```
<link crossorigin="anonymous" href="https://assets-cdn.github.com/assets/frameworks-d05617edbd0684501ca</pre>
<link crossorigin="anonymous" href="https://assets-cdn.github.com/assets/github-a5740f61dda90caedf6fe1f</pre>
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<link as="script" href="https://assets-cdn.github.com/assets/github-cb6ab72bc83e90e83a429b044f460572dc5</pre>
<meta http-equiv="X-UA-Compatible" content="IE=edge">
<meta http-equiv="Content-Language" content="en">
<meta name="viewport" content="width=device-width">
<title>caRpools/CaRpools-extended-PDF.Rmd at master · boutroslab/caRpools</title>
<link rel="search" type="application/opensearchdescription+xml" href="/opensearch.xml" title="GitHub">
<link rel="fluid-icon" href="https://github.com/fluidicon.png" title="GitHub">
<link rel="apple-touch-icon" href="/apple-touch-icon.png">
<link rel="apple-touch-icon" sizes="57x57" href="/apple-touch-icon-57x57.png">
<link rel="apple-touch-icon" sizes="60x60" href="/apple-touch-icon-60x60.png">
<link rel="apple-touch-icon" sizes="72x72" href="/apple-touch-icon-72x72.png">
<link rel="apple-touch-icon" sizes="76x76" href="/apple-touch-icon-76x76.png">
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<link rel="apple-touch-icon" sizes="120x120" href="/apple-touch-icon-120x120.png">
<link rel="apple-touch-icon" sizes="144x144" href="/apple-touch-icon-144x144.png">
<link rel="apple-touch-icon" sizes="152x152" href="/apple-touch-icon-152x152.png">
<link rel="apple-touch-icon" sizes="180x180" href="/apple-touch-icon-180x180.png">
<meta property="fb:app_id" content="1401488693436528">
   \verb|\content="https://avatars2.githubusercontent.com/u/2943832?v=3\&s=400" name="twitter:image:sright of the content of the
   <meta content="https://avatars2.githubusercontent.com/u/2943832?v=3&amp;s=400" property="og:image" />
   <meta name="browser-stats-url" content="https://api.github.com/_private/browser/stats">
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<link rel="assets" href="https://assets-cdn.github.com/">
<link rel="web-socket" href="wss://live.github.com/_sockets/NTgzMjAwNTo3MzQwMzBhNmION2RmNzk4MDdiMDAxOTB;</pre>
<meta name="pjax-timeout" content="1000">
<link rel="sudo-modal" href="/sessions/sudo modal">
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<meta name="msapplication-TileColor" content="#ffffff">
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<meta name="google-site-verification" content="KT5gs8h0wvaagLKAVWq8bbeNwnZZK1r1XQysX3xurLU">
       <meta name="hostname" content="github.com">
<meta name="user-login" content="francescojm">
       <meta name="expected-hostname" content="github.com">
   <meta name="js-proxy-site-detection-payload" content="OGQ5MTVkZjA5YjAyMjJkNjg0NzY2M2E1ZTY5YTR10DdmNDA</pre>
```

<meta charset='utf-8'>

```
<link rel="mask-icon" href="https://assets-cdn.github.com/pinned-octocat.svg" color="#4078c0">
 <link rel="icon" type="image/x-icon" href="https://assets-cdn.github.com/favicon.ico">
<meta name="html-safe-nonce" content="bb8dc8b60b7aedf7e2fd339d7e3a906259f78418">
<meta content="ab9ab8d96b0e82079f8c12a4f1e328ef0531a8c2" name="form-nonce" />
<meta http-equiv="x-pjax-version" content="46f108ca66e01da98c38cf52e08c76c9">
 <link rel="canonical" href="https://github.com/boutroslab/caRpools/blob/master/CaRpools-extended-PDF.</pre>
<div id="js-pjax-loader-bar" class="pjax-loader-bar"></div>
<a href="#start-of-content" tabindex="1" class="accessibility-aid js-skip-to-content">Skip to content/
   <div class="header header-logged-in true" role="banner">
<a class="header-logo-invertocat" href="https://github.com/" data-hotkey="g d" aria-label="Homepage" da</pre>
   <div class="header-search scoped-search site-scoped-search js-site-search" role="search">
<label class="form-control header-search-wrapper js-chromeless-input-container">
  <div class="header-search-scope">This repository</div>
 <input type="text"</pre>
   class="form-control header-search-input js-site-search-focus js-site-search-field is-clearable"
   data-hotkey="s"
   name="q"
   placeholder="Search"
   aria-label="Search this repository"
   data-unscoped-placeholder="Search GitHub"
   data-scoped-placeholder="Search"
   tabindex="1"
   autocapitalize="off">
</label>
 <a href="/pulls" class="js-selected-navigation-item header-nav-link" data-ga-click="Header, click</pre>
       Pull requests
   <a href="/issues" class="js-selected-navigation-item header-nav-link" data-ga-click="Header, click
       Issues
     <a class="header-nav-link" href="https://gist.github.com/" data-ga-click="Header, go to gist, t</pre>
```

```
<a href="/notifications" aria-label="You have unread notifications" class="header-nav-link notification</p>
    <span class="mail-status unread"></span>
    <svg aria-hidden="true" class="octicon octicon-bell" height="16" version="1.1" viewBox="0 0 14 16"</pre>
<a class="header-nav-link tooltipped tooltipped-s js-menu-target" href="/new"</pre>
   aria-label="Create new..."
   data-ga-click="Header, create new, icon:add">
  <svg aria-hidden="true" class="oction oction-plus left" height="16" version="1.1" viewBox="0 0 12 1</pre>
  <span class="dropdown-caret"></span>
<div class="dropdown-menu-content js-menu-content">
  New repository
Import repository
New organization
<span title="boutroslab/caRpools">This repository</span>
<a class="dropdown-item" href="/boutroslab/caRpools/issues/new" data-ga-click="Header, create new issue</pre>
 New issue
</a>
  </div>
<a class="header-nav-link name tooltipped tooltipped-sw js-menu-target" href="/francescojm"</pre>
   aria-label="View profile and more"
   data-ga-click="Header, show menu, icon:avatar">
  <img alt="@francescojm" class="avatar" height="20" src="https://avatars2.githubusercontent.com/u/5832</pre>
  <span class="dropdown-caret"></span>
</a>
<div class="dropdown-menu-content">
  <div class="dropdown-menu dropdown-menu-sw">
    <div class="dropdown-header header-nav-current-user css-truncate">
     Signed in as <strong class="css-truncate-target">francescojm</strong>
    </div>
    <div class="dropdown-divider"></div>
    <a class="dropdown-item" href="/francescojm" data-ga-click="Header, go to profile, text:your profil</pre>
     Your profile
    <a class="dropdown-item" href="/stars" data-ga-click="Header, go to starred repos, text:your stars"</pre>
     Your stars
    </a>
    <a class="dropdown-item" href="/explore" data-ga-click="Header, go to explore, text:explore">
    </a>
```

```
<a class="dropdown-item" href="/integrations" data-ga-click="Header, go to integrations, text:int</pre>
        Integrations
    <a class="dropdown-item" href="https://help.github.com" data-ga-click="Header, go to help, text:help.github.com" data-ga-click="Header, go to help, text:help.github.com"</pre>
      Help
    </a>
    <div class="dropdown-divider"></div>
    <a class="dropdown-item" href="/settings/profile" data-ga-click="Header, go to settings, icon:settings</pre>
      Settings
    </a>
    <!-- </textarea> --><!-- '"` --><form accept-charset="UTF-8" action="/logout" class="logout-form" d
      <button class="dropdown-item dropdown-signout" data-ga-click="Header, sign out, icon:logout">
        Sign out
      </button>
</div>
<div id="start-of-content" class="accessibility-aid"></div>
  <div id="js-flash-container">
<div role="main" class="main-content">
    <div itemscope itemtype="http://schema.org/SoftwareSourceCode">
<div id="js-repo-pjax-container" data-pjax-container>
    <!-- </textarea> --><!-- ""` --><form accept-charset="UTF-8" action="/notifications/subscribe" clas
    <div class="select-menu js-menu-container js-select-menu">
      <a href="/boutroslab/caRpools/subscription"</pre>
        class="btn btn-sm btn-with-count select-menu-button js-menu-target" role="button" tabindex="0"
        data-ga-click="Repository, click Watch settings, action:blob#show">
        <span class="js-select-button">
          <svg aria-hidden="true" class="octicon octicon-eye" height="16" version="1.1" viewBox="0 0 16</pre>
          Watch
        </span>
      <a class="social-count js-social-count" href="/boutroslab/caRpools/watchers">
      </a>
    <div class="select-menu-modal-holder">
      <div class="select-menu-modal subscription-menu-modal js-menu-content" aria-hidden="true">
        <div class="select-menu-header js-navigation-enable" tabindex="-1">
          <svg aria-label="Close" class="octicon octicon-x js-menu-close" height="16" role="img" version</pre>
          <span class="select-menu-title">Notifications</span>
        </div>
          <div class="select-menu-list js-navigation-container" role="menu">
```

```
<svg aria-hidden="true" class="octicon octicon-check select-menu-item-icon" height="16" v</pre>
              <div class="select-menu-item-text">
                <input checked="checked" id="do_included" name="do" type="radio" value="included" />
                <span class="select-menu-item-heading">Not watching</span>
                <span class="description">Be notified when participating or @mentioned.
                <span class="js-select-button-text hidden-select-button-text">
                  <svg aria-hidden="true" class="octicon octicon-eye" height="16" version="1.1" viewBox</pre>
                  Watch
                </span>
              </div>
            </div>
            <div class="select-menu-item js-navigation-item " role="menuitem" tabindex="0">
              <svg aria-hidden="true" class="octicon octicon-check select-menu-item-icon" height="16" v</pre>
              <div class="select-menu-item-text">
                <input id="do_subscribed" name="do" type="radio" value="subscribed" />
                <span class="select-menu-item-heading">Watching</span>
                <span class="description">Be notified of all conversations.
                <span class="js-select-button-text hidden-select-button-text">
                  <svg aria-hidden="true" class="octicon octicon-eye" height="16" version="1.1" viewBox</pre>
                  Unwatch
                </span>
              </div>
            </div>
            <div class="select-menu-item js-navigation-item " role="menuitem" tabindex="0">
              <svg aria-hidden="true" class="oction oction-check select-menu-item-icon" height="16" v</pre>
              <div class="select-menu-item-text">
                <input id="do_ignore" name="do" type="radio" value="ignore" />
                <span class="select-menu-item-heading">Ignoring</span>
                <span class="description">Never be notified.</span>
                <span class="js-select-button-text hidden-select-button-text">
                  <svg aria-hidden="true" class="oction oction-mute" height="16" version="1.1" viewBo.</pre>
                  Stop ignoring
                </span>
              </div>
            </div>
          </div>
        </div>
      </div>
<!-- </textarea> --><!-- ""` --><form accept-charset="UTF-8" action="/boutroslab/caRpools/unstar" class
    class="btn btn-sm btn-with-count js-toggler-target"
    aria-label="Unstar this repository" title="Unstar boutroslab/caRpools"
    data-ga-click="Repository, click unstar button, action:blob#show; text:Unstar">
    <svg aria-hidden="true" class="octicon octicon-star" height="16" version="1.1" viewBox="0 0 14 16"</pre>
   Unstar
  </button>
```

<div class="select-menu-item js-navigation-item selected" role="menuitem" tabindex="0">

```
<a class="social-count js-social-count" href="/boutroslab/caRpools/stargazers">
      4
    </a>
<!-- </textarea> --><!-- ""` --><form accept-charset="UTF-8" action="/boutroslab/caRpools/star" class="
    class="btn btn-sm btn-with-count js-toggler-target"
    aria-label="Star this repository" title="Star boutroslab/caRpools"
    data-ga-click="Repository, click star button, action:blob#show; text:Star">
    <svg aria-hidden="true" class="octicon octicon-star" height="16" version="1.1" viewBox="0 0 14 16"</pre>
    Star
  </button>
    <a class="social-count js-social-count" href="/boutroslab/caRpools/stargazers">
    </a>
      <a href="#fork-destination-box" class="btn btn-sm btn-with-count"</pre>
          title="Fork your own copy of boutroslab/caRpools to your account"
          aria-label="Fork your own copy of boutroslab/caRpools to your account"
          data-ga-click="Repository, show fork modal, action:blob#show; text:Fork">
          <svg aria-hidden="true" class="octicon octicon-repo-forked" height="16" version="1.1" viewBox</pre>
        Fork
      </a>
      <div id="fork-destination-box" style="display: none;">
        <h2 class="facebox-header" data-facebox-id="facebox-header">Where should we fork this repositor
        <include-fragment src=""</pre>
            class="js-fork-select-fragment fork-select-fragment"
            data-url="/boutroslab/caRpools/fork?fragment=1">
          <img alt="Loading" height="64" src="https://assets-cdn.github.com/images/spinners/octocat-spi.</pre>
        </include-fragment>
      </div>
<a href="/boutroslab/caRpools/network" class="social-count">
  2
</a>
<h1 class="public ">
boutroslab/caRpools
Code
<span itemscope itemtype="http://schema.org/ListItem" itemprop="itemListElement">
  <a href="/boutroslab/caRpools/issues" class="js-selected-navigation-item reponav-item" data-hotkey="g</pre>
    <svg aria-hidden="true" class="octicon octicon-issue-opened" height="16" version="1.1" viewBox="0 0</pre>
    <span itemprop="name">Issues</span>
    <span class="counter">1</span>
    <meta itemprop="position" content="2">
```

```
<a href="/boutroslab/caRpools/wiki" class="js-selected-navigation-item reponav-item" data-hotkey="g w" -
  <svg aria-hidden="true" class="octicon octicon-book" height="16" version="1.1" viewBox="0 0 16 16" wing
</pre>
  Wiki
Pulse Graphs
Permalink
<i>Branch:</i>
<span class="js-select-button css-truncate-target">master</span>
<div class="select-menu-modal">
  <div class="select-menu-header">
    <svg aria-label="Close" class="oction oction-x js-menu-close" height="16" role="img" version="1.1</pre>
    <span class="select-menu-title">Switch branches/tags</span>
  </div>
  <div class="select-menu-filters">
    <div class="select-menu-text-filter">
      <input type="text" aria-label="Filter branches/tags" id="context-commitish-filter-field" class="f</pre>
    </div>
    <div class="select-menu-tabs">
        <a href="#" data-tab-filter="branches" data-filter-placeholder="Filter branches/tags" class="</pre>
       <a href="#" data-tab-filter="tags" data-filter-placeholder="Find a tag..." class="js-select-m
        </div>
  </div>
  <div class="select-menu-list select-menu-tab-bucket js-select-menu-tab-bucket" data-tab-filter="branc</pre>
    <div data-filterable-for="context-commitish-filter-field" data-filterable-type="substring">
        <a class="select-menu-item js-navigation-item js-navigation-open selected"</pre>
          href="/boutroslab/caRpools/blob/master/CaRpools-extended-PDF.Rmd"
          data-name="master"
          data-skip-pjax="true"
          rel="nofollow">
          <svg aria-hidden="true" class="octicon octicon-check select-menu-item-icon" height="16" versi</pre>
          <span class="select-menu-item-text css-truncate-target js-select-menu-filter-text" title="mas</pre>
           master
          </span>
        </a>
    </div>
      <div class="select-menu-no-results">Nothing to show</div>
  </div>
  <div class="select-menu-list select-menu-tab-bucket js-select-menu-tab-bucket" data-tab-filter="tags"</pre>
```

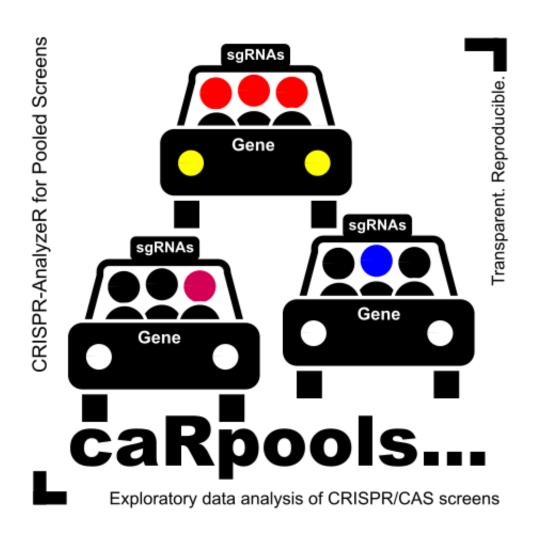
```
<div data-filterable-for="context-commitish-filter-field" data-filterable-type="substring">
        <a class="select-menu-item js-navigation-item js-navigation-open "</pre>
          href="/boutroslab/caRpools/tree/v0.83/CaRpools-extended-PDF.Rmd"
          data-name="v0.83"
          data-skip-pjax="true"
          rel="nofollow">
          <svg aria-hidden="true" class="oction oction-check select-menu-item-icon" height="16" versi</pre>
          <span class="select-menu-item-text css-truncate-target" title="v0.83">
          </span>
        </a>
        <a class="select-menu-item js-navigation-item js-navigation-open "</pre>
          href="/boutroslab/caRpools/tree/1.0241-alpha-1/CaRpools-extended-PDF.Rmd"
          data-name="1.0241-alpha-1"
          data-skip-pjax="true"
          rel="nofollow">
          <svg aria-hidden="true" class="oction oction-check select-menu-item-icon" height="16" versi</pre>
          <span class="select-menu-item-text css-truncate-target" title="1.0241-alpha-1">
            1.0241-alpha-1
          </span>
        </a>
        <a class="select-menu-item js-navigation-item js-navigation-open "</pre>
          href="/boutroslab/caRpools/tree/1.025-alpha-1/CaRpools-extended-PDF.Rmd"
          data-name="1.025-alpha-1"
          data-skip-pjax="true"
          rel="nofollow">
          <svg aria-hidden="true" class="oction oction-check select-menu-item-icon" height="16" versi</pre>
          <span class="select-menu-item-text css-truncate-target" title="1.025-alpha-1">
            1.025-alpha-1
          </span>
        </a>
    </div>
    <div class="select-menu-no-results">Nothing to show</div>
  </div>
</div>
<a href="/boutroslab/caRpools/find/master"</pre>
      class="js-pjax-capture-input btn btn-sm"
      data-pjax
      data-hotkey="t">
  Find file
</a>
<button aria-label="Copy file path to clipboard" class="js-zeroclipboard btn btn-sm zeroclipboard-butto
```


 <a class="commit-tease-sha" href="/boutroslab/caRpools/commit/986a412062197d2354e9419b73a9f6d8e6433'
 986a412</pre>

<span</pre>

```
</a>
   <relative-time datetime="2015-10-14T11:48:17Z">Oct 14, 2015/relative-time>
 </span>
 <div>
    <img alt="@jwinter6" class="avatar" height="20" src="https://avatars3.githubusercontent.com/u/72035</pre>
    <a href="/jwinter6" class="user-mention" rel="contributor">jwinter6</a>
      <a href="/boutroslab/caRpools/commit/986a412062197d2354e9419b73a9f6d8e6433b98" class="message" da
added 4 replicate template updated latest pac page version 0.82.4">updated Rmd files to include latest
changes
<div class="commit-tease-contributors">
  <button type="button" class="btn-link muted-link contributors-toggle" data-facebox="#blob_contributor</pre>
    <strong>1</strong>
    contributor
 </button>
</div>
<div id="blob_contributors_box" style="display:none">
 <h2 class="facebox-header" data-facebox-id="facebox-header">Users who have contributed to this file//
 <img alt="@jwinter6" height="24" src="https://avatars1.githubusercontent.com/u/7203572?v=3&amp;</pre>
       <a href="/jwinter6">jwinter6</a>
      </div>
<div class="btn-group">
 <a href="/boutroslab/caRpools/raw/master/CaRpools-extended-PDF.Rmd" class="btn btn-sm " id="raw-url">
    <a href="/boutroslab/caRpools/blame/master/CaRpools-extended-PDF.Rmd" class="btn btn-sm js-update-u
 <a href="/boutroslab/caRpools/commits/master/CaRpools-extended-PDF.Rmd" class="btn btn-sm" rel="nofo</pre>
</div>
    <a class="btn-oction tooltipped tooltipped-nw"</pre>
      href="github-mac://openRepo/https://github.com/boutroslab/caRpools?branch=master&filepath=Ca
      aria-label="Open this file in GitHub Desktop"
      data-ga-click="Repository, open with desktop, type:mac">
        <svg aria-hidden="true" class="octicon octicon-device-desktop" height="16" version="1.1" viewBo</pre>
   </a>
   <!-- </textarea> --><!-- ""` --><form accept-charset="UTF-8" action="/boutroslab/caRpools/edit/mast
     <button class="btn-oction tooltipped tooltipped-nw" type="submit"</pre>
       aria-label="Fork this project and edit the file" data-hotkey="e" data-disable-with>
       <svg aria-hidden="true" class="octicon octicon-pencil" height="16" version="1.1" viewBox="0 0 1</pre>
      </button>
     <button class="btn-octicon btn-octicon-danger tooltipped tooltipped-nw" type="submit"</pre>
       aria-label="Fork this project and delete the file" data-disable-with>
        <svg aria-hidden="true" class="octicon octicon-trashcan" height="16" version="1.1" viewBox="0 0</pre>
      </button>
```

```
1473 lines (1013 sloc)
 <span class="file-info-divider"></span>
69.9 KB
<article class="markdown-body entry-content" itemprop="text">
title
output
CaRpools Report - CRISPR Screen Analysis
pdf\_document
fig\_height
fig\_width
highlight
keep\_tex
latex\_engine
toc
toc\_depth
6
11
true
xelatex
true
3
```



Screen

Screen

Information

Screen ID

r screen.id

Screening Date

r screen. date

Organism

r screen.organism

Cell Line

r screen.cell

Drug

r screen.drug

Control

r screen.control

Number of Target

r screen.targets

Designs per Gene

r screen.despergene

Library

r screen.library

Library Reference File

 ${\bf r}$ referencefile

NGS

r screen.NGS

MIACCS The MIACCS-file can be found at

r paste
("", $\mathit{datapath},"/",""$, sep="")

r paste
("___","MIACCS.xls" ,"___", sep="")

 $Description\ r\ screen. description$

Load Data

A brief look into the data passed on to the analysis scripts:

- r # make randoms for brief stats table r sample.sgrna = sample(1:nrow(CONTROL1),10)
- r d.CONTROL1 r fileCONTROL1 r knitr::kable(CONTROL1[sample.sgrna,])
- r d.CONTROL2 r fileCONTROL2 r knitr::kable(CONTROL2[sample.sgrna,])
- r d.TREAT1 r fileTREAT1 r knitr::kable(TREAT1[sample.sgrna,])
- r d.TREAT2 r fileTREAT2 r knitr::kable(TREAT2[sample.sgrna,])

Stats

All file-based output (e.g. tables) is stored in: r datapath.

${\rm General}$

General stats can be found in r paste (analysis.name, "STATS.xls", sep="-"). The following read count statistics were calculated for the single datasets.

Missing sgRNAs in Datasets

```
Information of how many sgRNA per gene were not present in the mapped datasets is stored in:
```

```
r paste("", datapath, "/", "", sep="")
```

```
r paste<br/>("___", paste(analysis.name,"DROPOUT.xls", sep="_") ,"___", sep="")
```

In brief, the following number of sgRNAs had a read count of 0 in the dataset:

```
r d.CONTROL1: r sum(U1.unmapped[,2]) missing sgRNAs
```

r d.CONTROL2: r sum(U2.unmapped[,2]) missing sgRNAs

r d.TREAT1: r sum(T1.unmapped[,2]) missing sgRNAs

r d.TREAT2: r sum(T2.unmapped[,2]) missing sgRNAs

All Stats

In depth dataset read count stats can be found in

```
r paste("", datapath,"/","", sep="")
```

r paste("", paste(analysis.name,"STATS.xls", sep="-") ,"", sep="")

Quality Control

Read Distribution

These plots show how the read count of the sgRNAs for each dataset is distributed. Depending on the treatment stringency, e.g. in resistance or dropout screens, the data can show asymmetry. However, the major population should be more or less normally distributed.

Read Depth

The following plot shows the read count for each gene normalized to the number of sgRNAs. Spikes indicate a higher read count per sgRNA for this particular gene. One would expect no outstanding spikes within the untreated data samples, however spikes within the treated datasets indicate a read count enrichment for this particular gene. If a non-targeting control has been set in the MIACCS file, this control is highlighted in orange color.

Designs per Gene

These plots provide an overview of the representation of sgRNAs per gene within your data. Depending on the number of sgRNAs per gene in the library, one would expect a representation of more than 80 % of sgRNAs per gene in the untreated samples. Moreover, genes with a low percentage of present sgRNAs will also show a reduced readcount.

Controls

Non-Targeting

Non-targeting controls are sgRNAs that do either not target the genome at all (so called random or scramble sgRNAs) or target a gene that does not show a phenotype in the screen. Therefore, the scatter for these, which are highlighted in blue, will be distributed within the main cloud of scatter points.

r if(is.null(controls.nontarget)) {print("Unfortunately, no Non-Targeting controls were set.")}

Positive Controls

SgRNAs targeting genes that will show a phenotype in the screening setup can be used as positive controls. These will show either an enrichment (in resistance screens) or a depletion (in dropout screens) in the treatment. Within the scatter

Hit Analysis

Hit analysis is performed using three different methods:

Wilcox

DESeq2

MAGeCK

For each analysis method, separate plots will be created and analysis files will be written to r paste(analysis.name, "HIT-CALLING.xls", sep="__"). See below for further information.

The following adjusted p-values are used to determine significance levels:

Method

p-value

Wilcox

r sig.pval.wilcox

DESeq2

r sig.pval.deseq

MAGeCK

r sig.pval.mageck

Wilcox

Within this approach, the read counts of all sgRNAs in one dataset are first normalized by the function set in the MIACCS file. By default, normalization is done by read count division with the dataset median. Then, the fold change of each population of sgRNAs for a gene is tested against the population of either the non-targeting controls or randomly picked sgRNAs, as defined by the random picks option within the MIACCS file, using a two-sided Mann-Whitney test with FDR correction.

DESeq2

For the DESeq2 analysis implementation, the read counts of all sgRNAs for a given gene are first summed up to increase the available read count. Then, DESeq2 analysis is performed, which includes the estimation of size-factors, the variance stabilization using a parametric fit and a Wald-Test for difference in log2 fold changes between the untreated and treated data. More information about this can be found in Love et al. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2 Genome Biology 2014

MAGeCK

MAGeCK analysis uses a rank-based model to test for a change in abundance of sgRNAs after median normalization of the dataset. Further information can be found at the MAGeCK Homepage.

Wilcox

All analysis data can be found in the r paste(analysis.name, "HIT-CALLING.xls", sep="_") file.

P-Value Distribution

For all genes present in the data, the -log10 corrected p-values are plotted to estimate how well the analysis method performed for this screen. A straight line of points with only small differences in the p-value indicates that the analysis method did not perform well. Genes that resulted in a p-value below the threshold set in the MIACCS file are highlighted in red color.

Enriched

The following genes showed enrichment in the treatment datasets with a p-value smaller than r sig.pval.wilcox:

According to the value set, the top r number.hits.plot.enriched % enriched genes were:

r knitr::kable(data.wilcox.plot.enriched[1:number.enriched.counter,])

Depleted

The following genes showed depletion in the treatment datasets with a p-value smaller than r sig.pval.wilcox: According to the value set, the top r number.hits.plot.depleted % depleted genes were:

DESeq2

All analysis data can be found in the r paste(analysis.name, "HIT-CALLING.xls", sep="_") file.

P-Value Distribution

For all genes present in the data, the -log10 corrected p-values are plotted to estimate how well the analysis method performed for this screen. A straight line of points with only small differences in the p-value indicates that the analysis method did not perform well. Genes that resulted in a p-value below the threshold set in the MIACCS file are highlighted in red color.

Enriched

The following genes showed enrichment in the treatment datasets with a p-value smaller than r sig.pval.deseq:

According to the value set, the top r number.hits.plot.enriched % enriched genes were:

r knitr::kable(data.deseq.plot.enriched[1:number.enriched.counter,c(2,3,6,8)])

Depleted

The following genes showed depletion in the treatment datasets with a p-value smaller than r sig.pval.deseq: According to the value set, the top r number.hits.plot.depleted % depleted genes were:

r knitr::kable(data.deseq.plot.depleted[1:number.depleted.counter,c(2,3,6,8)])

MAGeCK

All analysis data for MAGeCK can be found in the r paste(analysis.name, "HIT-CALLING.xls", sep="_") file

P-Value Distribution

For all genes present in the data, the -log10 corrected p-values are plotted to estimate how well the analysis method performed for this screen. A straight line of points with only small differences in the p-value indicates that the analysis method did not perform well. Genes that resulted in a p-value below the threshold set in the MIACCS file are highlighted in red color.

Enriched

The following genes showed enrichment in the treatment datasets with a p-value smaller than r sig.pval.mageck:

According to the value set, the top r number.hits.plot.enriched % enriched genes were:

r knitr::kable(data.mageck.plot.enriched[1:number.enriched.counter,c(2,3,4,5,6,7)])

Depleted

The following genes showed depletion in the treatment datasets with a p-value smaller than r sig.pval.mageck: According to the value set, the top r number.hits.plot.depleted % depleted genes were:

r knitr::kable(data.mageck.plot.depleted[1:number.depleted.counter,c(2,3,4,5,6,7)])

Hit Candidate Overview

Overview

Genes which showed enrichment or depletion within the individual analysis methods are presented in the following section. All genes that showed significant enrichment within Wilcox, DESeq2 and MAGeCK with the given p-value cutoffs are highlighted in red color. All genes that showed significant depletion within Wilcox, DESeq2 and MAGeCK with the given p-value cutoffs are highlighted in blue color.

Moreover, all genes that showed up as significantly enriched or depleted in any of the analysis methods are highlighted in orange color. Genes that showed no significant effect are presented in grey or black color.

Overlaps in Enrichment Analysis

Within the top enriched hits, the overlap of enriched hits per analysis method is displayed as follows:

Overlaps in Depletion Analysis

Within the top depleted hits, the overlap of depleted hits per analysis method is displayed as follows:

Enriched Overlapping Hit Candidates

All overlapping genes which showed enrichment are highlighted in orange color in the following plots.

Depleted Overlapping Hit Candidates

All overlapping genes which showed depletion are highlighted in orange color in the following plots.

Hit Candidates

Scatterplots representing the gene read count as well as sgRNA read count for all overlapping hits are plotted in this section.

If there were significantly enriched or depleted genes that overlapped in all analysis methods, they will be presented here. Therefore, the top overlapping hits of each Hit Analysis with a p-value below the thresholds for each analysis method are highlighted in r plot.labelcolor within the scatter plots for all four samples.

In the case that no significantly enriched or depleted genes did overlap in all methods, those that overlapped within the r number.hits.plot.enriched % of top enriched and the top r number.hits.plot.depleted % depleted genes are used. Therefore, the top overlapping hits of each Hit Analysis are highlighted in r plot.labelcolor within the scatter plots for all four samples.

This allows a fast and easy view for single genes and its individual sgRNAs. Moreover, individual sgRNA effects are plotted as well as the corresponding target sequence.

In this section, the following plots are generated for each hit candidate:

Scatterplot with gene read count within all datasets

Scatterplot with sgRNA read count within all datasets

sgRNA log2 fold changes

sgRNA log2 fold change distribution in comparison to all sgRNAs or given controls

sgRNA target sequence list

The scatter plots show the median normalized, log read count of each genes/sgRNA. Moreover, the blue lines indicate a read count foldchange of 2, the green lines indicate a read count foldchange of 4.

Enriched

Compare Analysis

On the following pages, a comparison between the hit analysis methods is generated.

Enriched

List

Moreover, the r number.hits.plot.enriched % top enriched hits sorted according to MAGeCK are stored in r paste(analysis.name, "-COMPARE-HITS.xls", sep="") and are listed below:

3D Scatterplot This plot shows you the p-value of overlapping enriched genes of all three methods. The best hits will show up in the upper right corner.

Depleted

 List

Moreover, the r number.hits.plot.depleted % top depleted hits sorted according to MAGeCK_ are stored in __r paste(analysis.name, "-COMPARE-HITS.xls", sep="") and are listed below:

3D Scatterplot

This plot shows you the p-value of overlapping depleted genes of all three methods. The best hits will show up in the upper right corner.

Visualize Analysis

The following pages present a visual representation of the p-value for single genes within all analysis methods. Significantly enriched genes, which had a corrected p-value below the threshold set for each method, are highlighted in red. Significantly depleted genes, which had a corrected p-value below the threshold set for each method, are highlighted in blue.

Moreover, the number of significant sgRNAs is presented for MAGeCK as well.

Wilcox

 ${\rm DESeq2}$

MAGeCK

Final Gene Table

A final table with all information for each gene is stored in r paste(datapath, paste(analysis.name, "FINAL.xls", sep="_"), sep="/").

Annotate Hit Candidates Data Extraction, Mapping and Files Dataset .fastq file name Description Control #1 r fileCONTROL1 r d.CONTROL1 Control #2 r fileCONTROL2r d.CONTROL2 Treatment #1r fileTREAT1 r d.TREAT1 Treatment #2r fileTREAT2 r d.TREAT2The data is located in r datapath and the script files for data extraction and mapping are located in r scriptpath. All file-based output (e.g. tables) from MAGeCK is stored in: r getwd(). Parameter Value Reverse Complement Sequence r reversecomplement Pattern of Data Extraction r seq.pattern Maschine Identifier FASTq r maschine.pattern Create Bowtie2 Index? r createindex

r referencefile

Reference .fasta File

 $\mathrm{Bt}2\ \mathrm{Threads}$

r threads

Bt2 Sensitivity

 ${\bf r}$ sensitivity

```
sgRNA Oligo Match
r match
Jump to Line
<input class="form-control linejump-input js-jump-to-line-field" type="text" placeholder="Jump to line&</pre>
<button type="submit" class="btn">Go</button>
</div>
</div>
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<a href="https://status.github.com/" data-ga-click="Footer, go to status, text:status">Status/
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 <a href="https://shop.github.com" data-ga-click="Footer, go to shop, text:shop">Shop</a>
   <a href="https://github.com/blog" data-ga-click="Footer, go to blog, text:blog">Blog</a>
   <a href="https://github.com/about" data-ga-click="Footer, go to about, text:about">About</a></l</pre>
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 © 2016 <span title="0.08820s from github-fe151-cp1-prd.iad.github.net">GitHub</span>, Inc.
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