

Package ‘CFAcoop’

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Type Package

Title Colony Formation Assay: Taking into Account Cellular Cooperation

Version 0.1.0

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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 mvtnorm, Hmisc

Suggests knitr, rmarkdown, testthat

VignetteBuilder knitr

NeedsCompilation no

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| | |
|------------------|-------------------------|
| analyze_survival | <i>analyze_survival</i> |
|------------------|-------------------------|

Description

wrapper function for robust analysis 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_range = c(5, 20, 100))
```

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_range | number or vector of numbers of colonies counted for which the survival fraction is to be calculated (default = c(5, 20, 100)) |

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_range = c(5,20,100))
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_range = c(5, 20, 100))
```

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_range | colony numbers for which the survival fraction is calculated (default = c(5, 20, 100)) |

Value

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

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](https://doi.org/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`)

```

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* **dataset**

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