

Introduction into machine learning and analysis of Breast Cancer Proteomes

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1 Dataset

1.1 About Dataset

information about the data set and the three given files :

Context: This data set contains published iTRAQ proteome profiling of 77 breast cancer samples generated by the Clinical Proteomic Tumor Analysis Consortium (NCI/NIH). It contains expression values for ~12,000 proteins for each sample, with missing values present when a given protein could not be quantified in a given sample. this data was sampled from 105 originally from the TCGA (The Cancer Genome Atlas Program - NCI), which was further filtered to 77 samples containing high quality protein expression data. **Content:**

- **File:** 77cancerproteomesCPTACitraq.csv
 - **RefSeqaccessionnumber:** RefSeq protein ID (each protein has a unique ID in a RefSeq database)
 - **gene_symbol:** a symbol unique to each gene (every protein is encoded by some gene)
 - **gene_name:** a full name of that gene
 - **Remaining columns:** log2 iTRAQ ratios for each sample (protein expression data, most important), three last columns are from healthy individuals
- **File:** clinicalatabreast_cancer.csv
 - **First column** “Complete TCGA ID” is used to match the sample IDs in the main cancer proteomes file (see example script).
 - **All other columns** have self-explanatory names, contain data about the cancer classification of a given sample using different methods. ‘PAM50 mRNA’ classification is being used in the example script.
- **File:** PAM50_proteins.csv
 - **Contains** the list of genes and proteins used by the PAM50 classification system. The column RefSeqProteinID contains the protein IDs that can be matched with the IDs in the main protein expression data set.

Past Research: Original research paper: https://www.researchgate.net/publication/303509927_Proteogenomics_connects_somatic_mutations_to_signaling_in_breast_cancer

Summary: the data were used to assess how the mutations in the DNA are affecting the protein expression landscape in breast cancer. Genes in our DNA are first transcribed into RNA molecules which then are translated into proteins. Changing the information content of DNA has impact on the behavior of the proteome, which is the main functional unit of cells, taking care of cell division, DNA repair, enzymatic reactions and signaling etc.

my question is: Are there different ways to categorize breast cancer based on protein expression data, with machine learning being used to classify them without using the pam50 proteins?

1.2 Exploratory Data Analysis

loading of the dataframes and showing the successful loading and its dimensions. note only the first 5 columns of “77_cancer_proteomes_CPTAC_itraq.csv” are shown since after column 4 they are the same type.

```

protein_exp_data <- read.csv(file = "Analysis//data//77_cancer_proteomes_CPTAC_itraq.csv")
clinical_data <- read.csv(file = "Analysis//data//clinical_data_breast_cancer.csv")
pam50_protein_data <- read.csv(file = "Analysis//data/PAM50_proteins.csv")

# showing succeseful loading of data

# only showing first 5 columns of proteomes
pander(head(protein_exp_data[1:5], n = 5))

```

Table 1: Table continues below

RefSeq_accession_number	gene_symbol	gene_name	AO.A12D.01TCGA
NP_958782	PLEC	plectin isoform 1	1.096
NP_958785	NA	plectin isoform 1g	1.111
NP_958786	PLEC	plectin isoform 1a	1.111
NP_000436	NA	plectin isoform 1c	1.108
NP_958781	NA	plectin isoform 1e	1.115

C8.A131.01TCGA
2.61
2.65
2.65
2.646
2.646

```

pander(head(clinical_data, n=5))

```

Table 3: Table continues below

Complete.TCGA.ID	Gender	Age.at.Initial.Pathologic.Diagnosis	ER.Status
TCGA-A2-A0T2	FEMALE	66	Negative
TCGA-A2-A0CM	FEMALE	40	Negative
TCGA-BH-A18V	FEMALE	48	Negative
TCGA-BH-A18Q	FEMALE	56	Negative
TCGA-BH-A0E0	FEMALE	38	Negative

Table 4: Table continues below

PR.Status	HER2.Final.Status	Tumor	Tumor..T1.Coded	Node	Node.Coded
Negative	Negative	T3	T_Other	N3	Positive
Negative	Negative	T2	T_Other	N0	Negative
Negative	Negative	T2	T_Other	N1	Positive
Negative	Negative	T2	T_Other	N1	Positive
Negative	Negative	T3	T_Other	N3	Positive

Table 5: Table continues below

Metastasis	Metastasis.Coded	AJCC.Stage	Converted.Stage
M1	Positive	Stage IV	No_Conversion
M0	Negative	Stage IIA	Stage IIA
M0	Negative	Stage IIB	No_Conversion
M0	Negative	Stage IIB	No_Conversion
M0	Negative	Stage IIIC	No_Conversion

Table 6: Table continues below

Survival.Data.Form	Vital.Status	Days.to.Date.of.Last.Contact
followup	DECEASED	240
followup	DECEASED	754
enrollment	DECEASED	1555
enrollment	DECEASED	1692
followup	LIVING	133

Table 7: Table continues below

Days.to.date.of.Death	OS.event	OS.Time	PAM50.mRNA
240	1	240	Basal-like
754	1	754	Basal-like
1555	1	1555	Basal-like
1692	1	1692	Basal-like
NA	0	133	Basal-like

Table 8: Table continues below

SigClust.Unsupervised.mRNA	SigClust.Intrinsic.mRNA	miRNA.Clusters
0	-13	3
-12	-13	4
-12	-13	5
-12	-13	5
0	-13	5

Table 9: Table continues below

methylation.Clusters	RPPA.Clusters	CN.Clusters
5	Basal	3
4	Basal	4
5	Basal	1
5	Basal	1
5	Basal	1

Table 10: Table continues below

Integrated.Clusters..with.PAM50.	Integrated.Clusters..no.exp.
2	2
2	1
2	2
2	2
2	2

Integrated.Clusters..unsup.exp.
2
1
2
2
2

```
pander(head(pam50_protein_data, n=5))
```

GeneSymbol	RefSeqProteinID	Species	Gene.Name
MIA	NP_006524	Homo sapiens	melanoma inhibitory activity
FGFR4	NP_002002	Homo sapiens	fibroblast growth factor receptor 4
FGFR4	NP_998812	Homo sapiens	fibroblast growth factor receptor 4
FGFR4	NP_075252	Homo sapiens	fibroblast growth factor receptor 4
GPR160	NP_055188	Homo sapiens	G protein-coupled receptor 160

```
# showing the structure/dimensions of dataframe
```

```
cat("77_cancer_proteomes_CPTAC_itraq [ number of rows:", nrow(protein_exp_data), "number of columns:", ncol(protein_exp_data))
```

```
## 77_cancer_proteomes_CPTAC_itraq [ number of rows: 12553 number of columns: 86
```

```
cat("clinical_data [ number of rows:", nrow(clinical_data), "number of columns:", ncol(clinical_data), "\n")
```

```
## clinical_data [ number of rows: 105 number of columns: 30
```

```
cat("pam50_protein_data [ number of rows:", nrow(pam50_protein_data), "number of columns:", ncol(pam50_protein_data))
```

```
## pam50_protein_data [ number of rows: 100 number of columns: 4
```

Everything seems to be loaded completely, but we shall look further if everything is also correctly interpreted in R

Now checking if the protein expression data has been correctly read.

```
str(protein_exp_data)
```

```

## 'data.frame':    12553 obs. of  86 variables:
## $ RefSeq_accession_number: chr  "NP_958782" "NP_958785" "NP_958786" "NP_000436" ...
## $ gene_symbol            : chr  "PLEC" NA "PLEC" NA ...
## $ gene_name              : chr  "plectin isoform 1" "plectin isoform 1g" "plectin isoform 1a" "plec
## $ A0.A12D.01TCGA         : num  1.1 1.11 1.11 1.11 1.12 ...
## $ C8.A131.01TCGA         : num  2.61 2.65 2.65 2.65 2.65 ...
## $ A0.A12B.01TCGA         : num  -0.66 -0.649 -0.654 -0.632 -0.64 ...
## $ BH.A18Q.02TCGA         : num  0.195 0.215 0.215 0.205 0.215 ...
## $ C8.A130.02TCGA         : num  -0.494 -0.504 -0.501 -0.51 -0.504 ...
## $ C8.A138.03TCGA         : num  2.77 2.78 2.78 2.8 2.79 ...
## $ E2.A154.03TCGA         : num  0.863 0.87 0.87 0.866 0.87 ...
## $ C8.A12L.04TCGA         : num  1.41 1.41 1.41 1.41 1.41 ...
## $ A2.AOEX.04TCGA         : num  1.19 1.19 1.19 1.19 1.2 ...
## $ A0.A12D.05TCGA         : num  1.1 1.1 1.1 1.1 1.09 ...
## $ AN.A04A.05TCGA         : num  0.385 0.371 0.371 0.378 0.375 ...
## $ BH.A0AV.05TCGA         : num  0.351 0.367 0.367 0.361 0.371 ...
## $ C8.A12T.06TCGA         : num  -0.205 -0.162 -0.167 -0.184 -0.167 ...
## $ A8.A06Z.07TCGA         : num  -0.496 -0.499 -0.496 -0.492 -0.488 ...
## $ A2.AOCM.07TCGA         : num  0.683 0.694 0.698 0.687 0.687 ...
## $ BH.A18U.08TCGA         : num  -0.265 -0.252 -0.252 -0.252 -0.252 ...
## $ A2.AOEQ.08TCGA         : num  -0.913 -0.928 -0.928 -0.932 -0.928 ...
## $ AR.AOU4.09TCGA         : num  -0.0332 -0.0302 -0.0272 -0.0302 -0.0302 ...
## $ A0.AOJ9.10TCGA         : num  0.02 0.012 0.012 0.0039 0.012 ...
## $ AR.A1AP.11TCGA         : num  0.461 0.461 0.461 0.461 0.461 ...
## $ AN.AOFK.11TCGA         : num  0.974 0.977 0.977 0.97 0.985 ...
## $ A0.AOJ6.11TCGA         : num  0.831 0.857 0.857 0.837 0.865 ...
## $ A7.A13F.12TCGA         : num  1.28 1.28 1.28 1.28 1.28 ...
## $ BH.AOE1.12TCGA         : num  0.762 0.762 0.766 0.758 0.766 ...
## $ A7.AOCE.13TCGA         : num  -1.12 -1.12 -1.12 -1.13 -1.13 ...
## $ A2.AOYC.13TCGA         : num  0.819 0.815 0.815 0.799 0.819 ...
## $ A0.AOJC.14TCGA         : num  -0.307 -0.307 -0.307 -0.307 -0.301 ...
## $ A8.A08Z.14TCGA         : num  0.569 0.569 0.569 0.569 0.569 ...
## $ AR.AOTX.14TCGA         : num  -0.583 -0.573 -0.567 -0.583 -0.573 ...
## $ A8.A076.15TCGA         : num  1.87 1.87 1.87 1.86 1.87 ...
## $ A0.A126.15TCGA         : num  0.196 0.196 0.196 0.219 0.2 ...
## $ BH.AOC1.16TCGA         : num  -0.518 -0.51 -0.507 -0.518 -0.513 ...
## $ A2.AOEY.16TCGA         : num  1.17 1.18 1.18 1.17 1.18 ...
## $ AR.A1AW.17TCGA         : num  0.578 0.582 0.578 0.59 0.586 ...
## $ AR.A1AV.17TCGA         : num  -0.76 -0.76 -0.749 -0.736 -0.749 ...
## $ C8.A135.17TCGA         : num  1.12 1.14 1.14 1.14 1.12 ...
## $ A2.AOEV.18TCGA         : num  0.453 0.473 0.473 0.459 0.473 ...
## $ AN.AOAM.18TCGA         : num  1.5 1.51 1.5 1.5 1.5 ...
## $ D8.A142.18TCGA         : num  0.539 0.542 0.542 0.535 0.542 ...
## $ AN.AOFL.19TCGA         : num  2.46 2.48 2.48 2.46 2.48 ...
## $ BH.AODG.19TCGA         : num  -0.206 -0.206 -0.206 -0.215 -0.206 ...
## $ AR.AOTV.20TCGA         : num  -1.51 -1.53 -1.53 -1.53 -1.51 ...
## $ C8.A12Z.20TCGA         : num  -0.787 -0.756 -0.756 -0.775 -0.772 ...
## $ A0.AOJJ.20TCGA         : num  0.757 0.781 0.774 0.764 0.771 ...
## $ A0.AOJE.21TCGA         : num  0.56 0.563 0.56 0.542 0.56 ...
## $ AN.AOAJ.21TCGA         : num  -0.428 -0.406 -0.406 -0.406 -0.406 ...
## $ A7.AOCJ.22TCGA         : num  -1.001 -1.005 -1.005 -0.998 -1.001 ...
## $ A0.A12F.22TCGA         : num  -1.95 -1.95 -1.96 -1.95 -1.96 ...
## $ A8.A079.23TCGA         : num  1.05 1.05 1.05 1.06 1.05 ...
## $ A2.AOT3.24TCGA         : num  0.584 0.581 0.581 0.587 0.587 ...

```

```
## $ A2.AOYD.24TCGA      : num  0.0638 0.0933 0.0845 0.0667 0.0845 ...
## $ AR.AOTR.25TCGA      : num  -1.1 -1.11 -1.11 -1.1 -1.11 ...
## $ AO.AO3O.25TCGA      : num  1.05 1.06 1.06 1.06 1.06 ...
## $ AO.A12E.26TCGA      : num  0.265 0.276 0.276 0.278 0.278 ...
## $ A8.AO6N.26TCGA      : num  0.239 0.25 0.244 0.25 0.25 ...
## $ A2.AOYG.27TCGA      : num  -0.0782 -0.0681 -0.0714 -0.0579 -0.0647 ...
## $ BH.A18N.27TCGA      : num  1.1 1.1 1.1 1.09 1.11 ...
## $ AN.AOAL.28TCGA      : num  0.324 0.327 0.327 0.33 0.327 ...
## $ A2.AOT6.29TCGA      : num  0.794 0.818 0.815 0.801 0.818 ...
## $ E2.A158.29TCGA      : num  -1.09 -1.1 -1.1 -1.1 -1.1 ...
## $ E2.A15A.29TCGA      : num  2.18 2.18 2.18 2.18 2.18 ...
## $ AO.AOJM.30TCGA      : num  1.4 1.41 1.41 1.41 1.41 ...
## $ C8.A12V.30TCGA      : num  0.674 0.689 0.689 0.678 0.689 ...
## $ A2.AOD2.31TCGA      : num  0.1075 0.1042 0.1075 0.0975 0.1042 ...
## $ C8.A12U.31TCGA      : num  -0.482 -0.478 -0.482 -0.471 -0.482 ...
## $ AR.A1AS.31TCGA      : num  1.22 1.22 1.22 1.2 1.22 ...
## $ A8.AO9G.32TCGA      : num  -1.52 -1.51 -1.51 -1.52 -1.51 ...
## $ C8.A131.32TCGA      : num  2.71 2.73 2.74 2.73 2.75 ...
## $ C8.A134.32TCGA      : num  0.14 0.126 0.133 0.112 0.126 ...
## $ A2.AOYF.33TCGA      : num  0.311 0.296 0.296 0.296 0.296 ...
## $ BH.AODD.33TCGA      : num  -0.692 -0.659 -0.664 -0.657 -0.662 ...
## $ BH.AOE9.33TCGA      : num  1.47 1.48 1.47 1.46 1.47 ...
## $ AR.AOTT.34TCGA      : num  -0.511 -0.526 -0.526 -0.533 -0.53 ...
## $ AO.A12B.34TCGA      : num  -0.964 -0.938 -0.944 -0.935 -0.935 ...
## $ A2.AOSW.35TCGA      : num  -0.488 -0.488 -0.488 -0.488 -0.504 ...
## $ AO.AOJL.35TCGA      : num  -0.107 -0.107 -0.107 -0.107 -0.107 ...
## $ BH.AOBV.35TCGA      : num  -0.0658 -0.0559 -0.0658 -0.0559 -0.0625 ...
## $ A2.AOYM.36TCGA      : num  0.656 0.658 0.656 0.656 0.651 ...
## $ BH.AOC7.36TCGA      : num  -0.552 -0.548 -0.552 -0.552 -0.557 ...
## $ A2.AOSX.36TCGA      : num  -0.399 -0.393 -0.393 -0.393 -0.396 ...
## $ X263d3f.I.CPTAC      : num  0.599 0.607 0.604 0.604 0.604 ...
## $ blcdb9.I.CPTAC      : num  -0.191 -0.184 -0.186 -0.186 -0.167 ...
## $ c4155b.C.CPTAC      : num  0.567 0.579 0.577 0.577 0.577 ...
```

Nothing strange about the Proteomes dat everything seems to be read correct.

Checking if the clinical data has been correctly read.

```
str(clinical_data)
```

```
## 'data.frame':   105 obs. of  30 variables:
## $ Complete.TCGA.ID      : chr  "TCGA-A2-AOT2" "TCGA-A2-AOCM" "TCGA-BH-A18V" "TCGA-BH-A
## $ Gender                : chr  "FEMALE" "FEMALE" "FEMALE" "FEMALE" ...
## $ Age.at.Initial.Pathologic.Diagnosis: int  66 40 48 56 38 57 74 60 61 67 ...
## $ ER.Status             : chr  "Negative" "Negative" "Negative" "Negative" ...
## $ PR.Status             : chr  "Negative" "Negative" "Negative" "Negative" ...
## $ HER2.Final.Status     : chr  "Negative" "Negative" "Negative" "Negative" ...
## $ Tumor                 : chr  "T3" "T2" "T2" "T2" ...
## $ Tumor..T1.Coded       : chr  "T_Other" "T_Other" "T_Other" "T_Other" ...
## $ Node                  : chr  "N3" "N0" "N1" "N1" ...
## $ Node.Coded            : chr  "Positive" "Negative" "Positive" "Positive" ...
## $ Metastasis            : chr  "M1" "M0" "M0" "M0" ...
## $ Metastasis.Coded      : chr  "Positive" "Negative" "Negative" "Negative" ...
## $ AJCC.Stage            : chr  "Stage IV" "Stage IIA" "Stage IIB" "Stage IIB" ...
```



```
## $ Converted.Stage           : chr "No_Conversion" "Stage IIA" "No_Conversion" "No_Conversion" ...
## $ Survival.Data.Form       : chr "followup" "followup" "enrollment" "enrollment" ...
## $ Vital.Status             : chr "DECEASED" "DECEASED" "DECEASED" "DECEASED" ...
## $ Days.to.Date.of.Last.Contact : int 240 754 1555 1692 133 309 425 643 775 964 ...
## $ Days.to.date.of.Death     : int 240 754 1555 1692 NA NA NA NA NA NA ...
## $ OS.event                 : int 1 1 1 1 0 0 0 0 0 0 ...
## $ OS.Time                  : int 240 754 1555 1692 133 309 425 643 775 964 ...
## $ PAM50.mRNA               : chr "Basal-like" "Basal-like" "Basal-like" "Basal-like" ...
## $ SigClust.Unsupervised.mRNA : int 0 -12 -12 -12 0 0 0 -12 -12 -12 ...
## $ SigClust.Intrinsic.mRNA   : int -13 -13 -13 -13 -13 -13 -13 -13 -13 -13 ...
## $ miRNA.Clusters           : int 3 4 5 5 5 5 3 5 2 5 ...
## $ methylation.Clusters      : int 5 4 5 5 5 5 5 5 5 5 ...
## $ RPPA.Clusters            : chr "Basal" "Basal" "Basal" "Basal" ...
## $ CN.Clusters              : int 3 4 1 1 1 1 1 1 1 3 ...
## $ Integrated.Clusters..with.PAM50. : int 2 2 2 2 2 2 2 2 2 2 ...
## $ Integrated.Clusters..no.exp. : int 2 1 2 2 2 2 2 2 2 2 ...
## $ Integrated.Clusters..unsup.exp. : int 2 1 2 2 2 2 2 2 2 2 ...
```

Nothing strange about the clinical data everything seems to be read correct.

Checking if the pam50 protein data has been correctly read.

```
str(pam50_protein_data)
```

```
## 'data.frame': 100 obs. of 4 variables:
## $ GeneSymbol : chr "MIA" "FGFR4" "FGFR4" "FGFR4" ...
## $ RefSeqProteinID: chr "NP_006524" "NP_002002" "NP_998812" "NP_075252" ...
## $ Species : chr "Homo sapiens" "Homo sapiens" "Homo sapiens" "Homo sapiens" ...
## $ Gene.Name : chr "melanoma inhibitory activity" "fibroblast growth factor receptor 4" "fibroblast growth factor receptor 4" ...
```

Nothing strange about the pam50 protein data everything seems to be read correct.

codebook

loading of the created codebooks for the three dataframes. showing also its contents and successful loading

```
cancer_proteomes_CPTAC_codebook <- read.csv2("Analysis//data/77_cancer_proteomes_CPTAC_codebook.txt")
clinical_data_codebook <- read.csv2("Analysis//data/clinical_data_breast_cancer_codebook.txt")
PAM50_protein_codebook <- read.csv2("Analysis//data/PAM50_protein_codebook.txt", sep = ";")
```

```
pander(cancer_proteomes_CPTAC_codebook)
```

Column	Description	data.type	unit
RefSeq_accession_number	RefSeq protein ID	string	NA
gene_symbol	Gene abbreviation code	string	NA
gene_name	Name of the gene	string	NA
Remaining columns	log2 iTRAQ ratios	float	NA

pander(clinical_data_codebook)

Table 14: Table continues below

Column	Description
Complete_TCGA_ID	TCGA ID
Gender	Gender
Age_at_Initial_Pathologic_Diagnosis	Age at Initial Pathologic Diagnosis
ER Status	Estrogen receptor Status
PR Status	Progesterone receptor Status
HER2 Final Status	Human Epidermal growth factor Receptor 2
Tumor	Tumor
Tumor-T1 Coded	Tumor-T1 Coded
Node	Node
Node-Coded	Node-Coded
Metastasis	Metastasis
Metastasis-Coded	Metastasis-Coded
AJCC Stage	American Joint Committee on Cancer Stage
Converted Stage	Converted Stage
Survival Data Form	Survival Data Form
Vital Status	Vital Status
Days to Date of Last Contact	Days to Date of Last Contact
Days to date of Death	Days to date of Death
OS event	OS event 0= NO, 1= YES
OS Time	OS Time
PAM50 mRNA	PAM50 mRNA
SigClust Unsupervised mRNA	SigClust Unsupervised mRNA
SigClust Intrinsic mRNA	SigClust Intrinsic mRNA
miRNA Clusters	miRNA Clusters
methylation Clusters	methylation Clusters
RPPA Clusters	RPPA Clusters
CN Clusters	CN Clusters
Integrated Clusters (with PAM50)	Integrated Clusters (with PAM50)
Integrated Clusters (no exp)	Integrated Clusters (no exp)
Integrated Clusters (unsup exp)	Integrated Clusters (unsup exp)

type	data.type	unit
name	chr	NA
name	chr	NA
Descriptive	int	Years
Descriptive	chr	NA
Descriptive	chr	NA
Descriptive	chr	NA
Descriptive	chr	NA
Descriptive	chr	NA
Descriptive	chr	NA
Descriptive	chr	NA
Descriptive	chr	NA
Descriptive	chr	NA
Descriptive	chr	NA

type	data.type	unit
Descriptive	chr	NA
Descriptive	chr	NA
Descriptive	chr	NA
Time	int	Days
Time	int	Days
Descriptive	int	NA
Time	int	Hours
Descriptive	chr	NA
Count	int	NA
Count	int	NA
Count	int	NA
Count	int	NA
Descriptive	chr	NA
Count	int	NA
Count	int	NA
Count	int	NA
Count	int	NA

```
pander(PAM50_protein_codebook)
```

Column	Description	type	unit
GeneSymbol	Gene abbreviation	chr	NA
RefSeqProteinID	Unique reference identifier	chr	NA
Species	Species	chr	latin name
Gene.Name	Name of the gene	chr	NA

Here we can also see that everything has been successfully loaded into R

1.3 Data observation

there are 12553 rows in the data, these are proteins identifiable with a RefSeq ID number and have 86 columns of which the last 83 are samples (with their identifiers as their name and the last three from healthy individuals, but these shall not be used for the machine learning part since 3 samples is too little to use for analyses. to further use the data I shall reshape it to make the rows samples and each column a protein.

1.4 Data cleaning and altering

altering sample names

the alteration of sample names to correspondent to the clinical data names is needed for further comparison and analyses. this is done by changing the column names to that of the same format of the clinical data. this is done with some regex magic.

```
# storing a list of the column names
column_names <- names(protein_exp_data)

# function
```

```
change_sample_name <- function (x){
  #search for TCGA name,if found split and make new name
  if(grepl("TCGA",x) == TRUE){
    temp_list <- as.list(strsplit(x, '[_|-|.]')[[1]])
    x <- str_c(c('TCGA',temp_list[[1]],temp_list[[2]]),collapse = '-')
  }
  return (x)
}

# changing of the colnames
colnames(protein_exp_data) <- lapply(column_names, change_sample_name)
cat("Old name:",column_names[[4]],",New name:",names(protein_exp_data)[[4]])
```

```
## Old name: A0.A12D.01TCGA ,New name: TCGA-A0-A12D
```

this output show to conversion has been successful

numerical data frame

now we need to make a data frame with only the numerical data for the ease of analyzes

```
# first making a data frame with only the numerical data, samples start at column number 4 til the end
protein_exp_numerical <- protein_exp_data[4:86]
```

Transposing

transposing the created data frame “protein_exp_numerical”, and adding the refseq ID as column name

```
# transposing of the old dataframe to a new one
protein_exp__numerical_transposed <- as.data.frame(t(protein_exp_numerical))
colnames(protein_exp__numerical_transposed) <- protein_exp_data$RefSeq_accession_number

# checking if succesfull
cat("protein_exp_numerical number of rows:", nrow(protein_exp_numerical),
    "number of columns:", ncol(protein_exp_numerical), '\n')
```

```
## protein_exp_numerical number of rows: 12553 number of columns: 83
```

```
cat("protein_exp__numerical_transposed number of rows:", nrow(protein_exp__numerical_transposed),
    "number of columns:", ncol(protein_exp__numerical_transposed), '\n')
```

```
## protein_exp__numerical_transposed number of rows: 83 number of columns: 12553
```

as we can see the row and column dimensions have been flipped

cleaning

since there are NA values in the data lets see how much

```
count_na_func <- function(x) sum(is.na(x))
# getting NA values per RefSeqID(column)
Na_per_col <- sapply(protein_exp__numerical_transposed, count_na_func)
```

```
ggplot() +
  aes(Na_per_col) +
  geom_histogram(color = "black", fill = "#F9C000", binwidth = 3) +
  xlab("Number of NA") +
  ylab("Frequency of columns") +
  ggtitle("Frequency of number of NA values per RefSeqID")
```

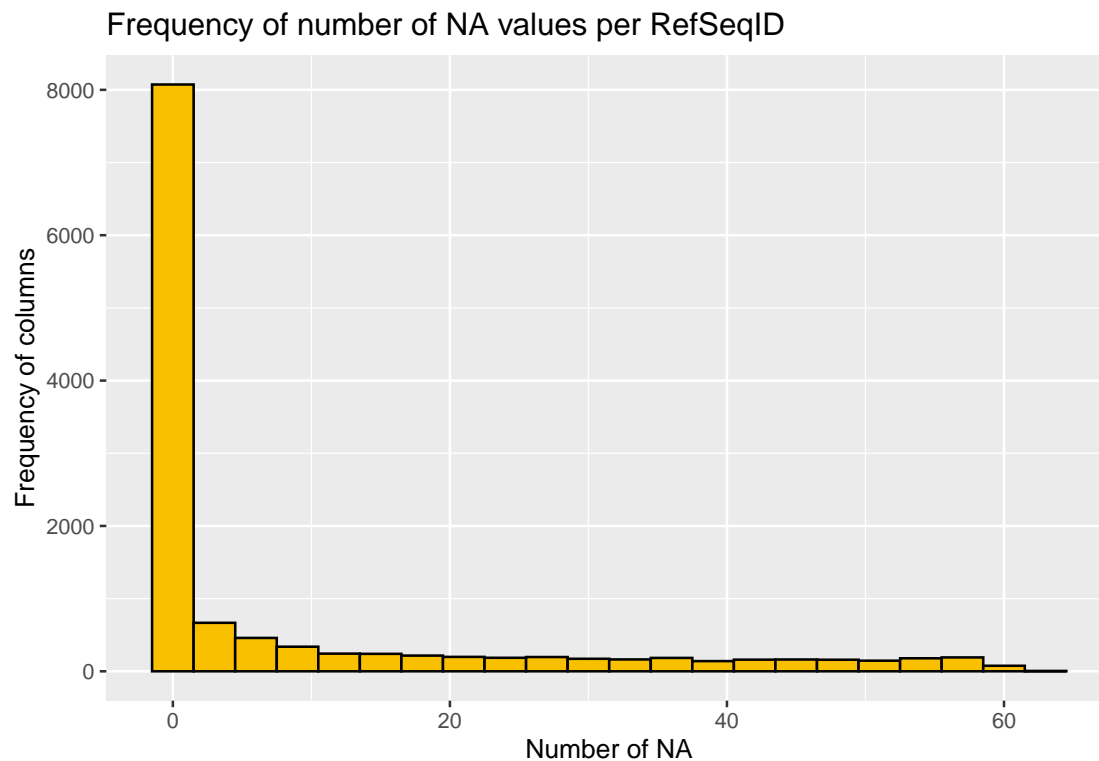


Figure 1: barplot with the frequency of columns with more than 0 NA in them

```
ggsave(
  filename = "figure1.png",
  plot = last_plot(),
  path = "data/figures")
```

Saving 6.5 x 4.5 in image

```
ggplot() +
  aes(Na_per_col[Na_per_col > 0]) +
  geom_histogram(color = "black", fill = "#0039F9", binwidth = 3) +
  xlab("Number of NA") +
  ylab("Frequency of columns") +
  ggtitle("Frequency of number of NA values per RefSeqID with 0 omitted")
```

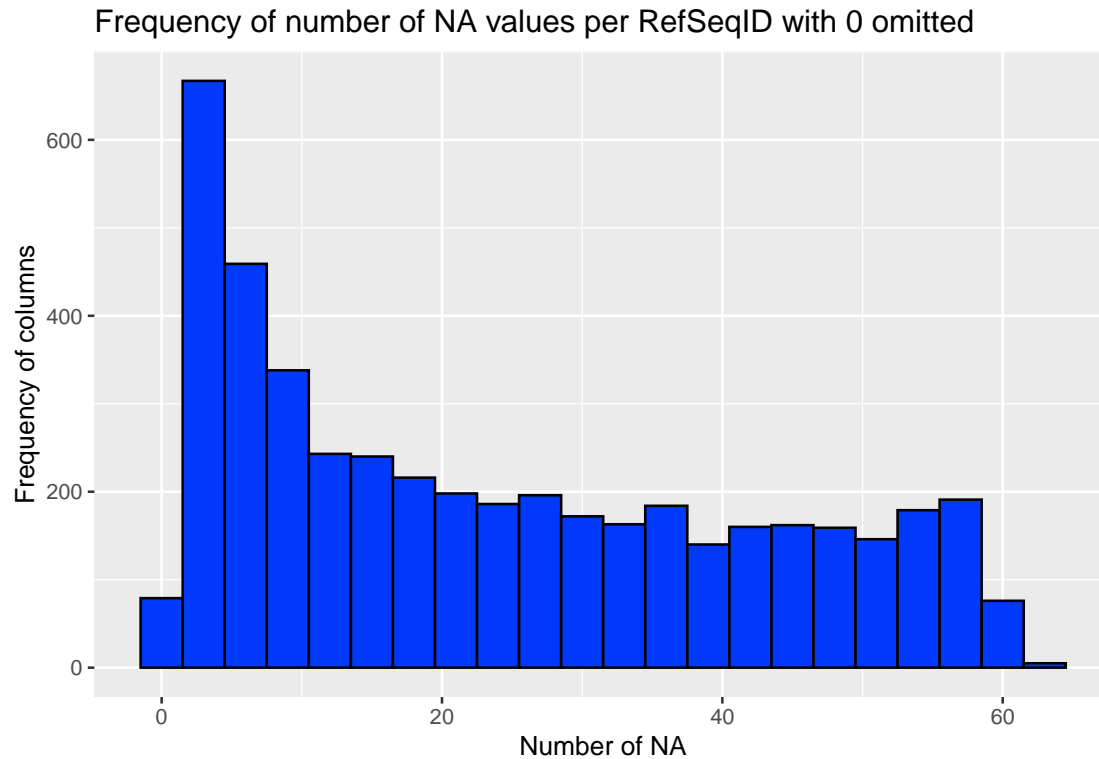


Figure 2: barplot with the frequency of columns with more than 0 NA in them

```
ggsave(
  filename = "figure2.png",
  plot = last_plot(),
  path = "data/figures")
```

Saving 6.5 x 4.5 in image

there are a lot of proteins with more than 10% of their samples with missing data, so i shall be removing.

```
cat("number of proteins with NA values in them:", sum(Na_per_col > 0), '\n' )
```

number of proteins with NA values in them: 4559

```
# deleting every protein with more than 10% NA values in them, since this is allot
proteomes_filtered_data <- protein_exp_numerical_transposed[Na_per_col < 8]
cat("number of proteins with 8 or more NA values in them and deleted from data:",
    sum(Na_per_col > 8), '\n')
```

number of proteins with 8 or more NA values in them and deleted from data: 3219

```
cat("proteomes_filtered_data[number of rows:",
    nrow(proteomes_filtered_data),
    "number of columns:",
    ncol(proteomes_filtered_data), '\n')
```

```
## proteomes_filtered_data[number of rows: 83 number of columns: 9199
```

as we can see from the reports generated by the code we can see that the filtering of NA was successful. and we now have a data set that contains data with less than 10% per protein of NA values

Merging clinical and protein expression dataframes

to be able to use the Clinical data we need to merge it to its corresponding row and sample in the Protein expressions

```
# first assigning row names to clinical data
rownames(clinical_data) <- clinical_data$Complete.TCGA.ID

# removing the used ID column to simplify it since it has been become redundend
clinical_data <- clinical_data[,-1]

# merge the two data frames according to the row names (TCGA Identification number),
merged_data <- merge(select(clinical_data, Tumor, Tumor..T1.Coded, AJCC.Stage, Vital.Status), protein_e
cleaned_merged_data <- merge(select(clinical_data, Tumor, Tumor..T1.Coded, AJCC.Stage, Vital.Status), p
cat("merged_data of rows:", nrow(merged_data),
    "number of columns:", ncol(merged_data), '\n')
```

```
## merged_data of rows: 77 number of columns: 12558
```

we can see that not every sample had an entry in the clinical data, so we end up with 6 rows of sample data that get left out of the merged data set

1.5 Data visualisation

Showing some examples of distributions of protein expression data since there are around 12 000 proteins found

```
# Open a png file
#png("../data/figures/figure3.png")
# 2. Create a plot
boxplot(merged_data[6:76],
        col = rainbow(ncol(merged_data[6:76])),
        xlab = "Protein",
        ylab = "log2 iTRAQ ratio",
        main = "distribution of Protein expression for first 70 Proteins",
        show.names= FALSE)
```

```
# Close the pdf file
#dev.off()
```

Since i can't be sure of the significance of every protein I can't simply throw away any sample

to fix this i shall get the standard deviation from every protein to prepare for a selection of the most deviant ones. and for illustration i will overlay the dataframe which has filtered out the proteins with more than 10% of their values being NA

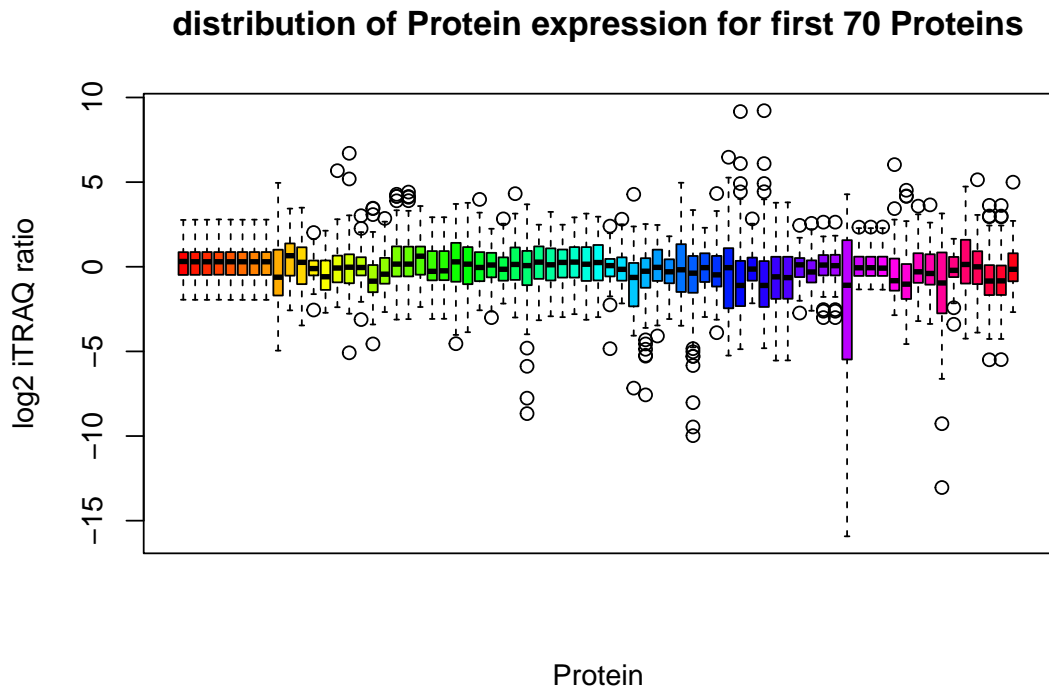


Figure 3: boxplot of protein expression distribution, first 70 proteins

```
fg <- colSds(as.matrix(merged_data[sapply(merged_data, is.numeric)]), na.rm = TRUE)
fg2 <- colSds(as.matrix(cleaned_merged_data[sapply(cleaned_merged_data, is.numeric)]), na.rm = TRUE)

fg <- as.data.frame(fg, row.names = colnames(merged_data[6:ncol(merged_data)]))
fg2 <- as.data.frame(fg2, row.names = colnames(cleaned_merged_data[6:ncol(cleaned_merged_data)]))

fg$count <- seq(from = 1, to = nrow(fg))

merged_fg <- merge(fg, fg2, all.x = TRUE, by = 0)
merged_fg <- merged_fg[order(merged_fg$count),]

# assigning 0 to every NA values in fg2 since that is the filtered one
merged_fg$fg2[is.na(merged_fg$fg2)] <- 0

# plotting
ggplot(data=merged_fg) +
  geom_point(size=0.0015, aes(x=count, y=fg, color="Non Na filtered")) +
  geom_point(size=0.0015, aes(x=count, y=fg2, color="Na filtered")) +
  labs(x = "Data Frame row number",
       y = "Standard deviation",
       color = "Legend") +
  ggtitle("Density plot for standard deviation of protein expression") +
  theme(legend.position = "bottom")
```




Figure 4: scatterplot of standard deviation for the protein expression data and for the NA filtered version

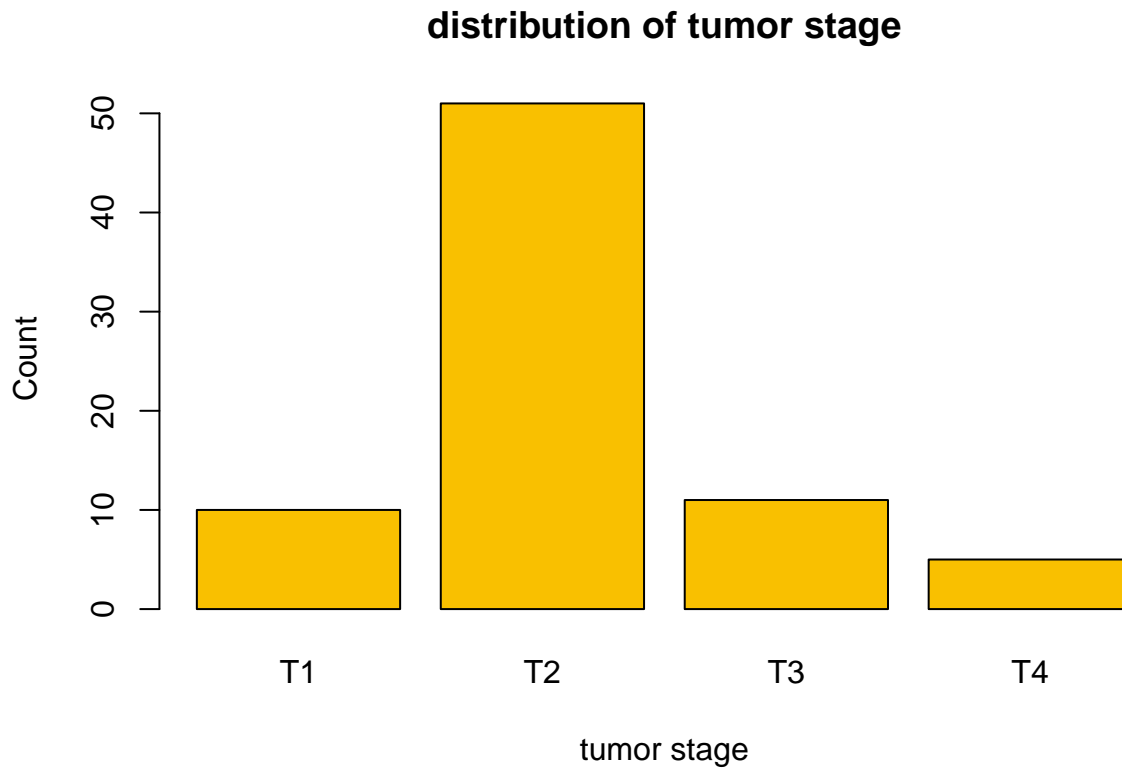
```
ggsave(
  filename = "figure4.png",
  plot = last_plot(),
  path = "data/figures")
```

Saving 6.5 x 4.5 in image

as we can see in the standard deviation plot from the proteins it is visible that the proteins with a lot of NA in them seem to be having a higher Standard deviation, but still there should be enough deviation in the filtered Data to use it for further analyse using machine learning.

```
# assigning as factors
merged_data$Tumor <- factor(merged_data$Tumor)
cleaned_merged_data$Tumor <- factor(cleaned_merged_data$Tumor)
#merged_data$Row.names <- factor(merged_data$Row.names, levels =)
#cleaned_merged_data$Row.names <- factor(cleaned_merged_data$Row.names)
```

```
plot(cleaned_merged_data$Tumor, ylab = "Count", xlab="tumor stage", col = "#F9C000", main = "distribution")
```



/newpage

2 Supervised Learning

In this chapter, we are looking at how we are going to train the machine learning algorithms to accurately predict the tumor stage of breast cancer according to the protein expressions found in breast tissue samples. after that there shall be a examination of the result and accuracy of the created models accordingly to different algorithms.

2.1 Weka

Weka is used for the data examination and machine learning part, Weka is a open source collection of machine learning algorithms for data mining tasks. It contains tools for data preparation, classification, regression, clustering, association rules mining, and visualization. java platform for Firstly the data is exported to a .arff file so it can be loaded into Weka

data exportation

```
train2 <- cleaned_merged_data[, -3:-5]
train3 <- train2[, -2]
train2$data.class <- as.factor(train2$Tumor)
```

```
#train2 <- train2[,-1]
write.arff(train3, file = "Analysis/data/train3.arff")
```

2.2 Models

experimenter

To firstly make a simple comparison for the effectiveness of the different algorithms we shall use the Weka experimenter. the algorithms compared are as follows in the tabel beneath. note ZeroR is left out since it throws a error when running in the experimenter.

Table 17: Tabel with the algorithms with default settings used in initial comparison .

- (1) rules.OneR '-B 6' -3459427003147861500
- (2) trees.RandomTree '-K 0 -M 1.0 -V 0.001 -S 38' -9051119597407395800
- (3) trees.RandomForest '-P 100 -I 100 -num-slots 1 -K 0 -M 1.0 -V 0.001 -S 38' 1116839470751428740
- (4) trees.J48 '-C 0.25 -M 2' -217733168393644448
- (5) meta.AttributeSelectedClassifier '-E CfsSubsetEval -P 6 -E 6:S GreedyStepwise -T -1.7976931348623157E308 -N -1 -num-slots 1=W trees.J48
- (6) meta.AttributeSelectedClassifier '-E CfsSubsetEval -P 6 -E 6:S BestFirst -D 2 -N 5=W trees.J48 - -C 0.25 -M 2' -1151805453487947520
- (7) meta.AttributeSelectedClassifier '-E CfsSubsetEval -P 6 -E 6:S BestFirst -D 2 -N 5=W trees.RandomForest - -P 100 -I 100 -num-slots 1 -K 0
- (8) meta.AttributeSelectedClassifier '-E CfsSubsetEval -P 6 -E 6:S BestFirst -D 2 -N 5=W trees.RandomTree - -K 0 -M 1.0 -V 0.001 -S 38' -115

the results are as follows

Table 18: Tabel with a T test performed on the percentage each algoritme correctly predicted.

Dataset	(1) rules.On	(2) trees	(3) trees	(4) trees	(5) meta.	(6) meta.	(7) meta.	(8) meta.
R data frame	59.74	50.65	66.23	35.06 *	45.45	42.86 *	62.34	46.75
significance	(v/ /*)	(0/1/0)	(0/1/0)	(0/0/1)	(0/1/0)	(0/0/1)	(0/1/0)	(0/1/0)

Conclusion

as we can see from these results nothing really stands out from the rest, the all perform quit bad especially if u compare it with the OneR and ZeroR. this is due to having a lot of attributes in my date with relatively a low number of instances, so overfitting is a major issue. This forces use to make some selection in atributes. this in turn forces us the meta.AttributeSelectedClassifier to first make a selection of the attributes and further refine these classifiers.

So for further testing we firstly make a good baseline with the zeroR and further make comparisons with mutiple combinations of the meta.AttributeSelectedClassifier and its parameters.

The next set of test where done in the weka explorer gui.

The first of these is a ZeroR and the rest are the results of a few of the best AttributeSelectedClassifier, since most of them are extremely poor performing and not worthy of mentioning the results at all. After showing these results we shall make a conclusion about which algorithm performs the best. All of the following test runs have been made with crossvallidation with the leave one out method to maximise the limited number of instances in the data

ZeroR

The first algorithm used is a zeroR one is produced the following results

Table 19: Tabel with the summary of results from zeroR

Correctly Classified Instances	51	66.2338
Incorrectly Classified Instances	26	33.7662
Kappa statistic	0	x
Mean absolute error	0.2665	x
Root mean squared error	0.3613	x
Relative absolute error	100	x
Root relative squared error	100	x
Total Number of Instances	77	x

Table 20: Confusion matrix

a	b	c	d	<- classified as
0	10	0	0	a = T1
0	51	0	0	b = T2
0	11	0	0	c = T3
0	5	0	0	d = T4

confusion matrix

algorithm conclusion

As we can see from the results of ZeroR that even classifing everything as T2 scores 66% good, thus using that as a base of evaluationg the classifiers used is not very reliable and we shall take the confusing matrix and ROC curves more as a indication for a good algoritme to use for oure data.

AttributeSelectedClassifier with cost sensitive J48

This is the second algorithm used, and this is using attribute selection with sub set evaluation based on the best first method. as a cost matrix I assigned every wrongly classified instance as class T2 extra heavy since that class is overrepresented, and further weight that every clas that is wrongly classified a little heavier

Relation: R_data_frame
Instances: 77
Attributes: 9200
Test mode: 77-fold cross-validation
Evaluation cost matrix:

0	5	2	2
1	0	1	1
1	5	0	2
2	5	2	0

=== Attribute Selection on all input data ===

Search Method:
Best first.
Start set: no attributes
Search direction: forward
Stale search after 5 node expansions
Total number of subsets evaluated: 211334
Merit of the best subset found: 0.601

Attribute Subset Evaluator (supervised, Class (nominal): 9200 data.class):
CFS Subset Evaluator
Including locally predictive attributes

Selected attributes: 338,905,1188,1230,1555,2172,2277,2821,3196,3333,3719,3932,5844,6802,7234,7490,7959,8149,8538
: 19
NP_008832 NP_056289 NP_001349 NP_055719 NP_001150 NP_079093 NP_000959 NP_004893
NP_004887 NP_065901 NP_058632 NP_002630 NP_060947 NP_065109 NP_848613 NP_001035147
NP_005639 NP_001092102 NP_001135757

Table 21: Tabel with the summary of results from zeroR

Correctly Classified Instances	22	28.5
Incorrectly Classified Instances	55	71.4
Kappa statistic	-0.1735	x
Total Cost	135	
Average Cost	1.7532	
Mean absolute error	0.3473	x
Root mean squared error	0.5733	x
Relative absolute error	129.1465	x
Root relative squared error	156.9896	x
Total Number of Instances	77	x

confusion matrix

Table 22: Confusion matrix

a	b	c	d	<- classified as
1	7	2	0	a = T1
8	19	19	5	b = T2
2	8	1	0	c = T3
0	4	0	1	d = T4

algorithm conclusion

in the above seen confusion matrix we are looking for a nicely made line from the top left to the bottom right, but we can clearly see that that is not the case. So this algorithm doesn't have a lot of merit for further exploration

AttributeSelectedClassifier HoeffdingTree

Relation: R_data_frame
Instances: 77
Attributes: 9200
Test mode: 77-fold cross-validation
Evaluation cost matrix:

```

0  5  2  2
1  0  1  1
1  5  0  2
2  5  2  0

```

=== Attribute Selection on all input data ===

Search Method:
Best first.
Start set: no attributes
Search direction: bi-directional
Stale search after 5 node expansions
Total number of subsets evaluated: 248373
Merit of best subset found: 0.604

Attribute Subset Evaluator (supervised, Class (nominal): 9200 data.class):
CFS Subset Evaluator
Including locally predictive attributes

Selected attributes: 338,1188,1230,1555,2172,2277,2844,3196,3333,3719,3932,5844,6802,7234,7490,7959,8149,8538
: 18
NP_008832 NP_001349 NP_055719 NP_001150 NP_079093 NP_000959 NP_877496 NP_004887
NP_065901 NP_058632 NP_002630 NP_060947 NP_065109 NP_848613 NP_001035147 NP_005639
NP_001092102 NP_001135757

confusion matrix

Table 23: Tabel with the summary of results from HoeffdingTree

Correctly Classified Instances	45	58.4
Incorrectly Classified Instances	32	41.5
Kappa statistic	-0.0584	x
Mean absolute error	0.2337	x
Root mean squared error	0.4332	x
Relative absolute error	86.9241	x
Root relative squared error	118.6126	x
Total Number of Instances	77	x

Table 24: labelHoeffdingTree confusionmatrixConfusion matrix

a	b	c	d	<- classified as
0	9	1	0	a = T1
5	45	1	0	b = T2
1	10	0	0	c = T3
0	5	0	0	d = T4

algorithm conclusion

in the above seen confusion matrix we are looking for a nicely made line from the top left to the bottom right, but we can clearly see that that is not the case, and it classifies them mostly as t1 and t2. So this algorithm doesn't have a lot of merit for further exploration

AttributeSelectedClassifier Ranker RandomTree

Relation: R_data_frame

Instances: 77

Attributes: 9200

Test mode: 77-fold cross-validation

Evaluation cost matrix:

0	5	2	2
1	0	1	1
1	5	0	2
2	5	2	0

=== Attribute Selection on all input data ===

Search Method:

Attribute ranking.

Attribute Evaluator (supervised, Class (nominal): 9200 data.class):

Gain Ratio feature evaluator

Ranked attributes:

0.735 4526 NP_071896

0.735 6015 NP_004840

0.735 1443 NP_006786

0.735 1312 NP_002829

0.65 8523 NP_002351

0.532 2172 NP_079093

0.49 1230 NP_055719
 0.481 5384 NP_006346
 0.481 2277 NP_000959
 0.481 4206 NP_000960
 0.481 8329 NP_000981
 0.439 1188 NP_001349

Selected attributes: 4526,6015,1443,1312,8523,2172,1230,5384,2277,4206,8329,1188 : 12

Header of reduced data:

@relation 'R_data_frame-weka.filters.unsupervised.attribute.Remove-V-R4526,6015,1443,1312,8523,2172,1230,5384,2277,4206,8329,1188'

@attribute NP_071896 numeric
 @attribute NP_004840 numeric
 @attribute NP_006786 numeric
 @attribute NP_002829 numeric
 @attribute NP_002351 numeric
 @attribute NP_079093 numeric
 @attribute NP_055719 numeric
 @attribute NP_006346 numeric
 @attribute NP_000959 numeric
 @attribute NP_000960 numeric
 @attribute NP_000981 numeric
 @attribute NP_001349 numeric

Table 25: Tabel with the summary of results from Randomtree

Correctly Classified Instances	42	54.5
Incorrectly Classified Instances	35	45.5
Kappa statistic	0.1126	x
Mean absolute error	0.226	x
Root mean squared error	0.4726	x
Relative absolute error	84.0	x
Root relative squared error	129.4	x
Total Number of Instances	77	x

Table 26: labelRandomTree confusionmatrixConfusion matrix

a	b	c	d	<- classified as
3	4	3	0	a = T1
3	38	7	3	b = T2
5	6	0	0	c = T3
0	4	0	1	d = T4

confusion matrix

algorithm conclusion

in the above seen confusion matrix we are looking for a nicely made line from the top left to the bottom right, but we can clearly see that that is not the case, and it classifies them mostly wrong as t2. So this algorithm doesn't have a lot of merit for further exploration

AttributeSelectedClassifier greedystepwise with OneR

Relation: R_data_frame
Instances: 77
Attributes: 9200
Test mode: 77-fold cross-validation
Evaluation cost matrix:

=== Attribute Selection on all input data ===

Search Method:
Greedy Stepwise (forwards).
Start set: no attributes
Merit of best subset found: 0.601

Attribute Subset Evaluator (supervised, Class (nominal): 9200 data.class):
CFS Subset Evaluator
Including locally predictive attributes

Selected attributes: 338,905,1188,1230,1555,2172,2277,2821,3196,3333,3719,3932,5844,6802,7234,7490,7959,8149,8539
: 19
NP_008832 NP_056289 NP_001349 NP_055719 NP_001150 NP_079093 NP_000959 NP_004893
NP_004887 NP_065901 NP_058632 NP_002630 NP_060947 NP_065109 NP_848613 NP_001035147
NP_005639 NP_001092102 NP_001017

Table 27: Tabel with the summary of results from OneR

Correctly Classified Instances	45	58.44
Incorrectly Classified Instances	32	41.55
Kappa statistic	-0.0788	x
Total Cost	133	
Average Cost	1.7273	
Mean absolute error	0.2078	x
Root mean squared error	0.4558	x
Relative absolute error	77.2714	x
Root relative squared error	124.8216	x
Total Number of Instances	77	x

Table 28: labelgreedystepwise with OneR confusionmatrixConfusion matrix

a	b	c	d	<- classified as
0	9	1	0	a = T1
2	45	4	0	b = T2
0	11	0	0	c = T3
0	5	0	0	d = T4

confusion matrix

algorithm conclusion

in the above seen confusion matrix we are looking for a nicely made line from the top left to the bottom right, but we can clearly see that that is not the case, we can see that is mostly classify them as T2. So this algorithm doesn't have a lot of merit for further exploration

2.3 Supervised Learning Conclusion

summary these are but the best of the multiple different settings that where tried, but all results where the same as these presented in the chapters here above. one if not the first things that stands out for every one of these results is that none of these results scored an accuracy of correctly predicting the class of the data better than ZeroR with its 66.2%. One got close but that was a RandomTree model without attributeselection, so it is questionable how accurate its truly is since overfitting with 9200 attributes is a major problem. But accuracy of correctly prediction the class is not everything, so we must also look at the confusion matrix's that where produced. As can be seen from the confusion matrix's (tables 20,22,24,26) there are two trends visible that firstly there is a huge bias towards t2, this was tried to be compensated with different SMOTE functions to boost the underrepresented classes but added more than 75 % of synthetic data is not very accurate and introduces a whole host of new problems and bias to the algorithm used to boost the unrepresented classes. Furthermore, under sampling the T2 class also was not an option since cutting out instances in a data set only containing 77 is not good since this is already a very low sample size. Thus, most of the models that where teste where with the meta learning AttributeSelectedClassifier , and yielded not much as explained above. The main thing that can be said of all the methods and algorithms used is they performed all bad or equally bad as just picking a random class. One more thing is that a significant portion the models that where tried with the AttributeSelectedClassifier is that they almost all chose these attributes :

NP_008832 ,NP_056289,NP_001349,NP_055719,NP_001150,NP_079093,NP_000959,NP_004893,NP_004887,NP_065903

These can be of interest for further research after collecting more instance / samples.

FINAL MODEL So after all this what was the final model ? it's a Cost sensitive classifiere using Adaboost M1 running a rondonTree with Seed 38. first off this model is not correct in any way and is not good. One it is likely overfitted since it uses all 9200 attributes. Two the cost matrix is wrong since it contains a mistake. Three its accuracy and cost matrix are still not good, but its ROC is one of the best found. it can be found here in the repositoryModels/Model-01_27_10.model

it results are follows: ==== Run information ====

Scheme: weka.classifiers.meta.CostSensitiveClassifier -cost-matrix "[0.0 20.0 20.0 20.0; 1.0 0.0 1.0 1.0; 20.0 20.0 0.0 20.0; 20.0 20.0 1.0 0.0]" -S 38 -W weka.classifiers.meta.AdaBoostM1 - -P 100 -S 38 -I 10 -W weka.classifiers.trees.RandomTree - -K 0 -M 1.0 -V 0.001 -S 38

Relation: R_data_frame

Instances: 77

Attributes: 9200

[list of attributes omitted]

Test mode: 77-fold cross-validation

==== Classifier model (full training set) ====

CostSensitiveClassifier using reweighted training instances

weka.classifiers.meta.AdaBoostM1 -P 100 -S 38 -I 10 -W weka.classifiers.trees.RandomTree - -K 0 -M 1.0 -V 0.001 -S 38

Classifier Model
AdaBoostM1: No boosting possible, one classifier used!

Cost Matrix
0 20 20 20
1 0 1 1
20 20 0 20
20 20 1 0

Time taken to build model: 0.05 seconds

==== Summary ====

Correctly Classified Instances 37 48.0519 %
Incorrectly Classified Instances 40 51.9481 %
Kappa statistic -0.0052
Total Cost 116
Average Cost 1.5065
Mean absolute error 0.2582
Root mean squared error 0.5062
Relative absolute error 96.
Root relative squared error 138.6
Total Number of Instances 77

==== Detailed Accuracy By Class ====

Table 29: labelFinal model accuracy final model accuracy									
TP Rate	FP Rate	Precision	Recall	F-Measure	MCC	ROC Area	PRC Area	Class	
	0,100	0,134	0,100	0,100	0,100	-0,034	0,494	0,133	T1
	0,647	0,692	0,647	0,647	0,647	-0,045	0,535	0,709	T2
	0,273	0,167	0,214	0,273	0,240	0,096	0,594	0,177	T3
	0,000	0,028	0,000	0,000	0,000	-0,043	0,631	0,112	T4
Weighted Avg.	0,481	0,502	0,472	0,481	0,476	-0,023	0,545	0,520	

=== Confusion Matrix ===

a b c d <- classified as

1 7 2 0 | a = T1

8 33 8 2 | b = T2

0 8 3 0 | c = T3

1 3 1 0 | d = T4