CyTOF gating-ML workflow

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CyTOF preprocessing workflow

1. Concatenate FCS files (R)
2. Bead normalization (immunologist)
3. Debarcode samples (immunologist)
4. Clean up your singlet FCS files (immunologist)
5. Batch normalization (R)

May be run as each batch of data is received from lab

Must be run once all batches are received from lab (previous normalization cannot be updated)

CyTOF analysis workflow

1. Generate and export Gating ML file on Cytobank (immunologist)

2. Apply Gating ML to individual samples (R)

3. Extract population counts (R)

4. Calculate population frequencies as proportion of parent population, as proportion of CD45+CD66-, etc. (R)

4. Extract expression data (R)

5. Visualize gates

Key R object classes

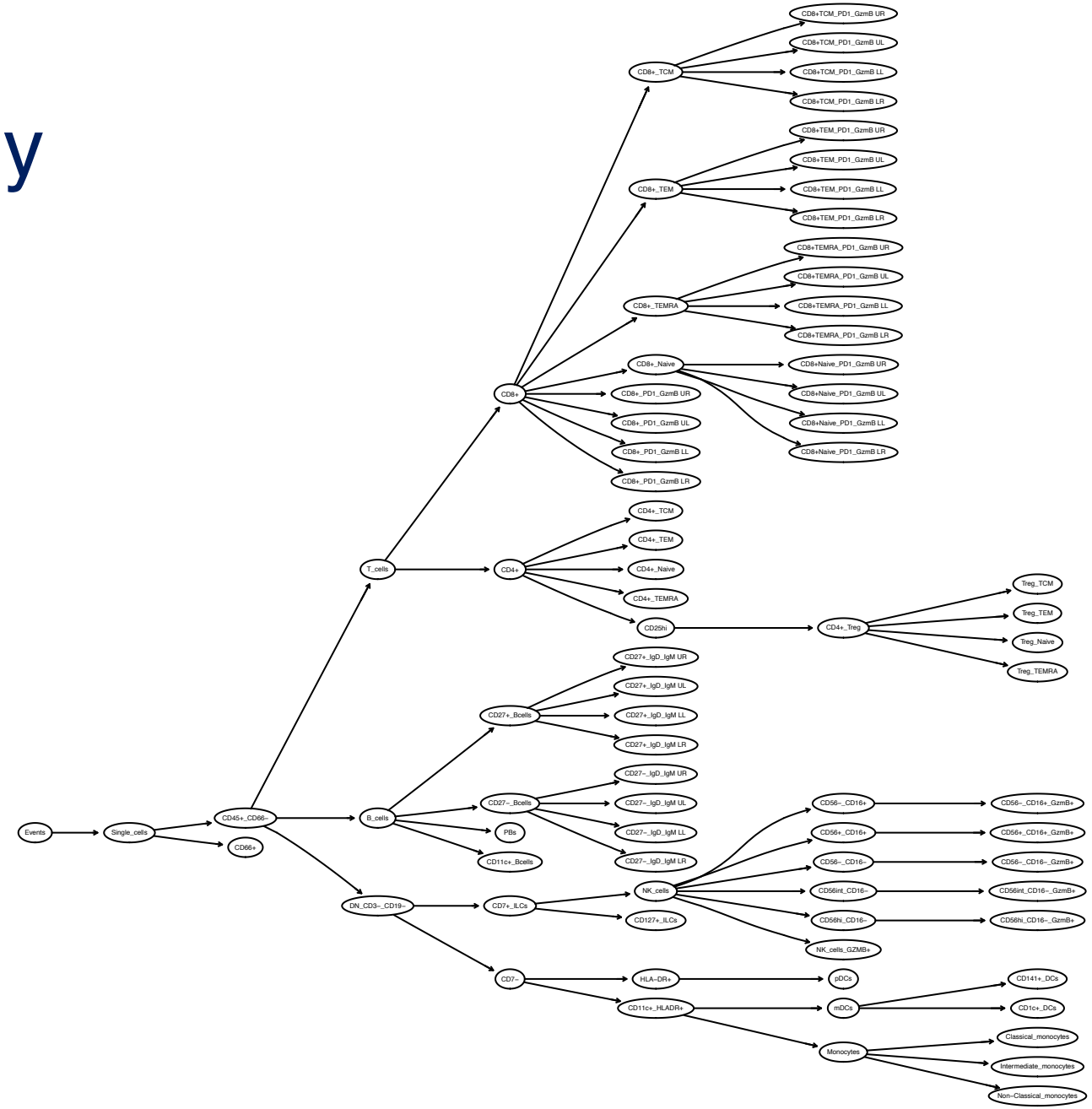
flowFrame = FCS data for one sample

flowSet = vector of flowFrames

ncdfFlowSet = vector of flowFrames for datasets too large for memory (not yet tested)

GatingSet = gated FCS data

Population hierarchy



Gate visualization