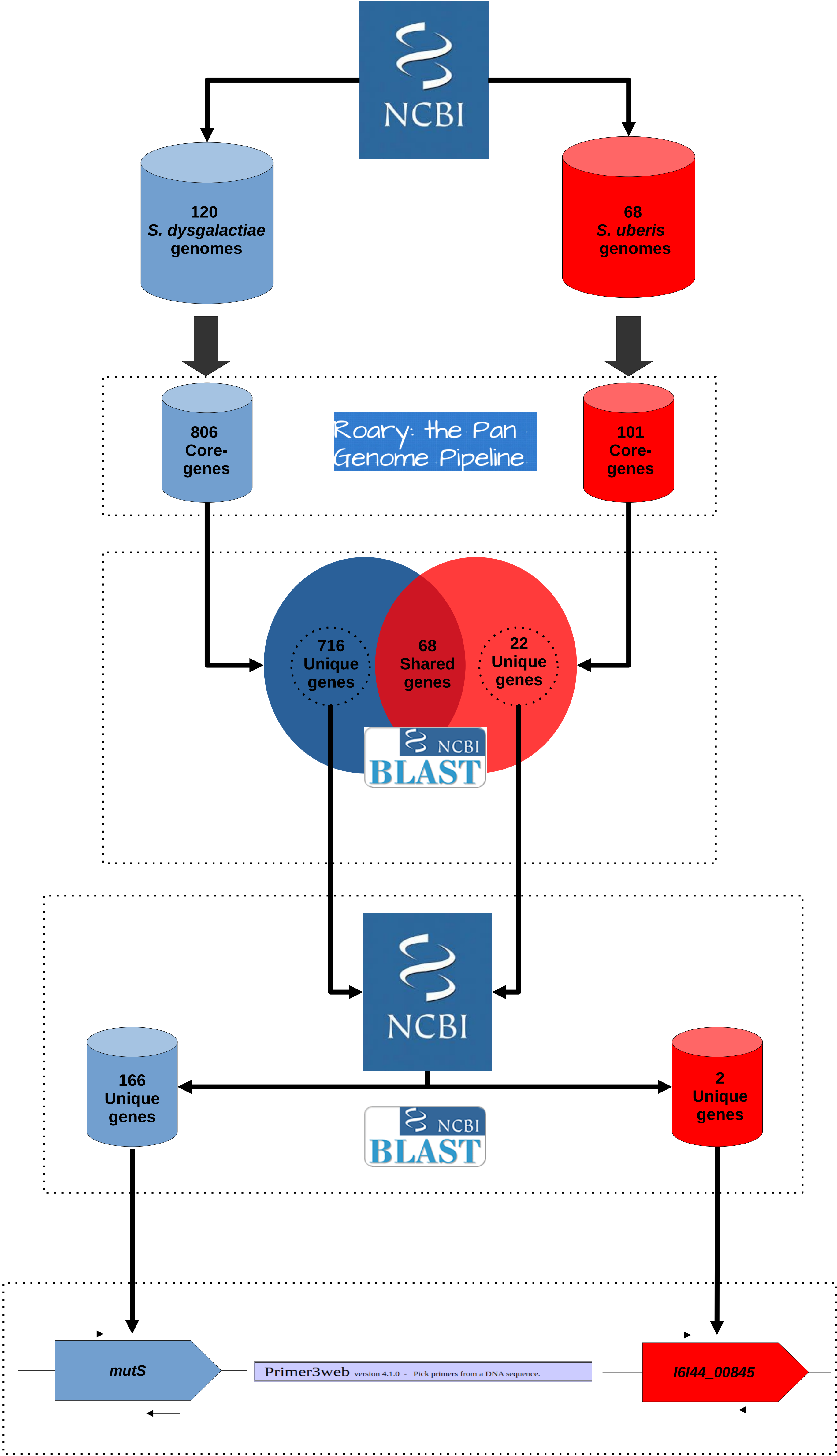**Supplementary Figure 1**

Download of *S. dysgalactiae* and *S. uberis* genomes from NCBI genomes database (June 2021)

Core-genome prediction by species
Software: Roary

Comparison of Core-genomes.
Software: tBLASTx
(parameter: Aminoacid identity % > 30, Coverage %> 70, E-value<10^5)

Searching of Unique-genes of *S. dysgalactiae* and *S. uberis* over Streptococcus genus
Software: Nucleotide BLAST
(parameter: Aminoacid identity % > 90, Coverage %> 70, E-value<10^5, Filters: include "*Streptococcus*", exclude:"*Streptococcus dysgalactiae*", "*Streptococcus uberis*")
Database: NCBI genomes

Gene Marker selection and Primer design.
Software: Primer3
(parameter: amplification product length< 180 bp, preference nucleotide start sequence: G or C, primers length: 18-24, probe length: 30-38 bp, GC%≥50 (only primers), NCBI blast hit:only with species to primers and probe design)

Supplementary Figure 1: Workflow for comparative genomics analysis and primers design implemented in the present study.