Frederick National Laboratory
for Cancer Research
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Preliminary Study of Classification of Pediatric Cancers Using Gene Expression Data from the TARGET Dataset

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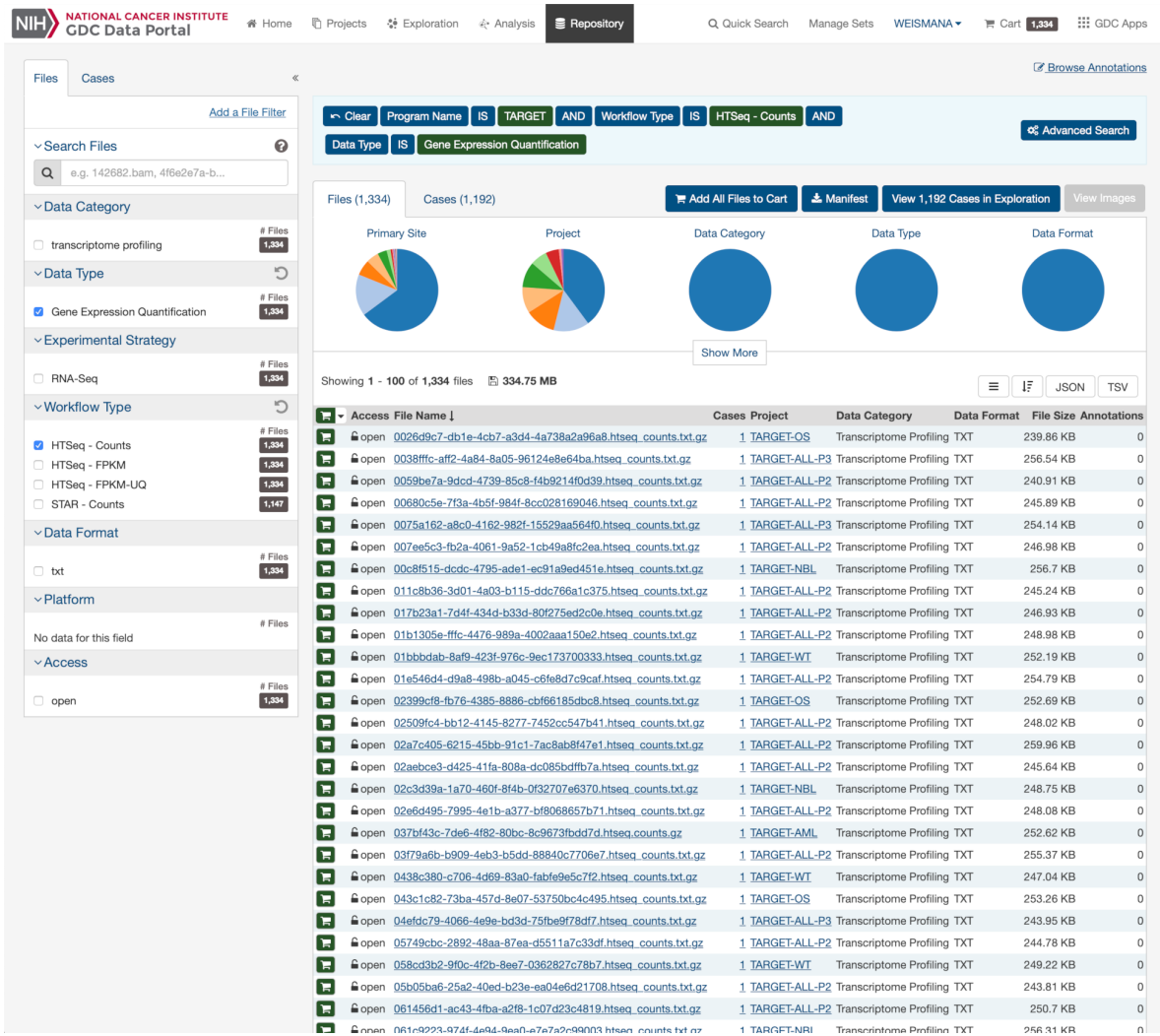
Overall project progression

- Initially tried using data from the TARGET project's data website https://target-data.nci.nih.gov
- Potentially helpful because there appears to be much more gene expression data there (~2x) than at the GDC Data Portal https://portal.gdc.cancer.gov
- However, we found great difficulties, especially in unifying all the gene names, IDs, accession numbers, etc., no matter the method
- Moved for the time being to using the data from the Data Portal, which is harmonized by the GDC
- Have since run initial studies using these data, described herein

Data download

- **Obtain all HTSeq-generated counts expression files in the TARGET program on Helix:**
  - Download the Manifest (blue button)
  - Use the GDC client tool (`module load gdc-client`)
- **Add all files to the cart, go to the cart, and download:**
  - Sample Sheet (blue button)
  - Metadata (blue button)
- **This yields data for 1,334 counts files and 1,192 cases (i.e., people)**

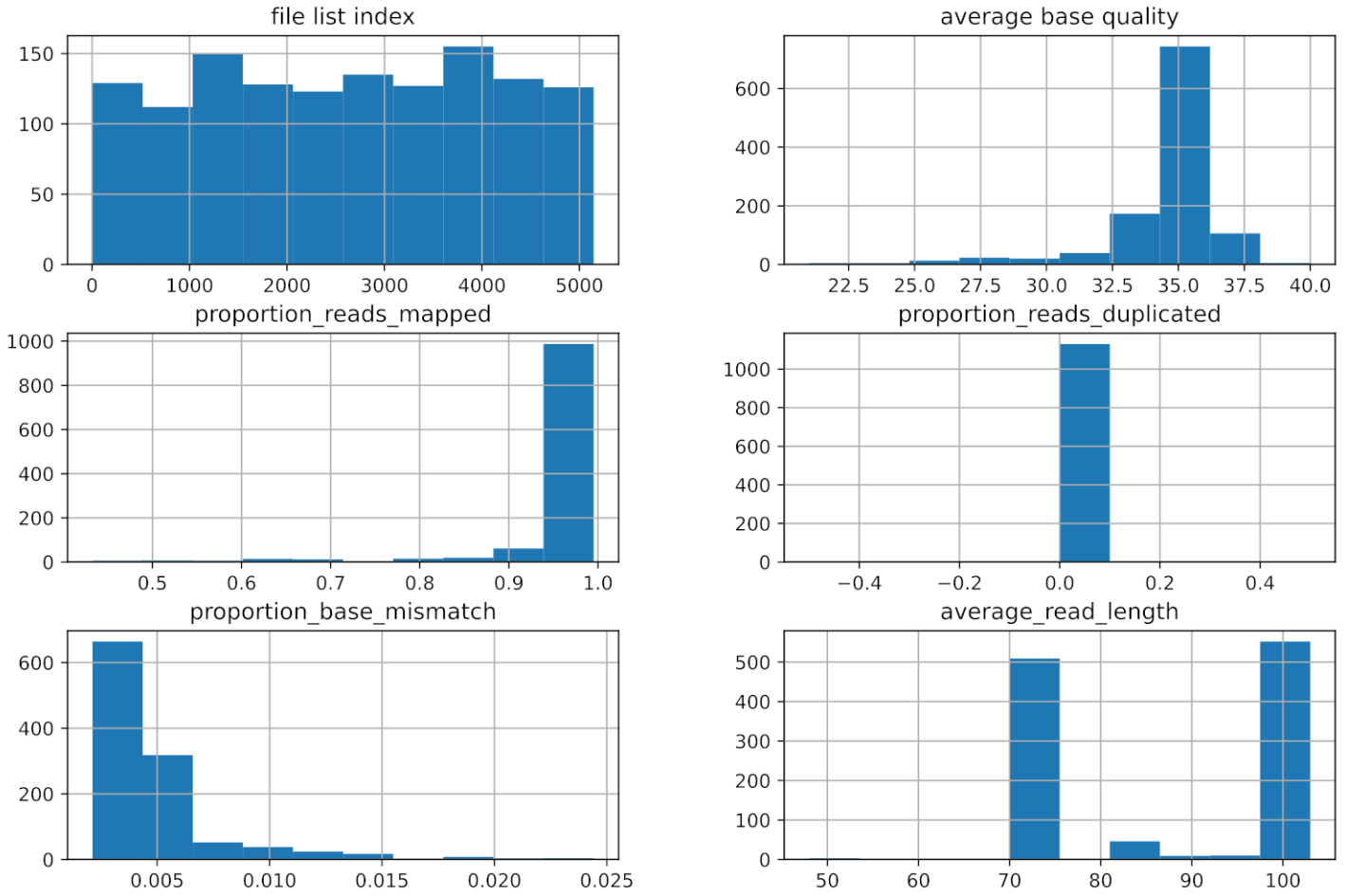


Data preprocessing

- **Read in and unify the counts files, sample sheet data, and metadata**
- **Found 1,321 unique samples in the sample sheet (instead of all 1,334)**
  - In 13 cases there are two HTSeq counts files for a single sample, i.e., multiple analyses of the same sample
  - In these cases, choose that with the best “average_base_quality” score (or choose the first in case of a tie)
- **Drop four samples that correspond to multiple cases (people), which doesn’t make sense → 1,317 samples remaining**
  - GDC said this was a bug and would be fixed in the future

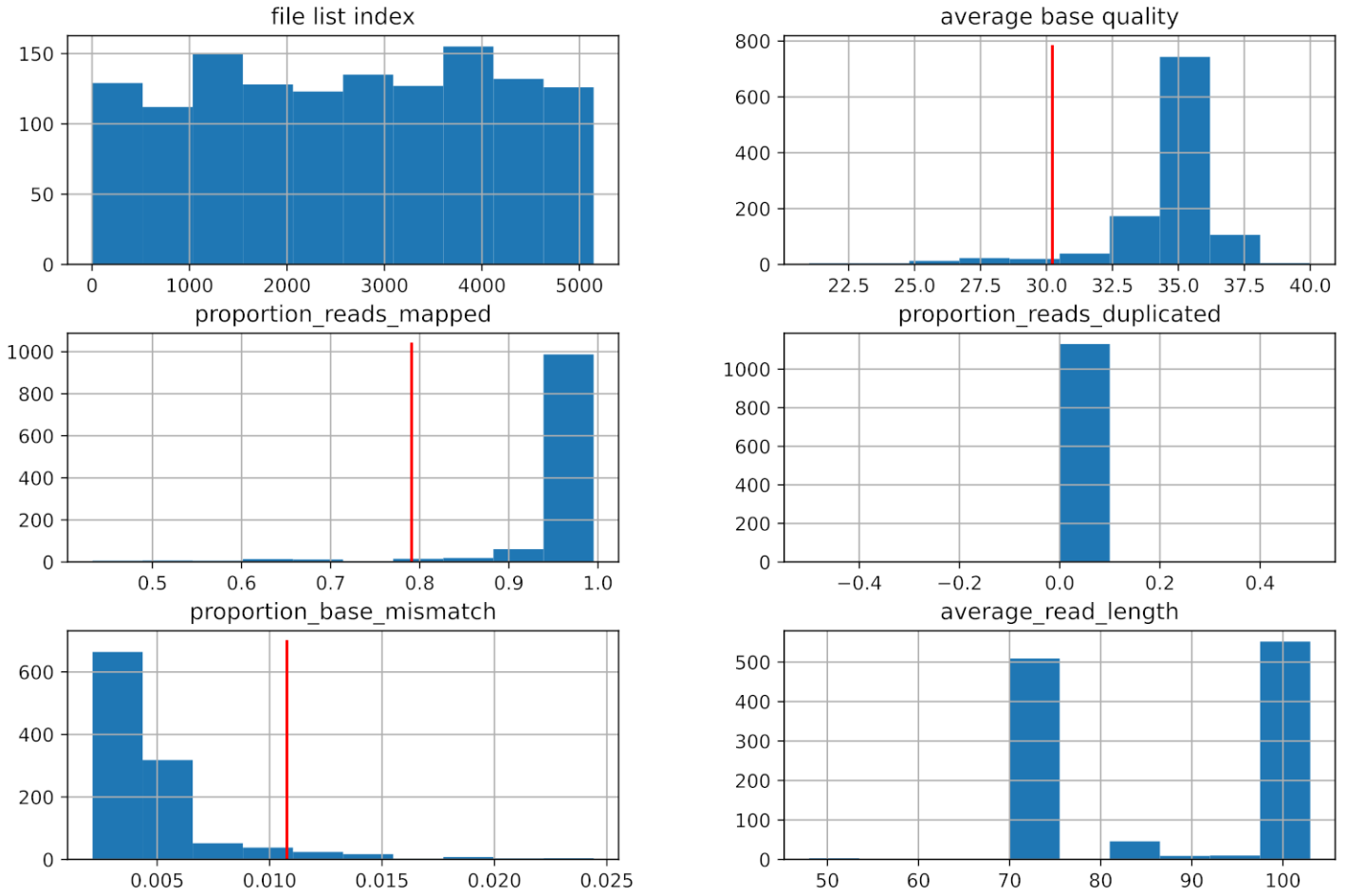
Numeric fields for each sample

- **Original:**



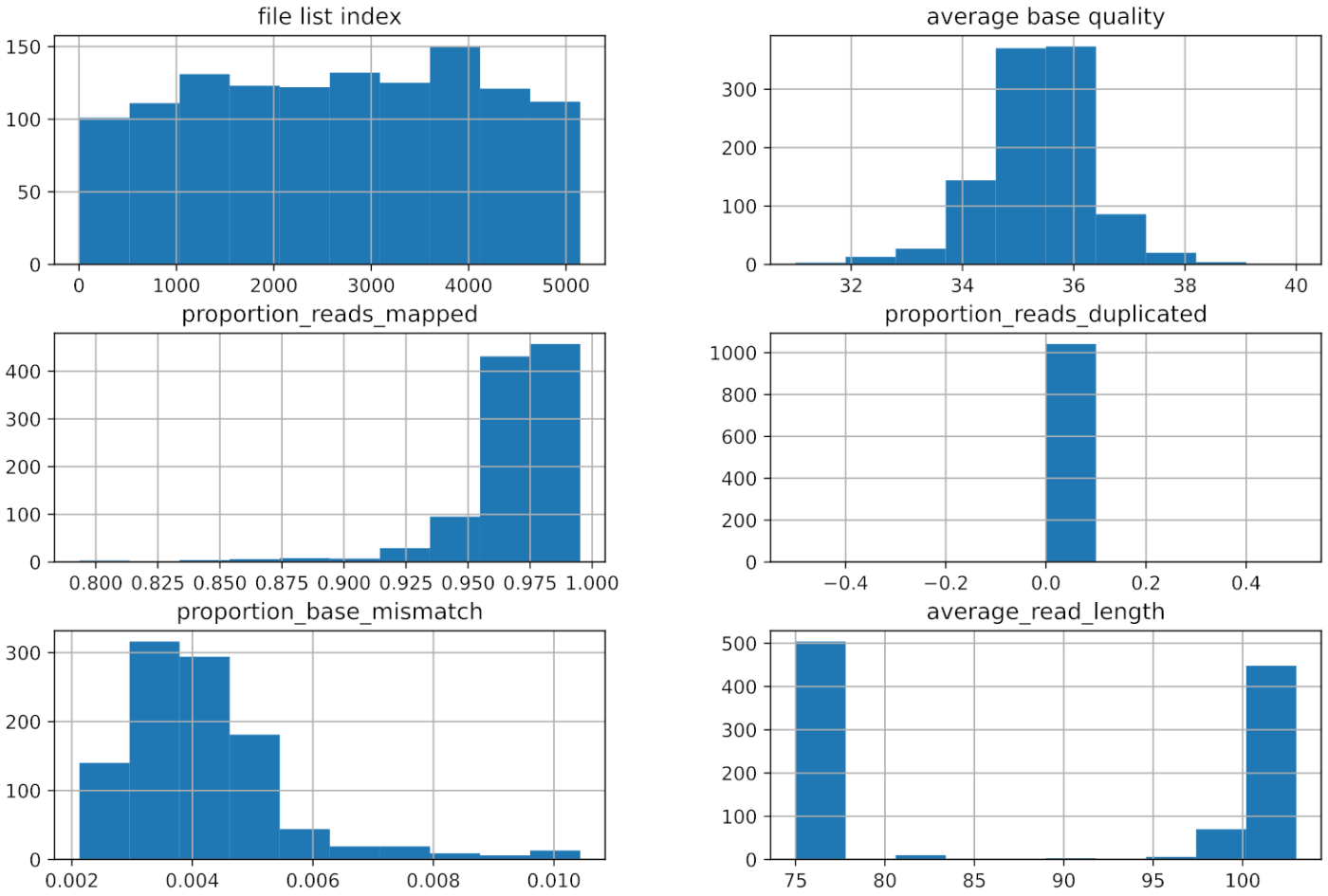
Numeric fields for each sample, ctd.

- **With cutoffs:**



Numeric fields for each sample, ctd.

- **After cutoffs applied, eliminating 89 samples → 1,228 remaining:**



Non-numeric fields for each sample

- **Columns with all unique values (1,228 of them), with example values:**
  - **counts file name:** de2b4915-95fc-45d9-b327-adde18559211.htseq_counts.txt.gz
  - **file id:** 677342f7-1f9a-4615-925c-746d2d460b49
  - **entity_submitter_id:** TARGET-10-PARTBP-09A-01R
  - **sample id:** TARGET-10-PARTBP-09A
- **Columns with uniform values:**
  - **contamination_error:** None
  - **contamination:** None
  - **state:** released
  - **platform:** Illumina

Non-numeric fields for each sample, ctd.

- **Columns with non-unique and non-uniform values:**
  - **case id** (1,099 unique values):
    - `TARGET-15-SJMPAL042946  3`
    - `TARGET-50-PAJNRL  2`
    - `TARGET-10-PARAKF  2`
    - `TARGET-15-SJMPAL041119  2`
    - `TARGET-10-PARPRW  2`
    - `...  ...`
    - `TARGET-50-PAJMKN  1`
    - `TARGET-15-SJMPAL012421  1`
    - `TARGET-10-PARNSP  1`
    - `TARGET-50-PAJLLF  1`
    - `TARGET-10-PANWEZ  1`

Non-numeric fields for each sample, ctd.

- **Columns with non-unique and non-uniform values, ctd.:**
  - **project id** (9 unique values):

TARGET-ALL-P2	**518**	Acute Lymphoblastic Leukemia – Phase II
TARGET-AML	**187**	Acute Myeloid Leukemia
TARGET-NBL	**143**	Neuroblastoma
TARGET-WT	**136**	Wilms Tumor
TARGET-ALL-P3	**135**	Acute Lymphoblastic Leukemia – Phase III (ALAL)
TARGET-RT	**69**	Rhabdoid Tumor
TARGET-OS	**24**	Osteosarcoma
TARGET-CCSK	**13**	Clear Cell Sarcoma of the Kidney
TARGET-ALL-P1	**3**	Acute Lymphoblastic Leukemia – Pilot

Non-numeric fields for each sample, ctd.

- **Columns with non-unique and non-uniform values, ctd.:**
  - **sample type** (8 unique values):

Primary Blood Derived Cancer - Bone Marrow	614
Primary Tumor	359
Recurrent Blood Derived Cancer - Bone Marrow	117
Primary Blood Derived Cancer - Peripheral Blood	109
Recurrent Tumor	12
Solid Tissue Normal	12
Recurrent Blood Derived Cancer - Peripheral Blood	4
Metastatic	1

Combine “project id” and “sample type” columns → most detailed set of labels

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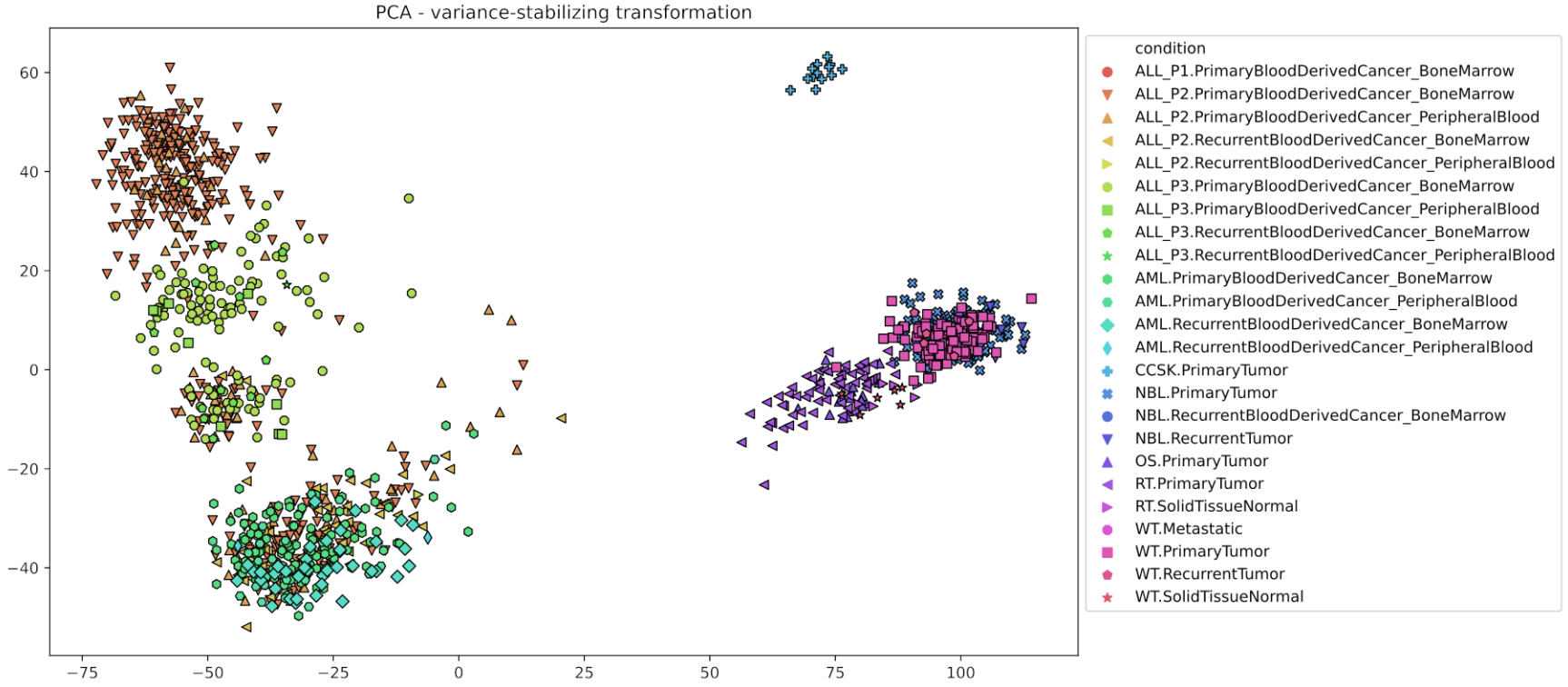
- TARGET-ALL-P2, Primary Blood Derived Cancer - Bone Marrow 379
- TARGET-NBL, Primary Tumor 135
- TARGET-WT, Primary Tumor 124
- TARGET-AML, Primary Blood Derived Cancer - Bone Marrow 119
- TARGET-ALL-P3, Primary Blood Derived Cancer - Bone Marrow 113
- TARGET-ALL-P2, Primary Blood Derived Cancer - Peripheral Blood 74
- TARGET-ALL-P2, Recurrent Blood Derived Cancer - Bone Marrow 64
- TARGET-RT, Primary Tumor 63
- TARGET-AML, Recurrent Blood Derived Cancer - Bone Marrow 40
- TARGET-AML, Primary Blood Derived Cancer - Peripheral Blood 26
- TARGET-OS, Primary Tumor 24
- TARGET-CCSK, Primary Tumor 13
- TARGET-ALL-P3, Recurrent Blood Derived Cancer - Bone Marrow 12
- TARGET-ALL-P3, Primary Blood Derived Cancer - Peripheral Blood 9
- TARGET-NBL, Recurrent Tumor 7
- TARGET-WT, Solid Tissue Normal 6
- TARGET-RT, Solid Tissue Normal 6
- TARGET-WT, Recurrent Tumor 5
- TARGET-ALL-P1, Primary Blood Derived Cancer - Bone Marrow 3
- TARGET-AML, Recurrent Blood Derived Cancer - Peripheral Blood 2
- TARGET-NBL, Recurrent Blood Derived Cancer - Bone Marrow 1
- TARGET-ALL-P3, Recurrent Blood Derived Cancer - Peripheral Blood 1
- TARGET-WT, Metastatic 1
- TARGET-ALL-P2, Recurrent Blood Derived Cancer - Peripheral Blood 1

Counts normalization

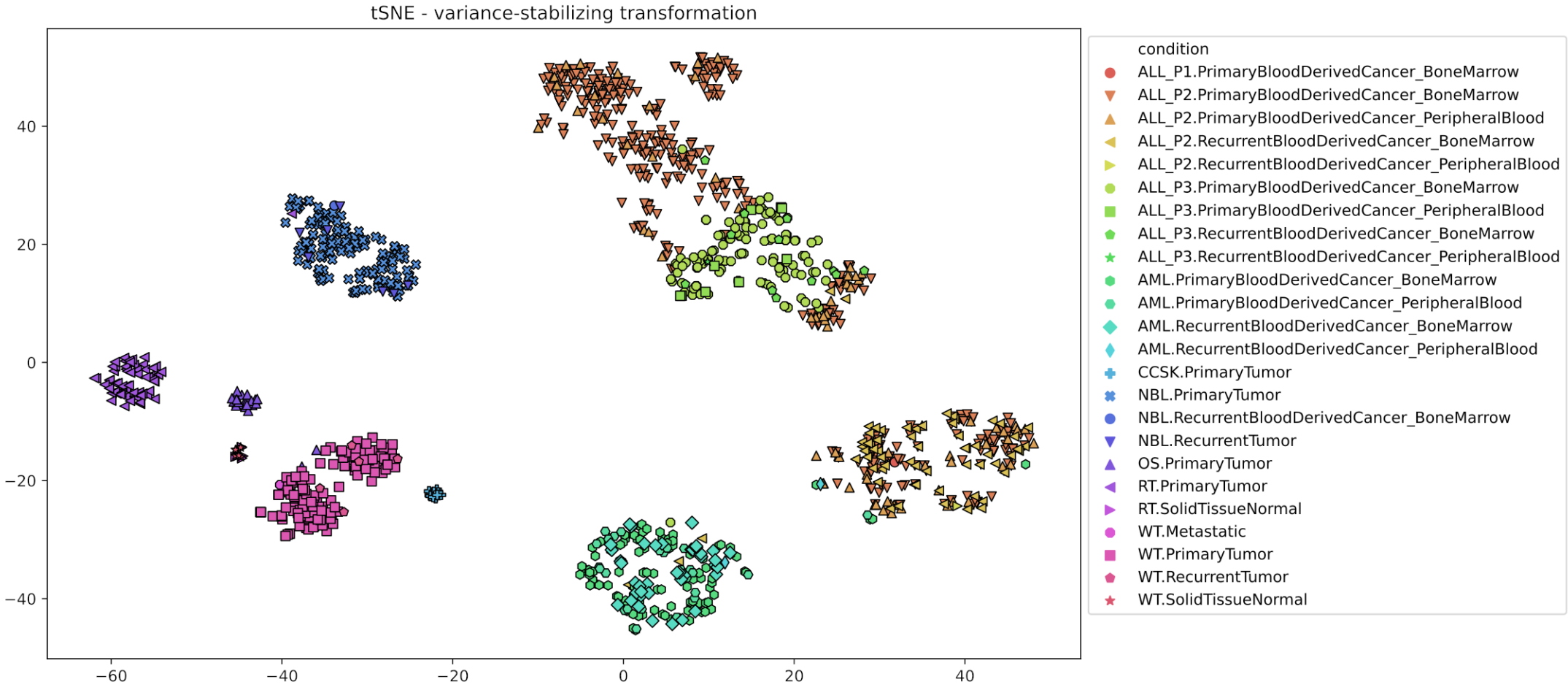
- **Most appropriate method is to perform a statistical analysis on the raw counts *C***
  - Sample packages: DESeq2, EdgeR, Limma Voom
  - We're choosing DESeq2 → popular and well-documented
- **Three main types of transformations in DESeq2 (they all transform the counts to the log-base-2 scale):**
  - **Normal transformation:** $\log_2(C^* + 1)$, where C^* represents the counts C divided by size factors (i.e., the normalized counts)
  - **Regularized log transformation:** makes count variances independent of their means using fitting to a GLM for each gene, taking library size into account
  - **Variance-stabilizing transformation (VST):** makes normalized count variances independent of their means using a monotonous mapping function based on the variance-mean dependence in a fitted negative binomial distribution, taking library size into account
    - We use this, as it is much faster and requires less memory than regularized log

Principal components analysis on the detailed dataset

- **Run VST on the counts and the detailed set of labels y_1, resulting in the data matrix X_1 consisting of VST-normalized counts**
- **Perform principal components analysis on X_1 (using y_1 as labels, and top-500-variance genes)**



tSNE on the detailed dataset



tSNE on the detailed dataset – observations

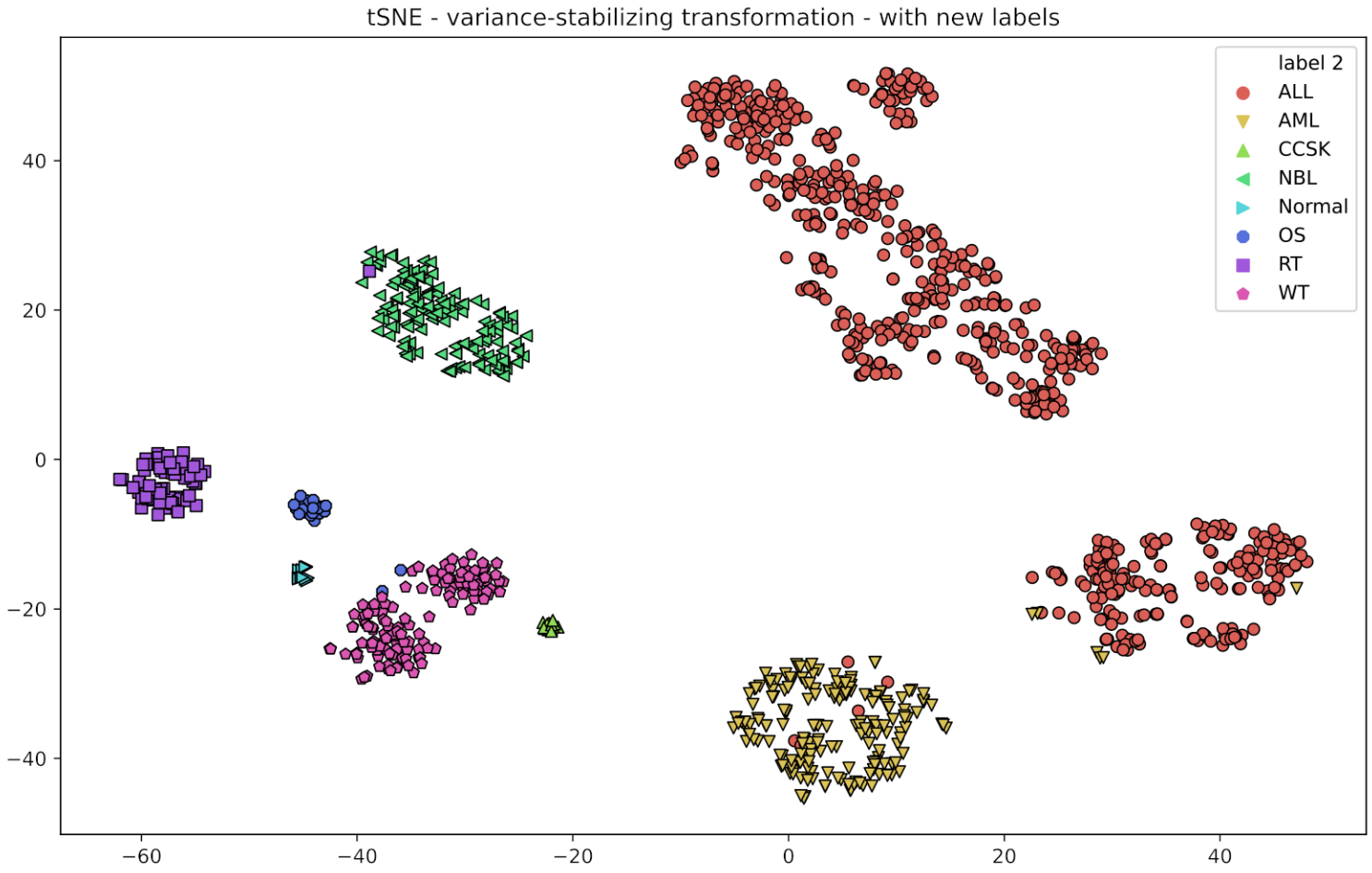
- ALL is spread out into four main clusters; in particular:
  - ALL/P2/PBDC (both /BM and /PB) is located in three of them
  - ALL/P2/RBDC (i.e., /BM) is basically isolated to one of them
  - ALL/P3 is basically in the fourth cluster
- AML has its own single cluster
- There are some ALL in an AML cluster and vice versa, and the two corresponding clusters are near each other
  - Note that even though ALL/P3 is supposed to be the "ambiguously myeloid" one (ALAL, acute leukemia of ambiguous lineage), it is primarily ALL/P2 that seems to be confused with AML
- CCSK is very tightly clustered
- NBL has its own cluster, with one RT instance that may be misclassified
- OS is tightly clustered, except for two samples in WT, which may indicate that they are misclassified
- Aside from the one possibly misclassified RT, they are all solidly clustered
- WT is clustered together
- The two normal species are very tightly clustered together
- Note the three kidney tumor types (RT, WT, CCSK), and OS and Normal, are relatively close together

Create a “less detailed” set of labels

- Combine the two normal species
- Combine all ALL projects into one
- Otherwise, use the project IDs as the labels

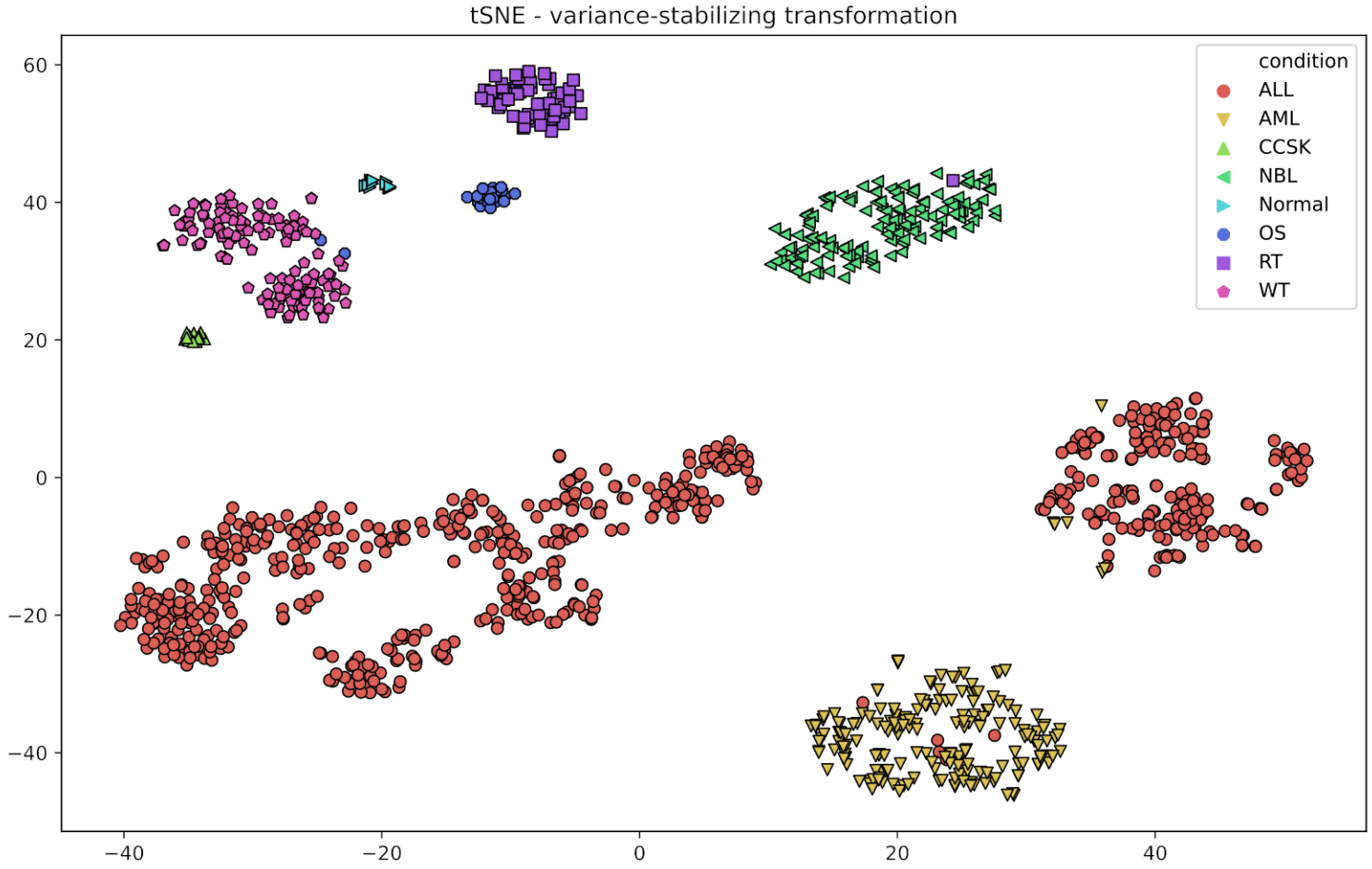
- New set of classes:
  - `ALL  656`
  - `AML  187`
  - `NBL  143`
  - `WT  130`
  - `RT  63`
  - `OS  24`
  - `CCSK  13`
  - `Normal  12`

Use the new set of labels on the previous tSNE plot



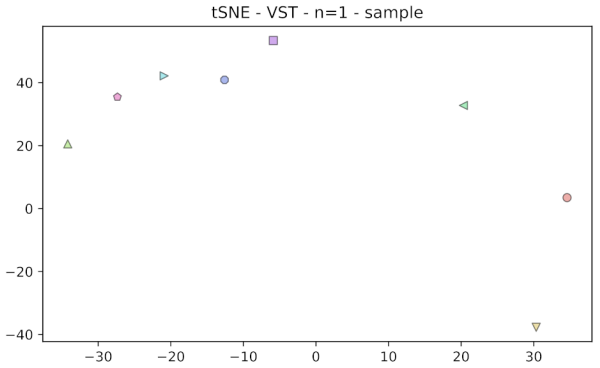
tSNE on the less-detailed dataset

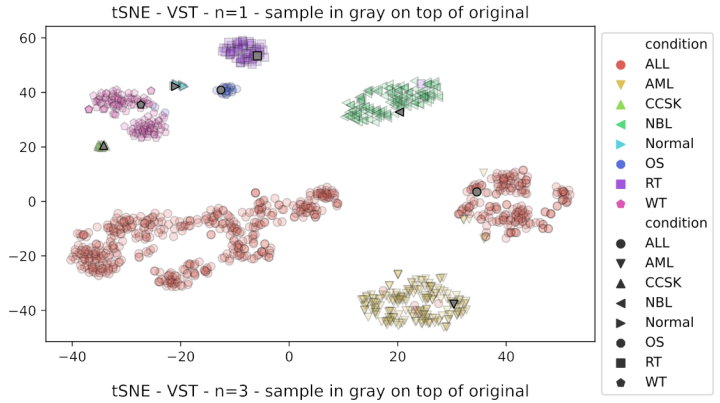
- **Must re-run VST on the raw counts using the new, less-detailed set of labels y_2, resulting in X_2**



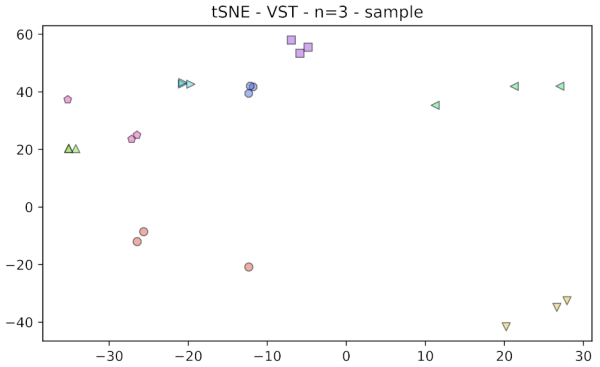
Sample with replacement from each class

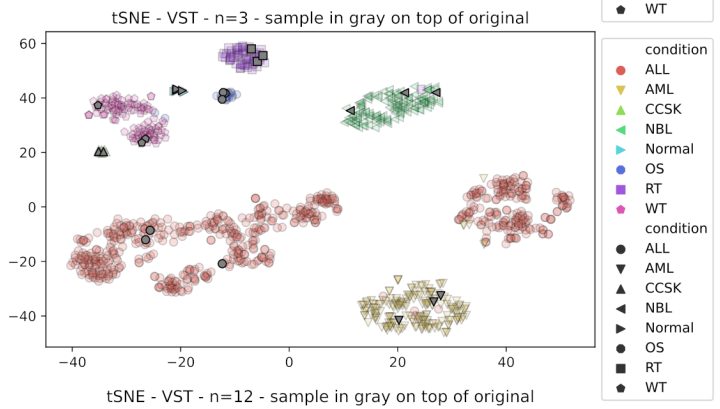
N=1



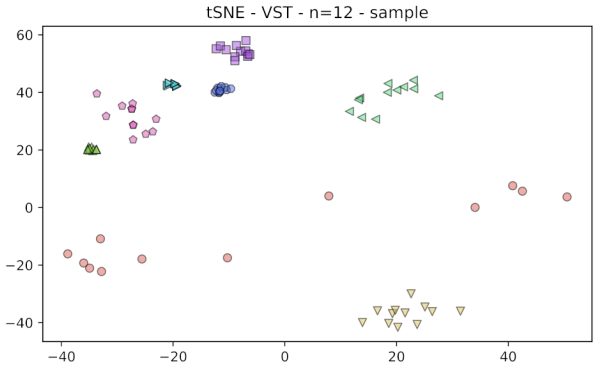


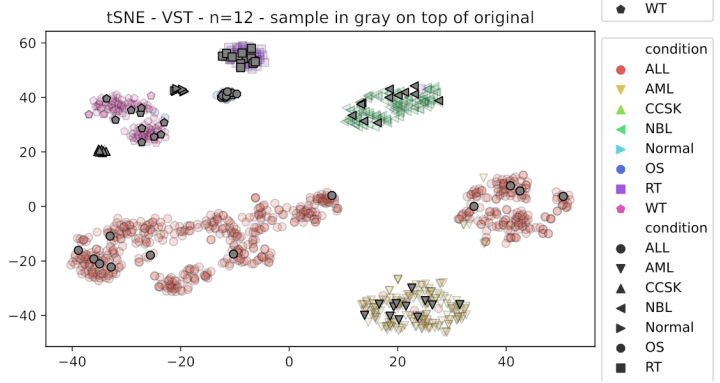
N=3





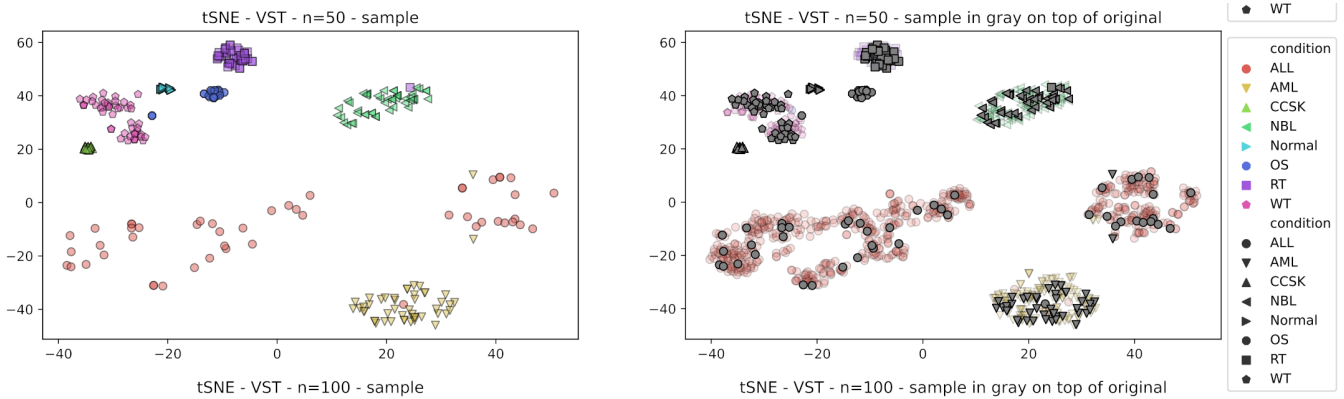
N=12



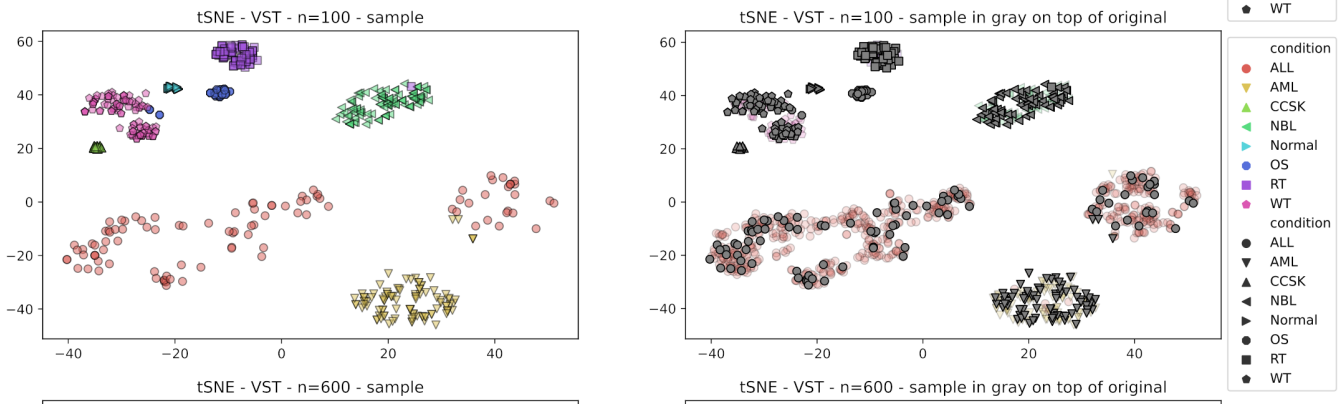


Sample with replacement from each class, ctd.

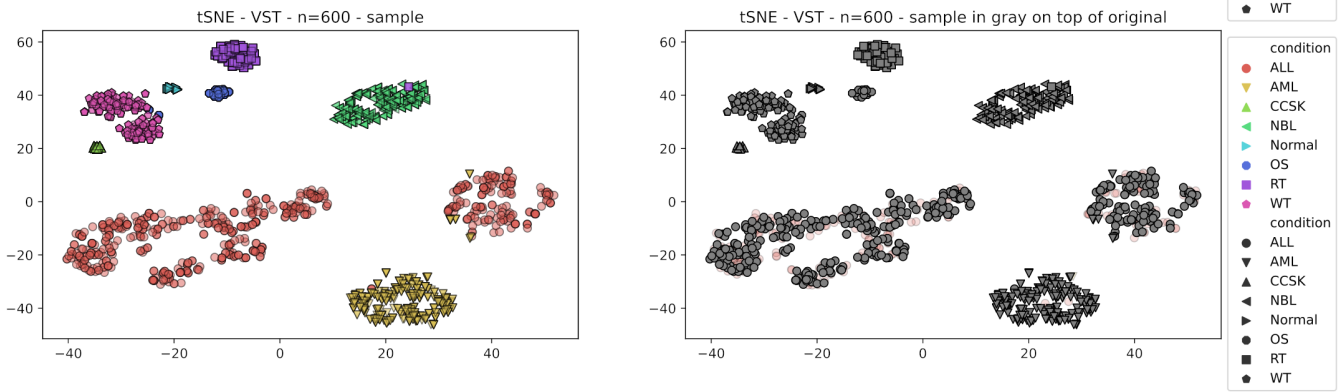
N=50



N=100

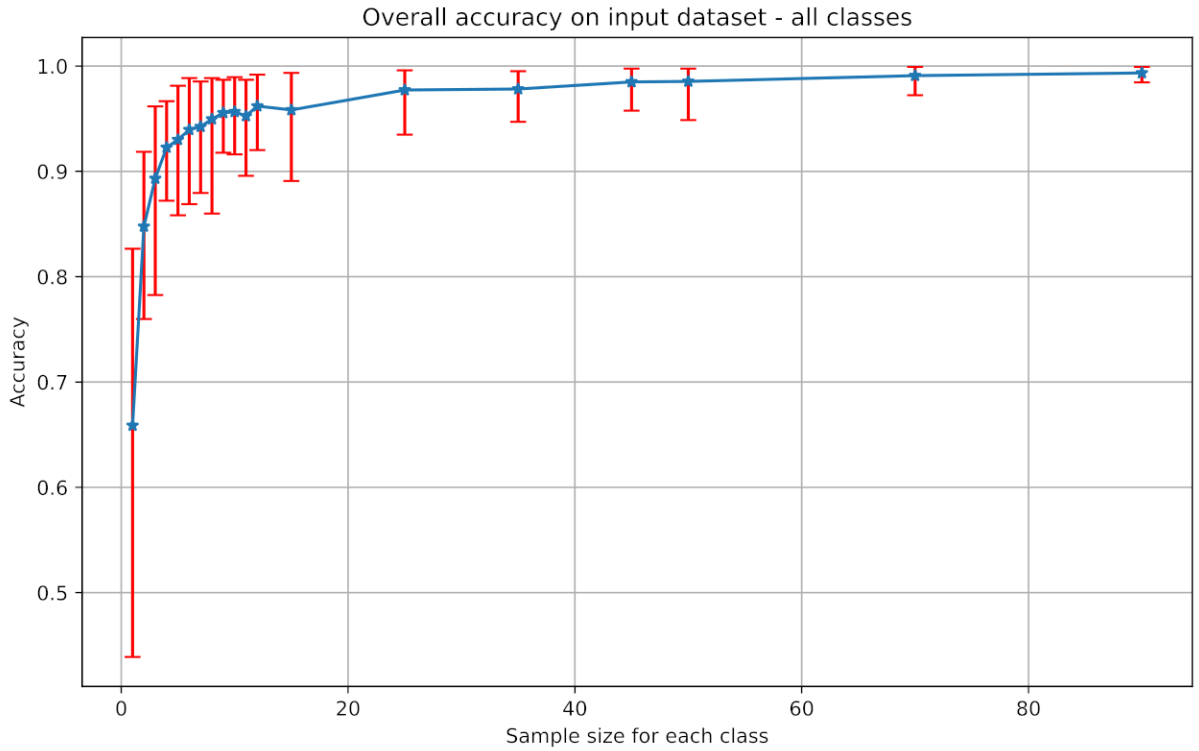


N=600



Learn from these balanced datasets of various sizes

- **For various sample sizes (*x*-axis), train a random forest classification model and compute the accuracy on the entire, imbalanced 1,228-sample dataset**
- **Run 30 different models for each differently-sized dataset**

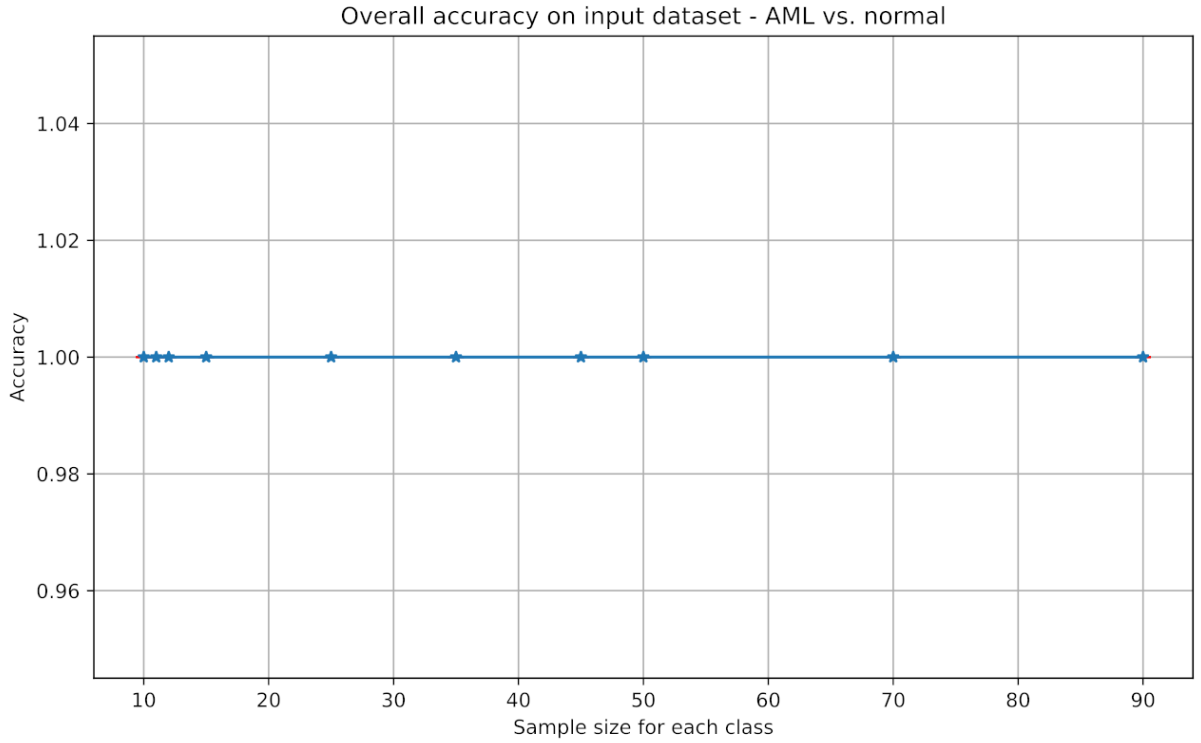


Roughly determine the most important genes

- **Running a crude feature importance study using the same random forest models, we find these most important genes for separating the eight classes (with score):**
  - 0.000953: UNC homeobox
  - 0.000903: WASP family member 3
  - 0.000896: matrix metallopeptidase 13
  - 0.000874: MyoD family inhibitor
  - 0.000806: DENN domain containing 11
  - 0.000805: small integral membrane protein 2
  - 0.000766: SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1
  - 0.000752: LY6/PLAUR domain containing 1
  - 0.000746: FZD10 antisense divergent transcript

Run the same methodology for AML vs. Normal

- **For various sample sizes, train a random forest classification model and compute the accuracy on the imbalanced dataset containing just AML and Normal samples**
- **Run 30 different models for each differently-sized dataset**

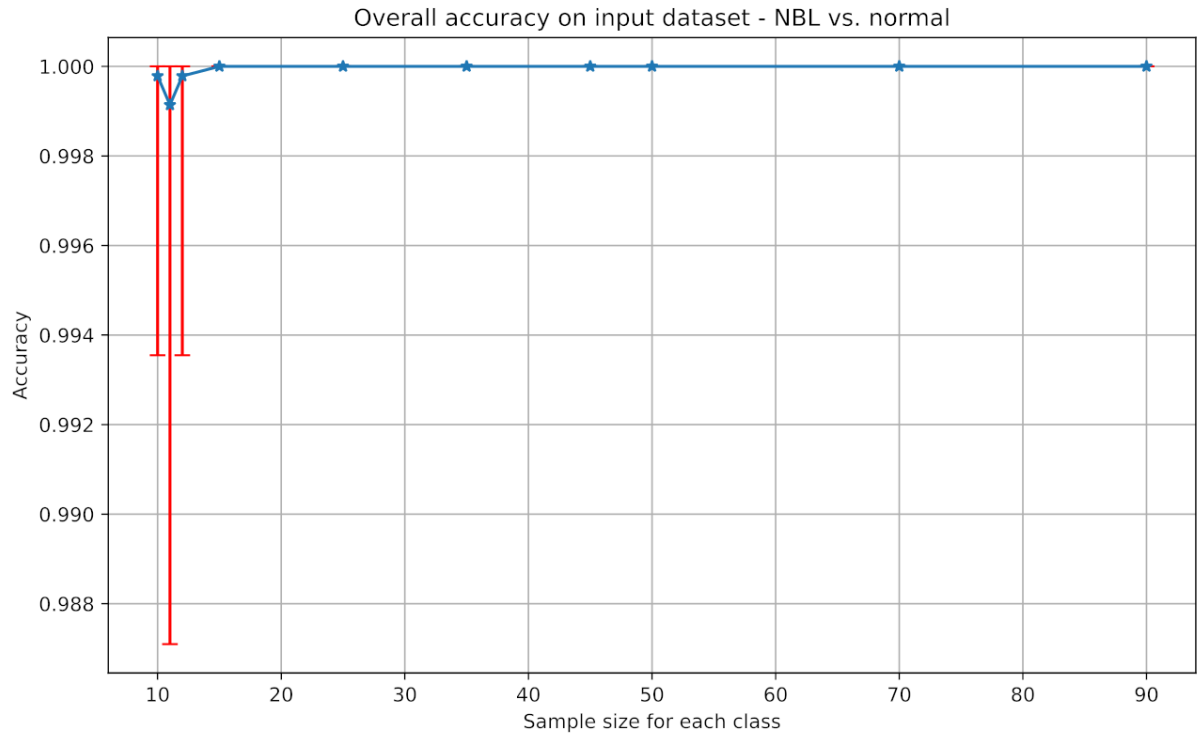


Roughly determine the most important genes

- **Running a crude feature importance study using the same random forest models, we find these most important genes for separating AML and Normal (with score):**
  - 0.000433: hyaluronidase 2
  - 0.000433: olfactory receptor family 7 subfamily E member 14 pseudogene
  - 0.000433: ERGIC and golgi 2
  - 0.000433: PDZ domain containing ring finger 3
  - 0.000433: FA complementation group B
  - 0.000433: laminin subunit gamma 2
  - 0.000433: LLGL scribble cell polarity complex component 2
  - 0.000400: POU class 3 homeobox 4
  - 0.000400: agrin
  - 0.000400: secretin receptor
  - 0.000400: adhesion G protein-coupled receptor F1
  - 0.000400: LOC339192
  - 0.000400: ADAM metallopeptidase with thrombospondin type 1 motif 9
  - 0.000400: plakophilin 4
  - 0.000400: solute carrier family 4 member 11
  - 0.000400: claudin 8
  - 0.000400: ADPGK antisense RNA 1
  - 0.000400: nucleolar protein 3
    - **Paper:** **“Expression of ARC [synonym] (apoptosis repressor with caspase recruitment domain), an antiapoptotic protein, is strongly prognostic in AML.”**

Run the same methodology for NBL vs. Normal

- **For various sample sizes, train a random forest classification model and compute the accuracy on the imbalanced dataset containing just NBL and Normal samples**
- **Run 30 different models for each differently-sized dataset**



Roughly determine the most important genes

- **Running a crude feature importance study using the same random forest models, we find these most important genes for separating NBL and Normal (with score):**
  - 0.000533: sulfatase modifying factor 1
  - 0.000533: keratinocyte differentiation factor 1
  - 0.000533: proline rich and Gla domain 1
  - 0.000533: paired like homeobox 2B
    - **Paper:** "Pleiotropic effect of common PHOX2B variants in Hirschsprung disease and neuroblastoma."
  - 0.000500: receptor accessory protein 2
  - 0.000500: methionyl-tRNA synthetase 1
  - 0.000500: DnaJ heat shock protein family (Hsp40) member C7
  - 0.000500: Nedd4 family interacting protein 2
  - 0.000500: tight junction protein 3
  - 0.000500: basigin (Ok blood group)
  - 0.000500: heterogeneous nuclear ribonucleoprotein K pseudogene 4

Conclusions

- Using even a small number of samples for training default random forest models, we obtain excellent predictability of cancer type
- The genes that are found to best discriminate are often associated with tumors, carcinoma, cell proliferation, etc.
- As a sanity check of AML vs. Normal and NBL vs. Normal, papers are easily found indicating that the differentiating genes are known indicators of these specific cancer types
- This gives us more confidence in our multiclassification model as well and indicates that we are getting the right answers for the right reasons

Potential next steps

- **Improve upon feature importance analysis:**
  - The random forest models used only default settings; they can likely be improved, particularly for determination of feature importance
  - Study the feature importance results more closely, perhaps even comparing with differential expression analyses
- **Run the same analysis on the more detailed design (i.e., X_1 and y_1) to try to learn what may differentiate between sample types**
  - In this case, the plotting of confusion matrices would likely be illuminating

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Secondary Slide Title

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- **Bullet point**
- **Bullet point**
- **Bullet point**

Section Title

Presenter