ProteinQuantification

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Protein Quantification

Bradford calibration curve

Mix well while pipeting. Holes with protein content must become blue.
Incubate 5 min.
Measure at 595 abs (TECAN). clear box.
5 ul y 50ul of protein. Take measures inside the curve range.
BSA on ug/ml

$$

BSA μl por ml	BSA μl	Bradford μl	Other
0	0	40	H_2O 160 μl
10	160	40	-
20	160	40	-
40	160	40	-
60	160	40	-
100	160	40	-
0	0	40	PriA 5 μl H_2O 155 μl
0	0	40	PriA 20 μl H_2O 130 μl

$$

```
## 160 ul of BSA on this concentrations on ug/ml were added to a 40 ul of Bradford
##BSA concentration on ug/ml
BSAConcentrationStock<-c(0,10,20,40,60,100)

#adding .16 ml on plates
vol<-160
Bradford_df = data.frame(BSAConcentrationStock,vol)

## quantity of micrograms on .16 ml
Bradford_df["Quantity"]<-BSAConcentrationStock*.160
## concentration after added 40 ul bradford (total 200 ul)
Bradford_df["Concentration200"]<-Bradford_df["Quantity"]/.2


## This are the absorbance measures for the different BSA concentrations
Abs1BSA<-c(0.2649999857,0.4329000115,0.6754000187,0.9449999928,1.24240005,1.432000041)
Abs2BSA<-c(0.2948000133,0.4298999906,0.7348999977,0.9965000153,1.225999951,1.494899988)

Bradford_df["Abs1BSA"]<-Abs1BSA
Bradford_df["Abs2BSA"]<-Abs2BSA
```

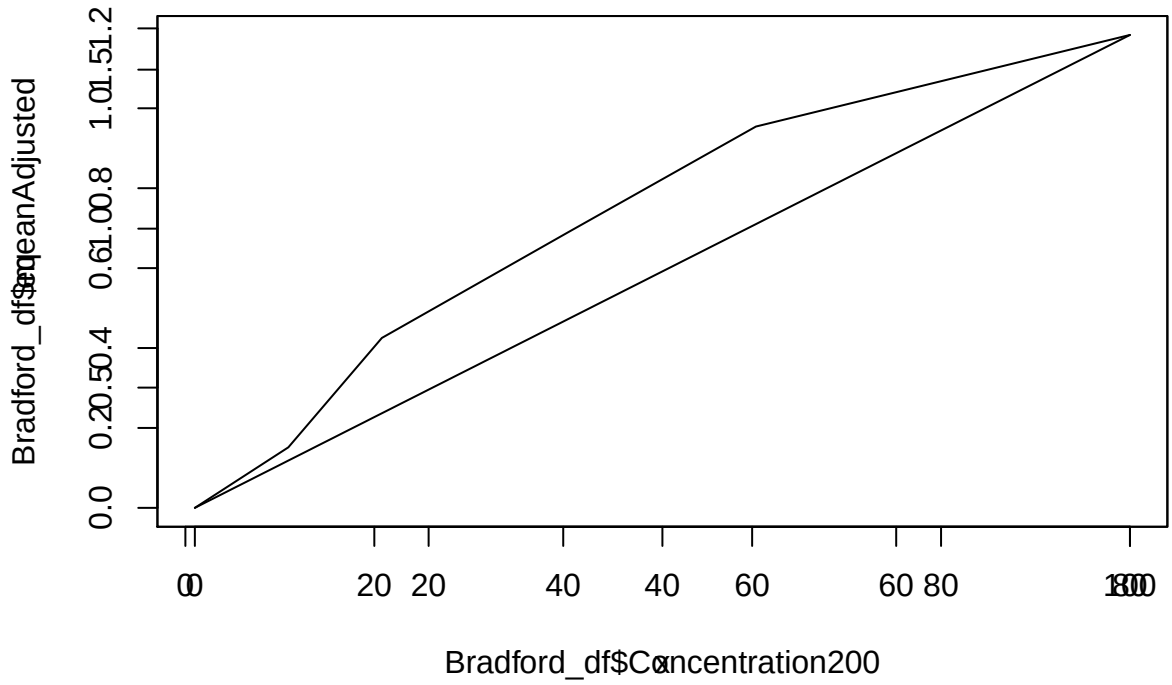
```r
## mean value of measures
Bradford_df$mean<-rowMeans(subset(Bradford_df, select = c(Abs1BSA, Abs2BSA)), na.rm = TRUE)

#adjusting blank
Bradford_df$meanAdjusted<-Bradford_df$mean-Bradford_df$mean[1]
BSA.lm = lm(meanAdjusted ~ Concentration200, data=Bradford_df)
coeffs = coefficients(BSA.lm); coeffs
```

```
##      (Intercept) Concentration200
##       0.10688027       0.01502265
```

```r
b=coeffs[1]
m=coeffs[2]

eq = function(x){x*m+b}
plot(Bradford_df$Concentration200,Bradford_df$meanAdjusted,type="l")
par(new=TRUE)
plot(eq, 1,100)
```



Enzyme quantification

For Bradford to work properly After protein concentration, 900 ul Buffer A (free of imidazol) must be added to 100 μl sample

```r
##  ul of
vol=5
type="Scoe"
ABS1=0.932099998
ABS2=1.048400044
Pria_df=data.frame(vol,type,ABS1,ABS2)
Pria_df$type<-as.character(Pria_df$type)
data2=c(20,"Scoe",1.639400005,1.569700003)
```

```
data3=c(5,"D11A",0.575600028,   0.576099992)
data4=c(20,"D11A",1.039499998,  1.067399979)
data5=c(160,"test",.69085,  .69085)

## add data to dataframe Pria_df
Pria_df=rbind(Pria_df,data2)
Pria_df=rbind(Pria_df,data3)
Pria_df=rbind(Pria_df,data4)
Pria_df=rbind(Pria_df,data5)

Pria_df$mean<-rowMeans(cbind(as.numeric(Pria_df$ABS1),as.numeric(Pria_df$ABS2)))

## Pasando absorbancia a concentration de proteina en 200 ul (ug/ml)
## kari values
#m=0.004679534923
#b=0.103674687

inter = function(y){(y-b)/m}
Pria_df$concentration200<-inter(Pria_df$mean)

## protein quantity on ug on 200 ul
Pria_df["Quantity"]=Pria_df$concentration200*.2

### Inicial concentration  C200_vol200=Cinicial_Vinicial
Pria_df["ConcentrationInitial"]=Pria_df$Quantity/(.001*as.numeric(Pria_df$vol))

kable(Bradford_df)
```

BSAConcentrationStock	vol	Quantity	Concentration200	Abs1BSA	Abs2BSA	mean	meanAdjusted
0	160	0.0	0	0.2650	0.2948	0.27990	0.00000
10	160	1.6	8	0.4329	0.4299	0.43140	0.15150
20	160	3.2	16	0.6754	0.7349	0.70515	0.42525
40	160	6.4	32	0.9450	0.9965	0.97075	0.69085
60	160	9.6	48	1.2424	1.2260	1.23420	0.95430
100	160	16.0	80	1.4320	1.4949	1.46345	1.18355

```
kable(Pria_df)
```

vol	type	ABS1	ABS2	mean	concentration200	Quantity	ConcentrationInitial
5	Scoe	0.932099998	1.048400044	0.99025	58.80251	11.760502	2352.10030
20	Scoe	1.639400005	1.569700003	1.60455	99.69408	19.938816	996.94081
5	D11A	0.575600028	0.576099992	0.57585	31.21750	6.243500	1248.70007
20	D11A	1.039499998	1.067399979	1.05345	63.00948	12.601897	630.09485
160	test	0.69085	0.69085	0.69085	38.87261	7.774521	48.59076

Since 1kDa = 1Kg/mol and PriA Scoe molecular weight is 25.7kDa we have that
$M \times V = \frac{g}{mw}$
$M = \frac{g}{V} \times \frac{1}{mw}$

```
## mas on Kda = kg/mol
mw<-25.7
```

```r
# converting to g/mol
mwgram<-mw*1000
## concentration on g/L (after convert ug/ml)
concentScoe<-2.352
concentD11A<-1.248
## Concentration on uM (10**6M)
molar = function(x){(10**6)*x/mwgram}
molar(concentScoe)
```

```
## [1] 91.51751
```

```r
molar(concentD11A)
```

```
## [1] 48.56031
```

Concentration on microMolar

So Scoe concentration is 91.5175097 and D11A is 48.5603113 μM

```r
## we want final concentration 2.4uM on a final volume of 160 ul
## ciVi=CfVf
## Vi =CfVf/Vi
## Cf= 80uM Vf=5ul Vi=?ul Ci=x

adjustMol = function(x){2.5*160/x}
## this are the ul need from stock cristal enzyme that will be diluted on buffer to complete 160 ul
adjustMol(molar(concentScoe))
```

```
## [1] 4.370748
```

```r
adjustMol(molar(concentD11A))
```

```
## [1] 8.237179
```