic protein coding sequences. The prokaryotic models are available with different redundancy cutoffs: 50%, 90%, and 100%. All three models were used to generate de novo genome annotations.

* Three models were generated using getKEGGModelForOrganism
* All three HMM models were merged using mergeModels.
* The HMM and KEGG models were combined using mergeModels
* The resulting HMM-KEGG model was combined with the previous Metacyc model using combineMetaCycKEGGModels

Note that a replication of this might not produce an exact copy of iBB151. This is because KEGG and Metacyc have been updated since I ran these in summer 2020, and HMMs have an element of machine learning which can result in slightly different results each time. Also, the draft model is a mess and has to be ‘curated’ before it’s usable.

A lot of manual curation followed to turn the combined draft model into iBB151. The curation steps included

* Addition of pseudoreactions including the biomass reaction
* Addition of reactions based on available literature
* Provision of extracellular metabolites and transport reactions. These were added to remove waste and provide reagents to the point that every metabolic reaction was able to carry flux. This was confirmed using the commands checkProduction and hasFlux
* Generally tidying up names and formatting, which are different across the various databases used.

**Analysis of iBB151**

Analysis was performed with COBRA. As with RAVEN above, COBRA was previously published at <https://doi.org/10.1038/s41596-018-0098-2>. Scripts and documentation are available at <https://github.com/opencobra/cobratoolbox/>. The COBRA Toolbox v3.0 was used in MATLAB R202A.

* iBB151 can be imported using readCbModel
* The model can be solved for biomass production using optimizeCbModel
* Essential reactions are identified using *singleRxnDeletion*
* Essential genes are identified using *singleGeneDeletion*
* Lethal double mutants are identified using *doubleGeneDeletion*
* The outputs from each of these yield a growth rate and a growth ratio (growth rate of mutant/growth rate of wild type)
* The essential reactions of *B. burgdorferi* were compared to those of *E. coli* and *S. aureus* to identify unique essential genes. For this, previously published reference models iML1515 (<https://doi.org/10.1038/nbt.3956>) and iYS854 (<https://doi.org/10.1371/journal.pcbi.1006644>) were used, analyzed as above