

This folder contains the necessary files needed to carry out cellular deconvolution of whole blood gene expression profiles to generate inferred neutrophil, lymphocyte, monocyte, and eosinophils using the CIBERSORTx java application (either locally or by web interface at <https://cibersortx.stanford.edu>) under the same settings used in the Technical Validation section of O’Connell et al. *cibersort_reference_samples.txt* is the expression matrix of TPM values originating from isolated human leukocyte samples that was used to construct the custom reference signature matrix that was used for deconvolution. *cibersort_reference_phenotypes.txt* is the associated phenotype file containing the cell type labels. *cibersort_reference_signature_matrix.txt* is the resultant reference signature matrix.

While we only used the java application to generate the custom reference signature used in the example analyses detailed in the manuscript (all actual deconvolution was carried out using the CIBERSORTx source code in R), we have also included a file named *whole_blood_rpm_values.txt* which is an abbreviated example input file illustrating the format whole blood gene expression data should be provided in if one also wanted to carry out deconvolution using the java application. Data should be normalized gene-level summarized read counts, ideally in the form of RPM or TPM values. The exact RNA sequencing data used for the analyses presented in the manuscript can be retrieved from the National Center for Biotechnology Information via BioProject accession number PRJNA949611.

The following settings were used to generate the custom reference signature matrix:

- **Job type:** Create Signature Matrix
- **Reference sample file:** cibersort_reference_samples.txt
- **Phenotype classes file:** cibersort_reference_phenotypes.txt
- **Disable quantile normalization:** true
- **kappa:** 999
- **q-value:** 0.01
- **No. barcode genes:** 300 to 500
- **Filter non-hematopoietic genes from signature matrix during construction:** false

The following settings can be used for deconvolution of whole blood gene expression profiles using the resultant reference signature matrix, and are identical to those that we used in the manuscript when implementing CIBERSORTx via R:

- **Job type:** Impute Cell Fractions
- **Signature matrix file:** cibersort_reference_signature_matrix.txt
- **Mixture file:** whole_blood_rpm_values.txt
- **Batch correction:** disabled
- **Disable quantile normalization:** true
- **Run mode (relative or absolute):** relative
- **Permutations:** 100