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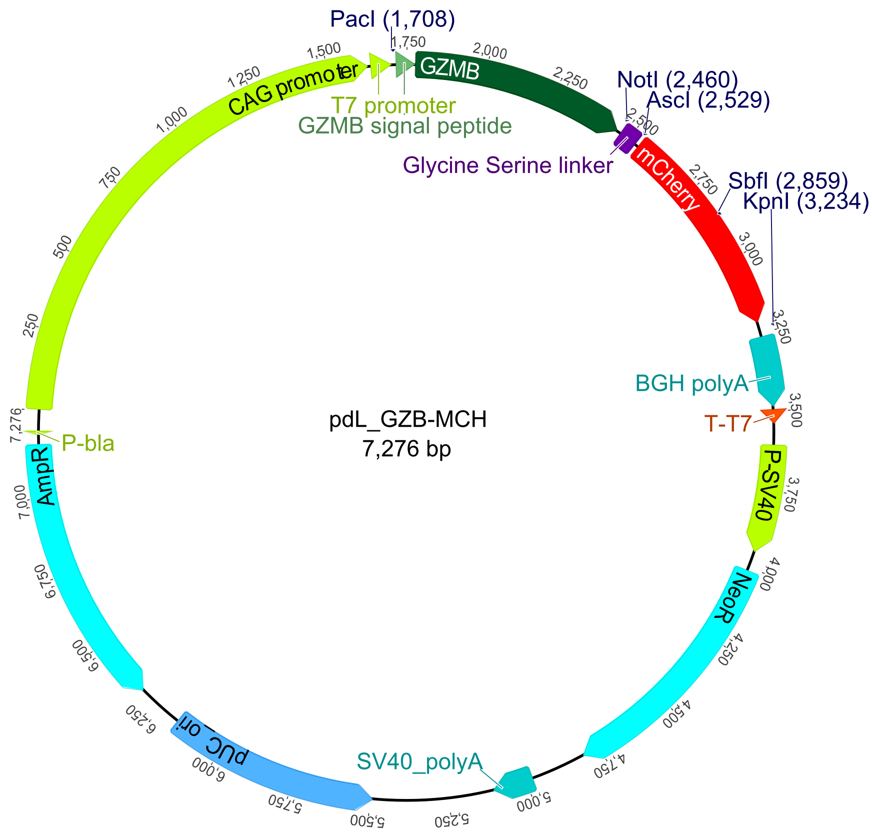
Supplemental Information

Targeted Cell-to-Cell Delivery of Protein Payloads via the Granzyme-Perforin Pathway

Daniel J. Woodsworth, Lisa Dreolini, Libin Abraham, and Robert A. Holt

1 Plasmids

1.1 Base pdL plasmid



Supplementary Figure 1: Base pdL vector. In this example the insert coding sequence is the granzyme B-crmCherry fusion protein (GZB-MCH). The annotations correspond to those in the genbank file.

1.2 Base pdL plasmid sequence

```
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DEFINITION  .
ACCESSION   .
VERSION     .
KEYWORDS    .
SOURCE
  ORGANISM
            .
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     misc_feature    complement(6307..7167)
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     promoter        complement(7202..7208)
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1.3 Coding sequence inserts

Coding sequences listed below extend from the PacI site to KpnI site of the pdL vector.

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ACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCC
CGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGA
TCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTG
CAGCTGCCCGGCGCCTACAACGTCAACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTA
CGAACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGGAATTCTAATAGCTCGAGGGTACC
```

2 Image filtration and colocalization analysis: MATLAB source code

```
%-------------------------------------------------%
%----Name: LAGfilter_batch.m
%----Author: Daniel Woodsworth
%----Date: March 3, 2017
%
%LAG = local and global filter
%
%Basic idea is to account for local variations in background signal, but
%also to achieve robust filtering of noise (so local and global filtering)
%
%To do this, first calculate local background for each pixel, subtract this
%background from original image.
%
```

```
%Then calculate global median absolute devation. Use this as estimate of
%variance of pixel noise, and define threshold as some multiple of this.
%Define all pixels below this threshold as noise, and set to zero.

%Local pixel intensity idea comes from Dunn et al 2011
%Median absolute deviation idea comes from Josh Scurl

clear all
close all
clc

%#####PARAM SET HERE######
%Set paths here for input and output directories
inPath = '/Volumes/DAN/coloc/RAW/';
outPath = '/Volumes/DAN/coloc/FILT/';

%#####PARAM SET HERE######
%length on either side of current pixel that to make box for median
%calculation
L = 12;

%#####PARAM SET HERE######
%Number of standard deviations above MAD to consider as above backcground
%These are empirical
C0_threshold = 3;
C1_threshold = 6;

%#####PARAM SET HERE######
%Regex pattern for extracting unique identifier of sample image (e.g.
%within given sample, whatever IDs the actual image files)
CaptureNumberPattern = '^Capture (\d+)_';


%Get directories in basepath
tmp = dir(inPath);
dirs = {tmp.name};
dirs([1,2]) = []; %Delete . and ..

for dirindex = 1:length(dirs)

    %Assume each directory name is sample name
    SampleName = dirs{dirindex};
    path = fullfile(inPath,dirs{dirindex});
    writePath = fullfile(outPath,dirs{dirindex});


    %Get all image file names, assuming tiff extension
    im_files = dir(fullfile(path,'*.tiff'));
    fnames = {im_files.name};

    %Get all unique captures. Each capture has separate tiff for each
    %channel. Assume format of Name_Cx.tiff, where x = 0,1 is channel id.
    %Strip channel id to get actual name, then strip duplicates.
    [temp, basenames, extensions] = cellfun(@fileparts,fnames, 'UniformOutput', false);
    ImageNames = cellfun(@(x) x(1:end-3), basenames, 'UniformOutput', false);
    ImageNames = unique(ImageNames);
```

```
mkdir(writePath);

SampleName

for i = 1:length(ImageNames)

    %Get unique identifier of image file name
    [tokens,matches] = regexp(ImageNames{i},CaptureNumberPattern,'tokens','match');
    CaptureNumber = cell2mat(tokens{1});

    CaptureNumber

    C0_img_name = fullfile(path,strcat(ImageNames{i},'_C0',extensions{1}));
    C1_img_name = fullfile(path,strcat(ImageNames{i},'_C1',extensions{1}));

    %% Read image
    imf = imfinfo(C0_img_name);
    C0_img = zeros(imf(1).Height, imf(1).Width, 'single');
    C0_img(:,:) = imread(C0_img_name);

    imf = imfinfo(C1_img_name);
    C1_img = zeros(imf(1).Height, imf(1).Width, 'single');
    C1_img(:,:) = imread(C1_img_name);

    %----C0 = green channel = lytic granule filter-----%
    %Useful for punctuate type objects

    %min and max pixels to consider, to account for edge cases
    min_pix = L+1;
    max_pix = size(C0_img,1)-L;



    %Initialize background matrix to maximum pixel intensity
    bg = repmat(max(C0_img(:)),size(C0_img,1),size(C0_img,1));

    %Calculate localized background pixel intensity for whole image
    for k = min_pix:max_pix
        for j = min_pix:max_pix

            sub = C0_img((k-L):(k+L),(j-L):(j+L));

            bg(k,j) = median(sub(:));

        end
    end

    %Subtract local background from image
    C0_im_bgfilt = C0_img-bg;

    %Set any negative pixels to 0
    C0_im_bgfilt(C0_im_bgfilt < 0) = 0;


    %Now subtract MAD from image to filter pixel noise
    %See below for more information
    %Since so many pixels are 0, use all nonzero pixels to calculate MAD
```

```
        %I don't know why using the original image works better, but it cleans up
        %pixel noise better (essentially a higher threshold)

        C0_pos = C0_img(C0_img > 0);
        mad_C0 = median(abs(C0_pos(:) - median(C0_pos(:))));
        noise_std_C0 = 1.4826 * mad_C0; %See below for explanation

        %Noise filter. Set all pixels below threshold to 0
        C0_mad_sub = C0_im_bgfilt;

        C0_mad_sub(C0_mad_sub < noise_std_C0 * C0_threshold) = 0;



        %C1 = RED = mCherry channel
        %-----Good for homogenous structures-----%
        %
        %Just do MAD threshold, but with 6sigma


        C1_pos = C1_img(C1_img > 0);
        mad_C1 = median(abs(C1_pos(:) - median(C1_pos(:))));
        noise_std_C1 = 1.4826 * mad_C1; %See below for explanation

        %Noise filter. Set all pixels below 6 sigma threshold to 0
        C1_mad_sub = C1_img;

        C1_mad_sub(C1_mad_sub < noise_std_C1 * C1_threshold) = 0;



        %Write images
        C0_OUT = fullfile(writePath,strcat(ImageNames{i},'_filt_C0',extensions{1}));
        C1_OUT = fullfile(writePath,strcat(ImageNames{i},'_filt_C1',extensions{1}));

        imwrite(uint16(C0_mad_sub),C0_OUT);
        imwrite(uint16(C1_mad_sub),C1_OUT);



    end

end

%------------Background on MAD derivation etc--------%

% Convert the median absolute deviation to something more resembling a
% standard deviation.  This is used instead of calculating the standard
% deviation directly because the median absolute deviation ignores outliers
% (and actual spots), giving a result after normalisation closer to the
% standard deviation of noise alone, with other image features ignored.
% (See http://en.wikipedia.org/wiki/Median_absolute_deviation#Relation_to_standard_deviation
% for the conversion factor)

%calculate the absolute median devation
%This is mad = median(abs(Intensity_i-median(Intensity)))
```

```
%In other words: calculate median intensity of images using all pixels.
%Subtract this from each pixel.
%Take absolute value of these values
%Calculate median of these values
%
%Sigma ~1.4mad (see wikipedia article on mad)
%To be very sure that retain only signal, take all pixels that have
%intensity that is 6*sigma above 1.4*mad
```