Validation of CellScanner 2.0

Data sets described in: https://www.nature.com/articles/s43705-022-00123-6
Monocultures mixed in known ratios in vitro in stationary phase
Monocultures paired with corresponding cocultures, e.g. RI_5.fcs with FP_95.fcs for co-culture RI-FP_5_95.fcs
Blank file: control_PBS.fcs
Data sets available at: https://flowrepository.org/id/FR-FCM-Z3TM

Settings

CellScanner 2.0 run with default values:
1000 events
Scaling constant: 150
number of neighbors: 50
UMAP min dist: 0
number of required blank neighbors: 20
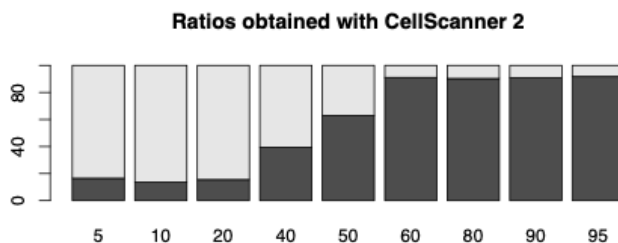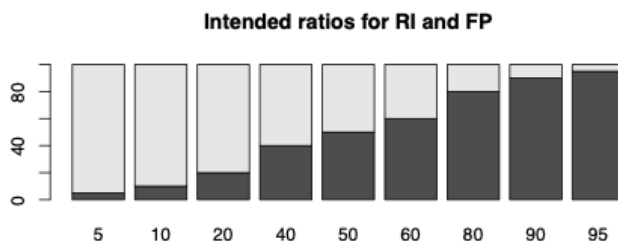number of required non-blank neighbors: 25
folds: 0
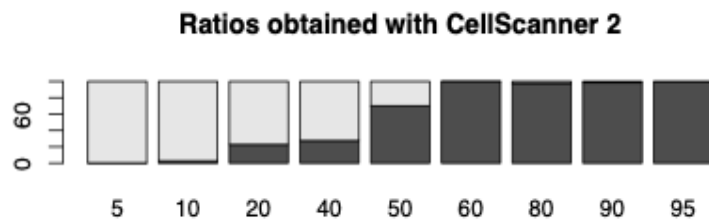epochs: 50
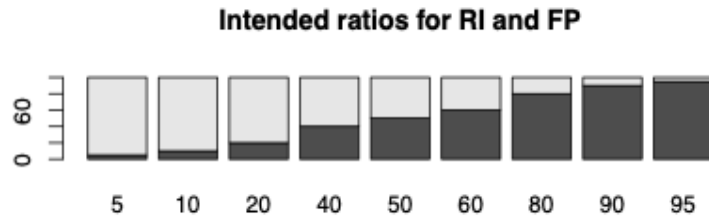early stopping patience: 10
batch size: 32

Result

Roseburia intestinalis (RI) and _Faecalibacterium prausnitzii_ (FP)

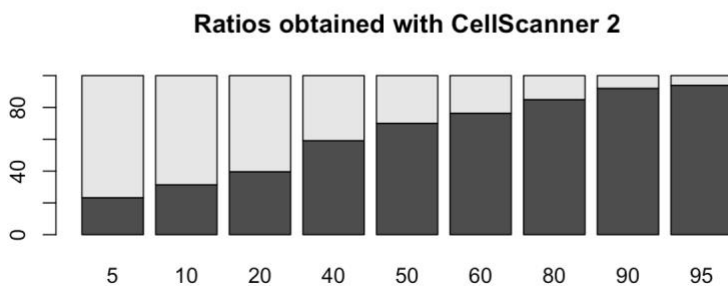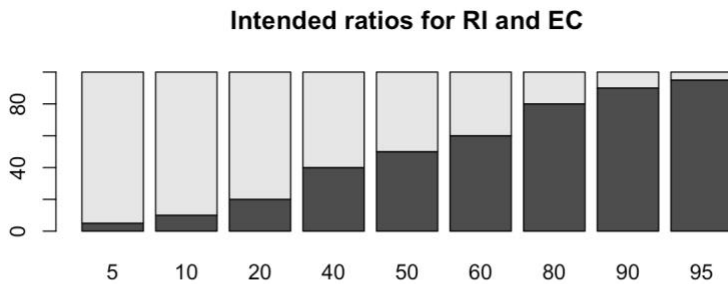Intended ratios for RI and FP

Ratios obtained with CellScanner 2

Roseburia intestinalis (RI) and *Faecalibacterium prausnitzii* (FP) with uncertainty filtering

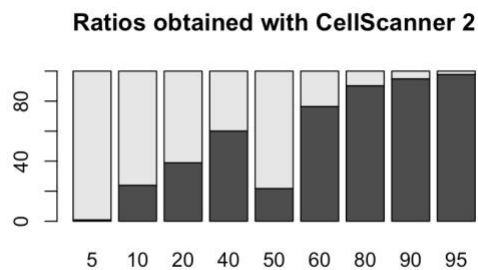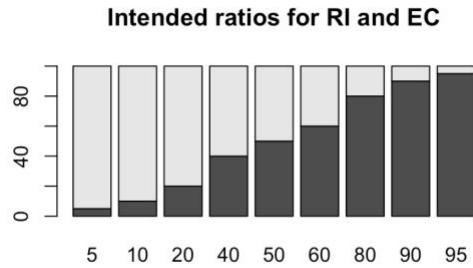Uncertainty filtering: events with entropy above a threshold set automatically are removed

Intended ratios for RI and FP

Ratios obtained with CellScanner 2

Roseburia intestinalis (RI) and *Escherichia coli* (EC)

This is a positive control since *E. coli* is labelled with mCherry.

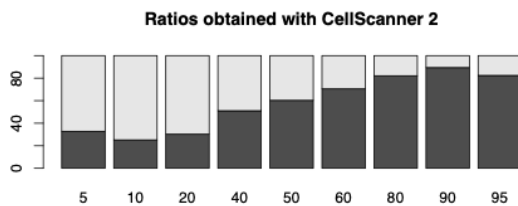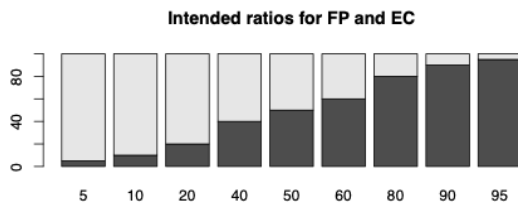### Intended ratios for RI and EC

Ratios obtained with CellScanner 2

Roseburia intestinalis (RI) and _Escherichia coli_ (EC) with uncertainty filtering

Intended ratios for RI and EC

Ratios obtained with CellScanner 2

The outlier at 50:50 in the results with uncertainty filtering goes to show that this filtering step is only effective if uncertain events are evenly distributed between species. Here, _E. coli_, being labelled with mCherry, had only few uncertain events, and thus _R. intestinalis_ was over-represented in the removed events, which biased the predicted proportion.
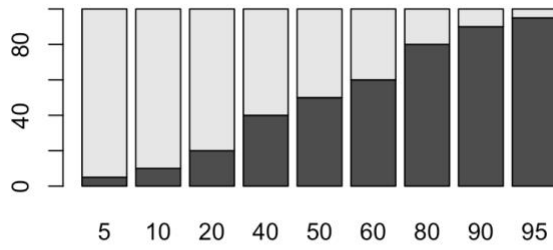
Faecalibacterium prausnitzii (FP) and _Escherichia coli_ (EC)
This is another positive control since _E. coli_ is labelled with mCherry.

Intended ratios for FP and EC

Ratios obtained with CellScanner 2

Faecalibacterium prausnitzii (FP) and _Escherichia coli_ (EC) with uncertainty filtering

Intended ratios for FP and EC

Ratios obtained with CellScanner 2