

Package ‘CFAcoop’

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Type Package

Title Colony Formation Assay: Taking into Account Cellular Cooperation

Version 1.0.0

Maintainer Daniel Samaga <daniel.samaga@helmholtz-muenchen.de>

Depends R (>= 3.5.0)

URL <https://github.com/ZytoHMGU/CFAcoop>

BugReports <https://github.com/ZytoHMGU/CFAcoop/issues>

Description Cellular cooperation compromises the plating efficiency-based analysis of clonogenic survival data. This tool provides functions that enable a robust analysis of colony formation assay (CFA) data in presence or absence of cellular cooperation.

The implemented method has been described

in Brix et al. (2020). (Brix, N., Samaga, D., Hennel, R. et al.

``The clonogenic assay: robustness of plating efficiency-based analysis is strongly compromised by cellular cooperation." Radiat Oncol 15, 248 (2020). <doi:10.1186/s13014-020-01697-y>)

Power regression for parameter estimation, calculation of survival fractions, uncertainty analysis and plotting functions are provided.

License GPL-3

Encoding UTF-8

LazyData true

RoxygenNote 7.1.1

Imports Hmisc

Suggests knitr, rmarkdown, testthat

VignetteBuilder knitr

NeedsCompilation no

Author Daniel Samaga [cre, aut],
Nikko Brix [aut],
Kirsten Lauber [aut],
Horst Zitzelsberger [aut]

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Contents

analyze_survival	2
calculate_sf	3
CFAdata	4
export_sf	4
plot_sf	5
pwr_reg	7
Index	8

analyze_survival	<i>analyze_survival</i>
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Description

wrapper function for robust analysis of clonogenic survival data from the colony formation assay according to Brix et al. (2020), Radiation Oncology. Mean values are calculated and used for power regression. Resulting coefficients are used for calculation of survival fractions and corresponding uncertainty analysis.

Usage

```
analyze_survival(RD, name = "no name", xtreat = NULL, C = 20)
```

Arguments

RD	data.frame or matrix containing a table of experiment data
name	optional: experiment name (e.g. name of cell line)
xtreat	optional: treatment dose of the colonies counted in the corresponding columns of RD
C	number of colonies counted for which the survival fraction is to be calculated (default = 20)

Value

list object containing several experiments and treatments organized for convenient plotting with `plot_sf`

Examples

```
seeded <- rep(10^(seq(1,5,0.5)),each = 3)
df.1 <- data.frame(
  "seeded" = seeded,
  "counted1" = 0.4 * seeded^1.1 * rnorm(n = length(seeded),1,0.05),
  "counted2" = 0.2 * seeded^1.125 * rnorm(n = length(seeded),1,0.05),
  "counted3" = 0.05 * seeded^1.25 * rnorm(n = length(seeded),1,0.05))
df.2 <- data.frame("seeded" = seeded,
```

```

"counted1" = 0.5 * seeded^1.01 * rnorm(n = length(seeded),1,0.05),
"counted2" = 0.4 * seeded^1.0125 * rnorm(n = length(seeded),1,0.05),
"counted3" = 0.2 * seeded^1.025 * rnorm(n = length(seeded),1,0.05))
SF <- vector("list",2)
SF[[1]] <- analyze_survival(RD = df.1,
                           name = "cell line a",
                           xtreat = c(0,1,4),
                           C = 20)
SF[[2]] <- analyze_survival(RD = df.2,
                           name = "cell line b",
                           xtreat = c(0,1,4))

```

calculate_sf

calculate_sf

Description

calculates the survival fraction according to the procedure presented in Brix et al. (2020), which is robust against cellular cooperation.

Usage

```
calculate_sf(par_ref, par_treat, C = 20)
```

Arguments

par_ref	summary.lm object or 2-column matrix for the treatment-free reference survival
par_treat	summary.lm object or 2-column matrix for the clonogenic survival after treatment
C	colony number for which the survival fraction is calculated (default = 20)

Value

survival fractions. If par_ref and par_treat are summary.lm objects, a scalar is returned. If par_ref and par_treat are matrices, a vector of the same length as nrow(par_treat) is returned

Examples

```

seeded <- 10^(seq(1, 5, 0.5))
counted.ref <- 0.4 * 10^(seq(1, 5, 0.5) + rnorm(n = 9, 0, 0.1))^1.1
counted.treat <- 0.01 * 10^(seq(1, 5, 0.5) + rnorm(n = 9, 0, 0.1))^1.2
fit_ref <- pwr_reg(seeded = seeded, counted = counted.ref)
fit_treat <- pwr_reg(seeded = seeded, counted = counted.treat)
calculate_sf(par_ref = fit_ref, par_treat = fit_treat)
data("CFadata")
D <- subset.data.frame(
  x = CFadata,
  subset = cell.line == levels(CFadata$cell.line)[1]
)

```

```
fit_ref <- pwr_reg(seeded = D$`Cells seeded`, counted = D$`0 Gy`)
fit_treat <- pwr_reg(seeded = D$`Cells seeded`, counted = D$`4 Gy`)
calculate_sf(par_ref = fit_ref, par_treat = fit_treat)
```

CFadata

Colony Formation Assay data on cellular cooperation

Description

Clonogenic survival data from seven cell lines T47D, MDA-MB231, A549, HCC1806, SKBR3, SKLU1 and BT20 as presented in Figure 2 in Brix et al. (2020).

Usage

```
data(CFadata)
```

Format

```
data.frame
```

References

Brix, N., Samaga, D., Hennel, R. et al. "The clonogenic assay: robustness of plating efficiency-based analysis is strongly compromised by cellular cooperation." *Radiat Oncol* 15, 248 (2020). <doi:10.1186/s13014-020-01697-y>

Examples

```
data(CFadata)
head(CFadata)
c11 <- levels(CFadata$cell.line)
```

export_sf

export_sf

Description

export table with results of clonogenic survival analysis from the colony formation assay considering cellular cooperation

Usage

```
export_sf(SF)
```

Arguments

SF list build of objects returned by analyze_survival

Value

data.frame containing all estimated coefficients and effects from all experiments contained in SF

Examples

```
seeded <- rep(10^(seq(1, 5, 0.5)), each = 3)
df.1 <- data.frame(
  "seeded" = seeded,
  "counted1" = 0.4 * seeded^1.1 * rnorm(n = length(seeded), 1, 0.05),
  "counted2" = 0.2 * seeded^1.125 * rnorm(n = length(seeded), 1, 0.05),
  "counted3" = 0.05 * seeded^1.25 * rnorm(n = length(seeded), 1, 0.05)
)
df.2 <- data.frame(
  "seeded" = seeded,
  "counted1" = 0.5 * seeded^1.01 * rnorm(n = length(seeded), 1, 0.05),
  "counted2" = 0.4 * seeded^1.0125 * rnorm(n = length(seeded), 1, 0.05),
  "counted3" = 0.2 * seeded^1.025 * rnorm(n = length(seeded), 1, 0.05)
)
SF <- vector("list", 2)
SF[[1]] <- analyze_survival(
  RD = df.1, name = "cell line a",
  xtreat = c(0, 1, 4)
)
SF[[2]] <- analyze_survival(
  RD = df.2, name = "cell line b",
  xtreat = c(0, 1, 4)
)
export_sf(SF)

data("CFAdata")
SF <- vector("list", 4)
ll <- levels(CFAdata$cell.line)[c(1, 3, 5, 7)]
for (i in seq_along(ll)) {
  cdat <- subset.data.frame(
    x = CFAdata,
    subset = CFAdata$cell.line == ll[i]
  )
  SF[[i]] <- analyze_survival(
    RD = cdat[, -1],
    name = ll[i],
    xtreat = c(0, 1, 2, 4, 6, 8)
  )
}
export_sf(SF)
```

plot_sf

*plot_sf***Description**

plot cellular cooperativity and clonogenic survival for colony formation assay data

Usage

```
plot_sf(SF, showUncertainty = TRUE)
```

Arguments

SF list build of objects returned by analyze_survival
 showUncertainty logical, switches on/off uncertainty bands for sf-values.

Value

none

Examples

```
seeded <- rep(10^(seq(1, 5, 0.5)), each = 3)
df.1 <- data.frame(
  "seeded" = seeded,
  "counted1" = 0.4 * seeded^1.1 * rnorm(n = length(seeded), 1, 0.05),
  "counted2" = 0.2 * seeded^1.125 * rnorm(n = length(seeded), 1, 0.05),
  "counted3" = 0.05 * seeded^1.25 * rnorm(n = length(seeded), 1, 0.05)
)
df.2 <- data.frame(
  "seeded" = seeded,
  "counted1" = 0.5 * seeded^1.01 * rnorm(n = length(seeded), 1, 0.05),
  "counted2" = 0.4 * seeded^1.0125 * rnorm(n = length(seeded), 1, 0.05),
  "counted3" = 0.2 * seeded^1.025 * rnorm(n = length(seeded), 1, 0.05)
)
SF <- vector("list", 2)
SF[[1]] <- analyze_survival(
  RD = df.1, name = "cell line a",
  xtreat = c(0, 1, 4)
)
SF[[2]] <- analyze_survival(
  RD = df.2, name = "cell line b",
  xtreat = c(0, 1, 4)
)
plot_sf(SF)

data("CFAdata")
SF <- vector("list", 4)
ll <- levels(CFAdata$cell.line)[c(1, 3, 5, 7)]
for (i in seq_along(ll)) {
  cdat <- subset.data.frame(
    x = CFAdata,
    subset = CFAdata$cell.line == ll[i]
  )
  SF[[i]] <- analyze_survival(
    RD = cdat[, -1],
    name = ll[i],
    xtreat = c(0, 1, 2, 4, 6, 8)
  )
}
```

```
)  
}  
plot_sf(SF)
```

pwr_reg

pwr_reg

Description

pwr_reg performs a power regression ($\log(C) = \log(a) + b * \log(S) + e$) for clonogenic assay data of experiments examining the cellular cooperation.

Usage

```
pwr_reg(seeded, counted)
```

Arguments

seeded numeric vector with number of cells seeded (S)
counted numeric vector with number of colonies counted (C, same length as seeded)

Value

summary.lm object as returned by [summary](#)

Examples

```
pwr_reg(  
  seeded = 10^(seq(1, 5, 0.5)),  
  counted = 0.4 * (10^seq(1, 5, 0.5))^1.25 * rnorm(n = 9, 1, 0.05)  
)  
data(CFadata)  
D <- subset.data.frame(  
  x = CFadata,  
  subset = cell.line == levels(CFadata$cell.line)[1]  
)  
pwr_reg(seeded = D$`Cells seeded`, counted = D$`0 Gy`)
```

Index

* **dataset**

CFAdata, [4](#)

analyze_survival, [2](#)

calculate_sf, [3](#)

CFAdata, [4](#)

export_sf, [4](#)

plot_sf, [5](#)

pwr_reg, [7](#)

summary, [7](#)