Supplementary Information

Construction of a genetic multiplexer to toggle between chemosensory pathways in *Escherichia coli*

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1. Chemotaxis Assay Protocol

This assay was developed by Goulian and co-workers (Derr *et al*, 2006). Here, we present an easy-to-follow protocol with minor modifications. We provide the manufacturer and model name for all equipment, although it may not be critical.

Equipment and materials

■ 37°C incubator shaker (Innova® 44, New Brunswick Scientific, Edison, NJ)
■ 30°C and 37°C static incubator (Isotemp Incubator, Fisher Scientific, Pittsburgh, PA)
■ 4°C refrigerator
■ square integrid Petri dishes (90 mm x 90 mm, PETRIDISH SQ ST CS300, BD Biosciences, San Jose, CA)
■ AlphaImager® (Cell Biosciences, Santa Clara, CA) or other equipment with camera
■ spectrophotometer (50 Bio, Varian, Palo Alto, CA)
■ culture tubes (Falcon® 14 mL Polypropylene Round-Bottom Tube, BD Biosciences, San Jose, CA)
■ centrifuge (Allegra™ X-12R Centrifuge, Beckman Coulter, Brea, CA)

■ 34 mg/mL chloramphenicol (in ethanol, Acros Organics, Morris Plains, NJ)
■ 10 mM L-aspartate (sterilized by filtration, Sigma Aldrich, St. Louis, MO)
■ 10 mM L-serine (sterilized by filtration, Sigma Aldrich, St. Louis, MO)
■ 1 M arabinose (sterilized by filtration, Sigma Aldrich, St. Louis, MO)
■ 20% (w/v) glucose (sterilized by filtration, Fisher Scientific, Pittsburgh, PA)
■ LB (Miller, BD Biosciences, San Jose, CA)
■ agar (Bacto Agar, BD Biosciences, San Jose, CA)
■ LB/Cm agar plates (LB + 34 μg/mL chloramphenicol)

■ 5x minA salts (per 1L, pH7.4, autoclaved)
  5g $(NH_4)_2SO_4$ (Fisher Scientific, Pittsburgh, PA)
  22.5g KH_2PO_4 (Fisher Scientific, Pittsburgh, PA)
  52.5g K_2HPO_4 (Fisher Scientific, Pittsburgh, PA)
  2.5g sodium citrate•$2H_2O$ (Fisher Scientific, Pittsburgh, PA)

■ 1 M $MgSO_4$ (sterilized by filtration, Fisher Scientific, Pittsburgh, PA)
■ 0.1 M $CaCl_2$ (sterilized by filtration, Fisher Scientific, Pittsburgh, PA)
■ 1M $ZnSO_4$ (sterilized by filtration, Fisher Scientific, Pittsburgh, PA)
■ 10% (v/v) glycerol (sterilized by filtration, Fisher Scientific, Pittsburgh, PA)
■ 10 mg/mL methionine (pH7, sterilized by filtration, Sigma Aldrich, St. Louis, MO)
■ 10 mg/mL histidine (pH7, sterilized by filtration, Acros Organics, Morris Plains, NJ)
■ 10 mg/mL leucine (pH7, sterilized by filtration, Acros Organics, Morris Plains, NJ)
■ 10 mg/mL threonine (pH7, sterilized by filtration, Acros Organics, Morris Plains, NJ)

■ minAaa medium (per 500 mL)
  Mix the following:
  86.9 mL 5x minA salts
  100 mL 10 mg/mL methionine (pH7)
  100 mL 10 mg/mL histidine (pH7)

100 mL 10 mg/mL leucine (pH7)
100 mL 10 mg/mL threonine (pH7)

Add while stirring:

0.5 mL 1 M $MgSO_4$
2.5 mL 0.1 M $CaCl_2$
0.1 mL 1 M $ZnSO_4$
10 mL 10% (v/v) glycerol

■ minAaa semisolid agar medium (per 500 mL)

Mix and autoclave the following:

86.9 mL 5x minA salts
1.5g agar

Add while stirring:

100 mL 10 mg/mL methionine (pH7)
100 mL 10 mg/mL histidine (pH7)
100 mL 10 mg/mL leucine (pH7)
100 mL 10 mg/mL threonine (pH7)
0.5 mL 1 M $MgSO_4$
2.5 mL 0.1 M $CaCl_2$
0.1 mL 1 M $ZnSO_4$
10 mL 10% (v/v) glycerol
0.5 mL 34 mg/mL chloramphenicol

Pour the mixture into square integrid Petri dishes before it solidifies, and store them in a refrigerator.

Materials that should be freshly prepared

■ Media for step 2

1.95 mL of minAaa media
2 μL of 34 mg/mL chloramphenicol
50 μL of 20% (w/v) glucose

■ Media for step 4

tube A
8 mL of minAaa media
8 μL of 34 mg/mL chloramphenicol
tube B
7.8 mL of minAaa media
8 μL of 34 mg/mL chloramphenicol
200 μL of 20% (w/v) glucose

■ Washing solution for step 8 and 9

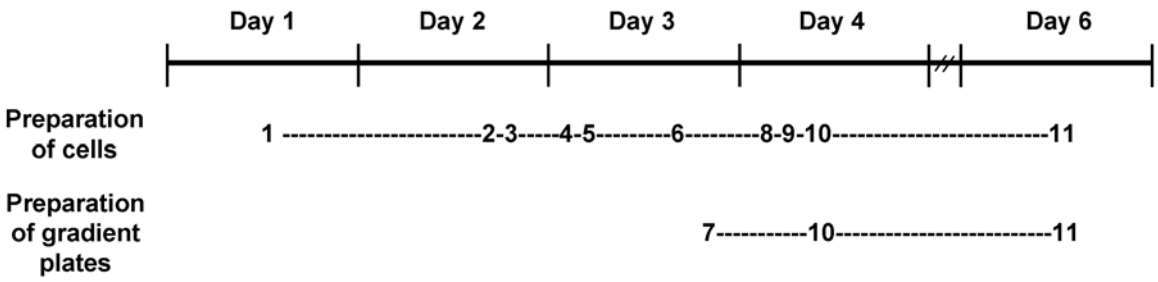
5 mL of minAaa media
5 μL of 34 mg/mL chloramphenicol

■ Preparation of culture with OD_{600} = 0.2 (the measured OD_{600} = X) for step 9

2 – 0.4/X mL of minAaa media
2 – 0.4/X μL of 34 mg/mL chloramphenicol
0.4/X mL of the resuspended cells

Procedure

Timeline (The numbers indicate those in the procedure below.)



Day 1

1. Transform the knockout strain CAV8 (BW28357 with the eight chromosomal genes deleted) with pChemoK and incubate on LB/Cm agar plates overnight.

Day 2

2. Inoculate a freshly transformed colony from the plate in step 1 into a Falcon® 14 mL tube containing 2 mL minAaa media supplemented with 34 μg/mL chloramphenicol and 0.5% (w/v) glucose.

3. Grow at 37°C and 250 rpm to OD_{600} = 3.

Day 3

4. Transfer 80 μL of the culture from step 3 into two different fresh media:
   tube A = 8 mL minAaa media supplemented with 34 μg/mL chloramphenicol
   tube B = 8 mL minAaa media supplemented with 34 μg/mL chloramphenicol and 0.5% (w/v) glucose

5. Grow at 37°C and 250 rpm to OD_{600} = 0.2

6. Induce (tube A only) using 5 mM arabinose (add 40 μL of the 1M arabinose stock) and grow the culture at 37°C and 250 rpm for 16 hr

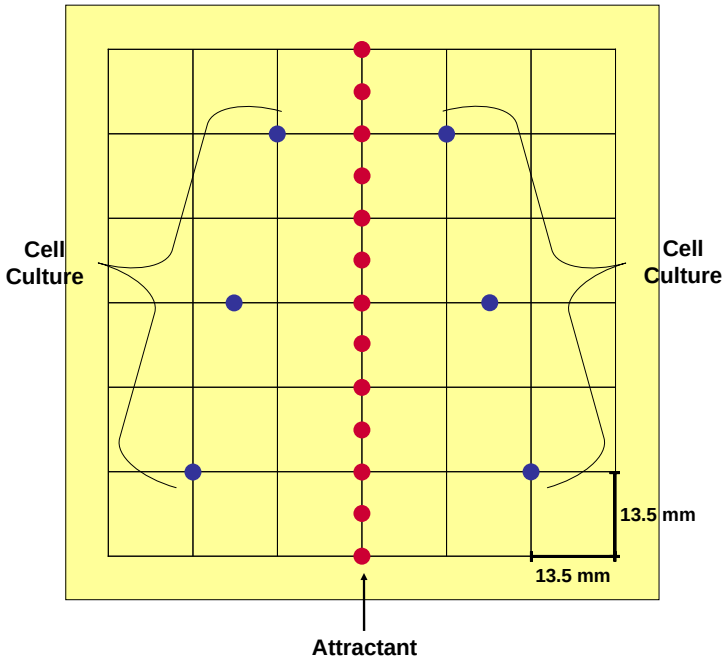
7. Place 10 μL of 10 mM attractant (either aspartate or serine) on minAaa semisolid agar every 7 mm down the middle of the plate (total 13 spots as shown below) and then allow attractant to dry at 4°C for 16 hr.

Day 4

8. After 16 hr culture, centrifuge the culture tubes at 3000xg and 25°C for 7 min, decant supernatant, and resuspend cells using 5 mL minAaa midia supplemented with 34 μg/mL chloramphenicol (washing solution).

9. Centrifuge the tubes at 3000xg and 25°C for 5 min and decant supernatant. Resuspend cells again using 5 mL minAaa midia supplemented with 34 μg/mL chloramphenicol (washing solution), and measure OD_{600} of the resuspended cells. Dilute cells to an OD_{600} of 0.2 and grow in minAaa media supplemented with 34 μg/mL chloramphenicol for additional 1 hr.

10. Place 10μL of culture 13.5, 20, and 27 mm from the center line of attractant (as shown below) and incubate at 30°C.



Day 6

11. Take images after 48 hr incubation at 30°C.

■ Camera (AlphaImager®) setting:
Distance between lens and plate = 50 cm
Position and zoom adjustment = to put the spot position (the blue dot in the figure above) to the center of each image and to cover 27x27 mm^2
Exposure time = 0.06-0.12 sec

References

Derr P, Boder E, Goulian M (2006) Changing the specificity of a bacterial chemoreceptor. *Journal of molecular biology* **355:** 923-932.

2. Supplementary Figures

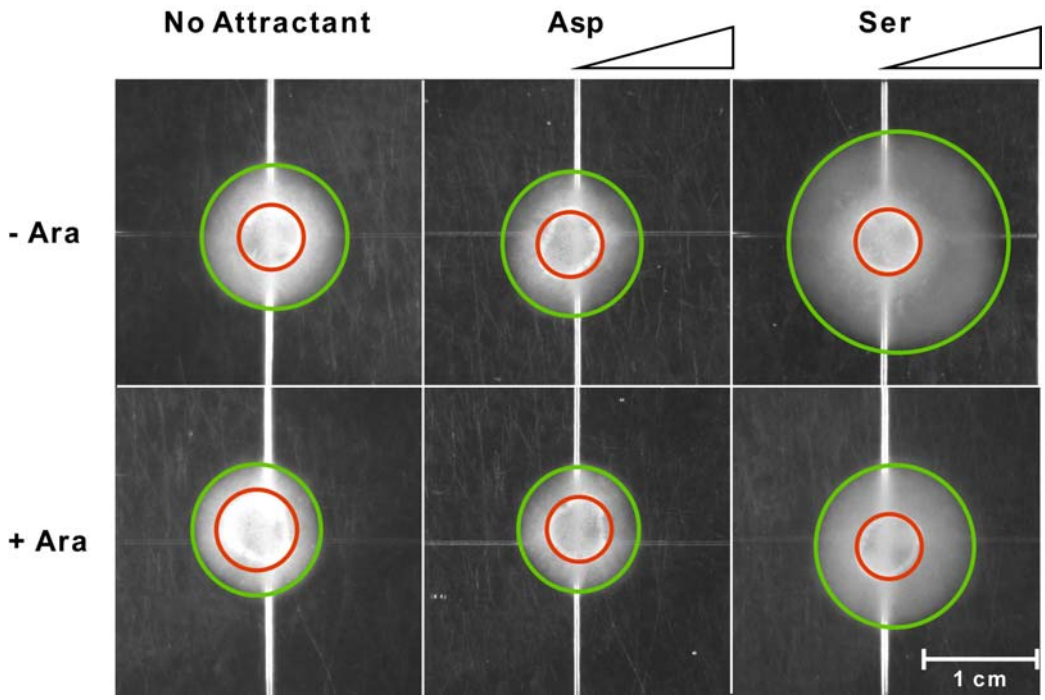


Figure S1A. Chemotaxis assay using the *cheW* deletion mutant. Images shown are representative of experiments that were performed at least three times (see Figure 4A).

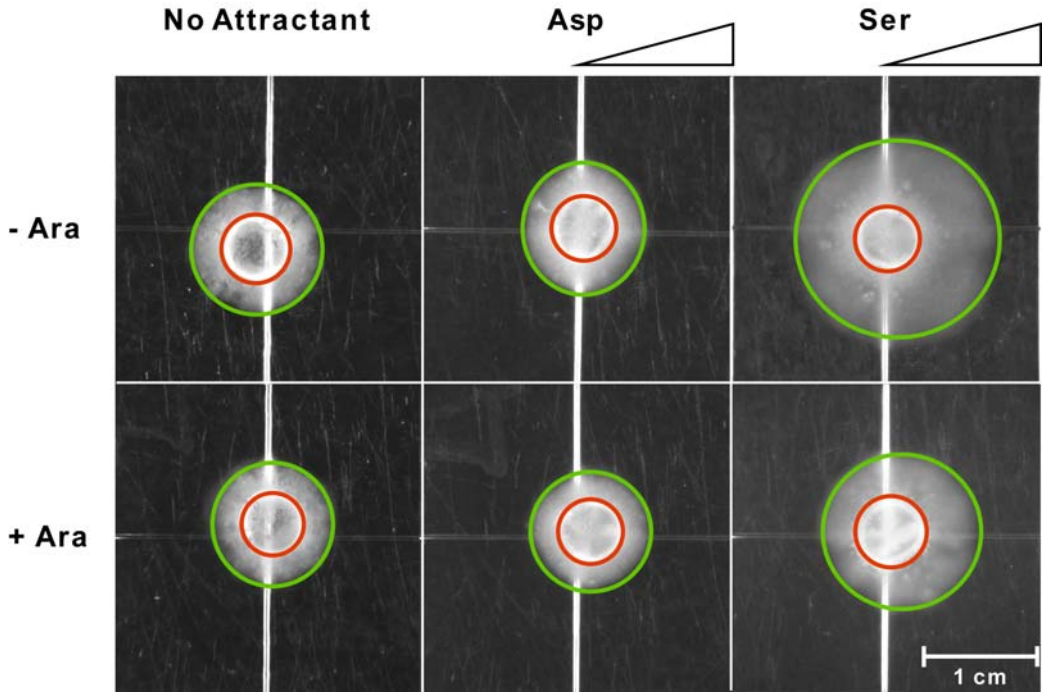


Figure S1B. Chemotaxis assay using the *tar* deletion mutant. Images shown are representative of experiments that were performed at least three times (see Figure 4B).

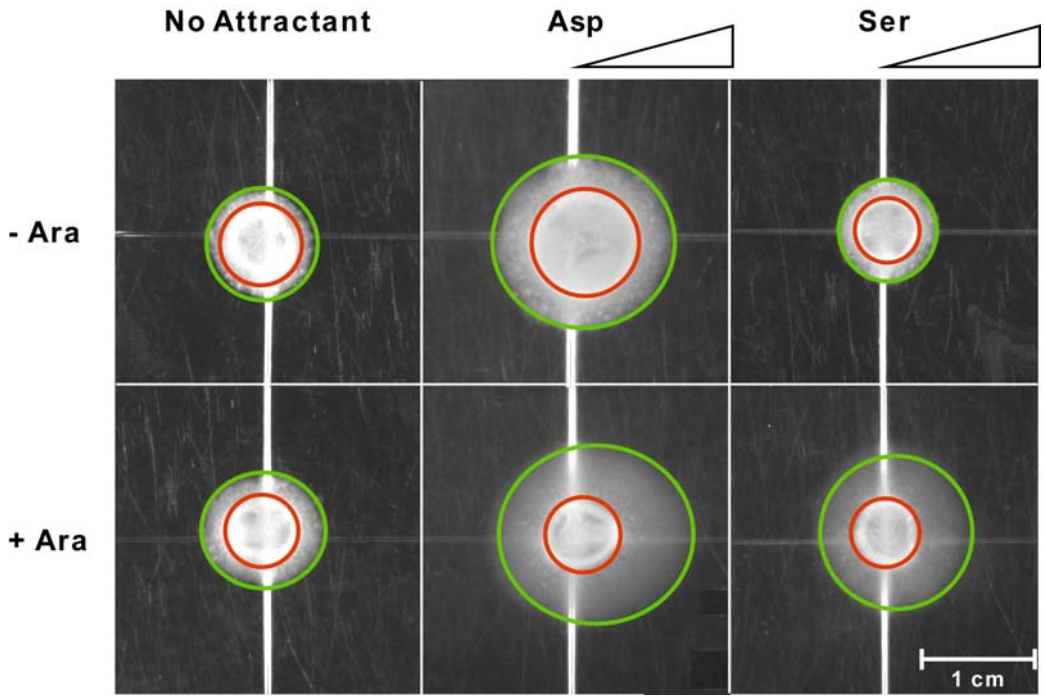


Figure S1C. Chemotaxis assay using the *cheW** deletion mutant. Images shown are representative of experiments that were performed at least three times (see Figure 4C).

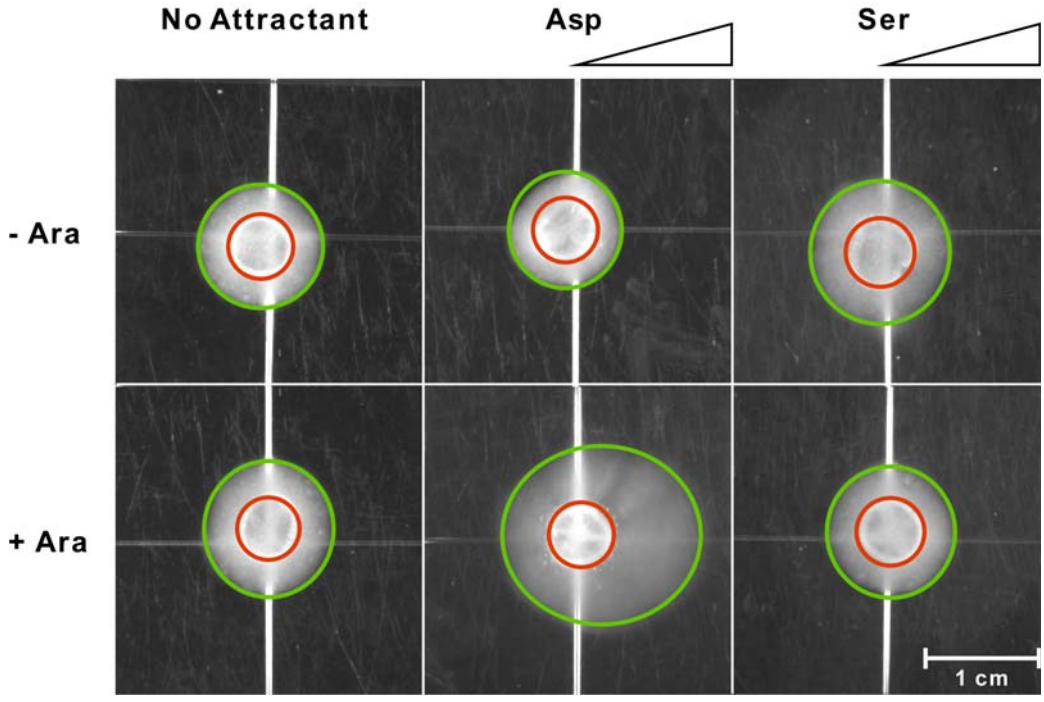


Figure S1D. Chemotaxis assay using the *tsr** deletion mutant. Images shown are representative of experiments that were performed at least three times (see Figure 4D).

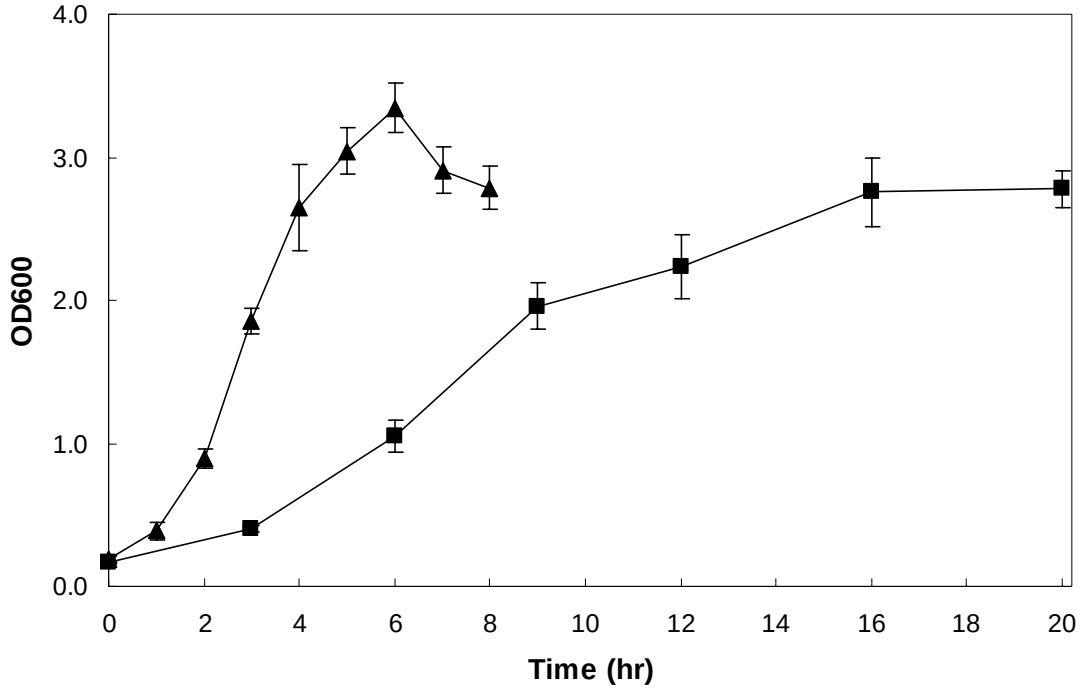


Figure S2. Growth curves for data presented in Figure 2C (▲ = LB, 5mM Ara) and 2F (■ = minAaa, 5mM Ara).

3. Image Analysis Algorithm (MATLAB Code)

```
clear all;
hold off;
imagefile = 'File_Name.png';    %------Image file type should be png.
im = imread(imagefile);
%-------Image Processing Inner 0.7-------------------------------------
%im = rgb2gray(im);
im = im2bw(im,0.7);     %------Change this parameter (by ±0.01) until the algorithm fits the coordinates.
im = imfill(im,'holes');
RE = strel('disk',25);
im = imerode(im,RE);
im = imdilate(im,RE);
imshow(imagefile); hold on;
%----------Calculate Perimeter-----------
found = 0;
for i = 1:size(im,1)
   for j = 1:size(im,2)
      if im(i,j) == 1 && found == 0
         initRow = i;
```

```
            initCol = j;
            found = 1;
        end
    end
end
contour = bwtraceboundary(im, [initRow,initCol], 'N', 8, 2000);
x = contour(:,2);
y = contour(:,1);
x = x(1:end-1);
y = y(1:end-1);
clear i j perim contour;
%-----------Ellipse Fitting---------------------
s = size(x);
xcen = sum(x) / size(x,1);
ycen = sum(y) / size(y,1);
t = zeros(size(x,1),1);
for b = 1:size(x,1)
    if (y(b)-ycen) > 0 && (x(b)-xcen) > 0
        t(b) = atan((y(b)-ycen)./(x(b)-xcen));
    elseif (y(b)-ycen) > 0 && (x(b)-xcen) <= 0
        t(b) = pi + atan((y(b)-ycen)./(x(b)-xcen));
    elseif (y(b)-ycen) <= 0 && (x(b)-xcen) <= 0
        t(b) = pi + atan((y(b)-ycen)./(x(b)-xcen));
    elseif (y(b)-ycen) <= 0 && (x(b)-xcen) > 0
        t(b) = (2*pi) + atan((y(b)-ycen)./(x(b)-xcen));
    end
end
W=[ones(s) zeros(s) cos(t);
   zeros(s) ones(s) sin(t) ] ;
af = W \ [x;y];
hold on
xnew = af(1) + af(3)*cos(t);
ynew = af(2) + af(3)*sin(t);
scatter(xnew, ynew ,'.r')
clear RE W b s t x y xcen xnew ycen ynew im found initRow initCol
%----------------------Outer Circle----------------------------------------
%--------------------------------------------------------------------------
im = imread(imagefile);
%-------Image Processing outer 0.25---------------------------------------
%im = rgb2gray(im);
im = im2bw(im,0.25);      %---- Change this parameter (by ±0.01) until the algorithm fits the coordinates.
im = imfill(im,'holes');
RE = strel('disk',25);
im = imerode(im,RE);
im = imdilate(im,RE);
%----------Calculate Perimeter-----------
found = 0;
for i = 1:size(im,1)
    for j = 1:size(im,2)
        if im(i,j) == 1 && found == 0
            initRow = i;
            initCol = j;
            found = 1;
        end
    end
end
```

```
contour = bwtraceboundary(im, [initRow,initCol], 'N', 8, 2000);
x = contour(:,2);
y = contour(:,1);
x = x(1:end-1);
y = y(1:end-1);
clear i j perim contour;
%----------Ellipse Fitting---------------------
s = size(x);
xcen = sum(x) / size(x,1);
ycen = sum(y) / size(y,1);
t = zeros(size(x,1),1);
for b = 1:size(x,1)
   if (y(b)-ycen) > 0 && (x(b)-xcen) > 0
      t(b) = atan((y(b)-ycen)./(x(b)-xcen));
   elseif (y(b)-ycen) > 0 && (x(b)-xcen) <= 0
      t(b) = pi + atan((y(b)-ycen)./(x(b)-xcen));
   elseif (y(b)-ycen) <= 0 && (x(b)-xcen) <= 0
      t(b) = pi + atan((y(b)-ycen)./(x(b)-xcen));
   elseif (y(b)-ycen) <= 0 && (x(b)-xcen) > 0
      t(b) = (2*pi) + atan((y(b)-ycen)./(x(b)-xcen));
   end
end
W=[ones(s) cos(t) zeros(s)  ;
   zeros(s) zeros(s) sin(t) ] ;
y = y - af(2);
bf = W \ [x;y];
xnew = bf(1) + bf(2)*cos(t);
ynew = af(2) + bf(3)*sin(t);
scatter(xnew, ynew ,'.b')
hold off;
A = bf(2) - (bf(1) - af(1));
B = bf(2);
C = bf(3);
J = min([(A^2 / (B*C)) ((2*B - A)^2 / (B*C))]);
K = min([(A / (2*B - A)) ((2*B - A) / A)]);
clear RE W b imagefile s t x y xcen xnew ycen ynew im found initRow initCol
%--------Important Variables-------
% af(1) is inner circle's X center
% af(2) is inner circle's Y center
% af(3) is inner circle's radius
% bf(1) is outer ellipse's X center
% bf(2) is outer ellipse's X radius
% bf(3) is outer ellipse's Y radius
% Outer Ellipse's Y center is set to be the same as the Inner Circle's Y center
%----------------------------------------
```

4. Sequence Alignment

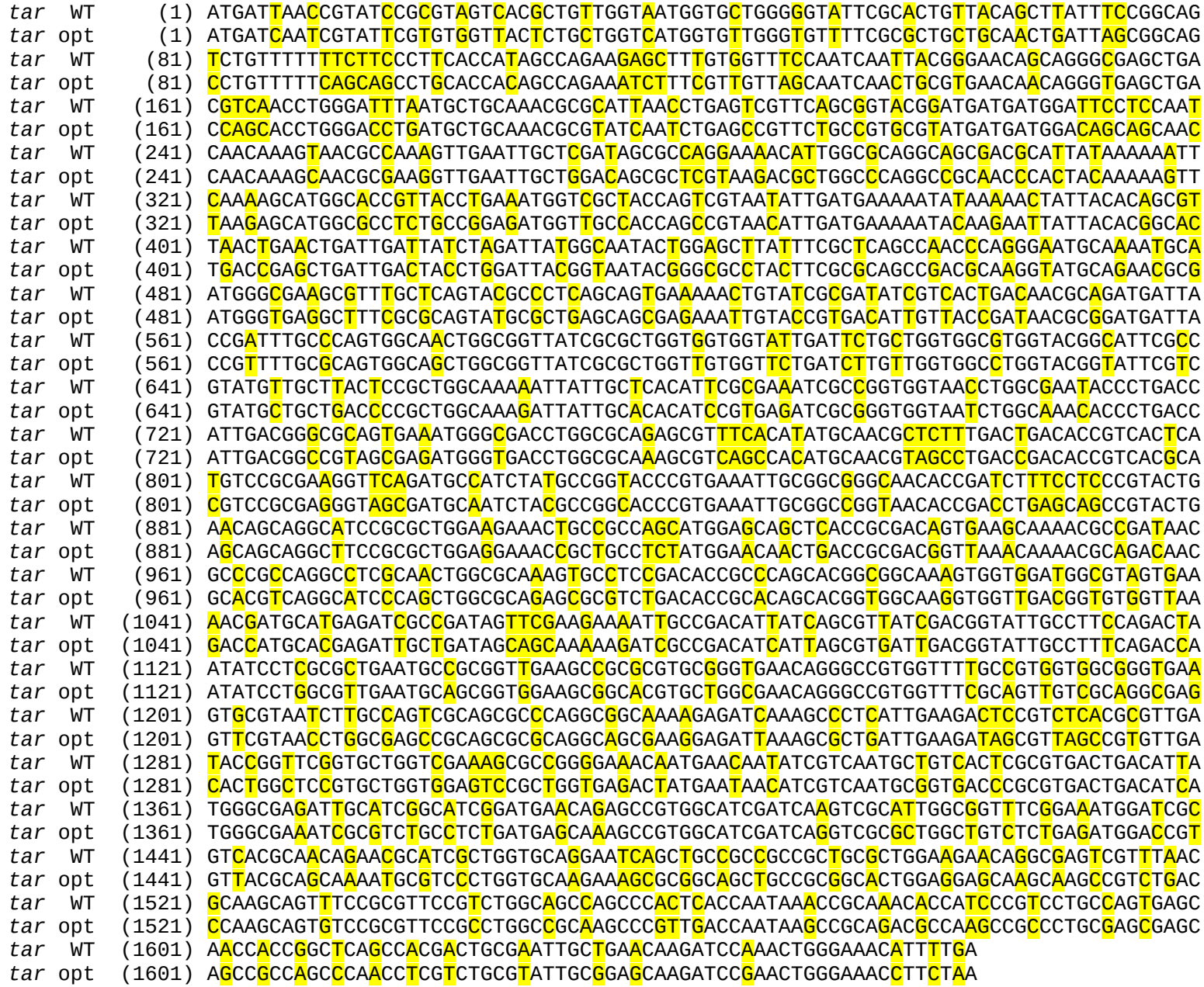


Figure S2A. Sequences of wild type *tar* (WT) and codon-optimized *tar* (opt). The codon-optimized gene was synthesized and used in this work.

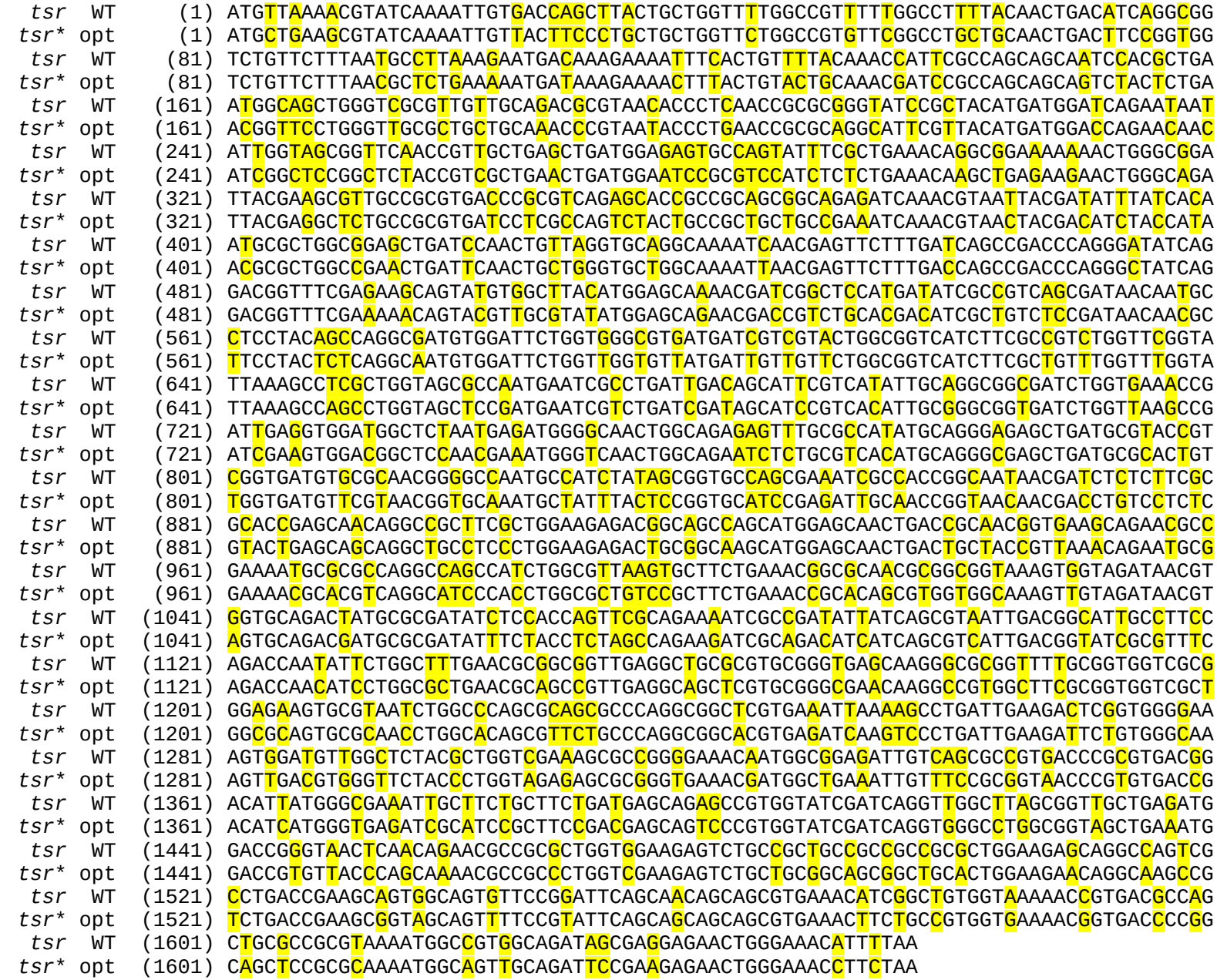


Figure S2B. Sequences of wild type *tsr* (WT) and codon-optimized *tsr** (opt). The codon-optimized gene was synthesized and used in this work.

```
 cheW  WT    (1) ATGACCGGTATGACGAATGTAACAAAGCTGGCCAGCGAGCCGTCAGGCCAGGAATTTCTGGTATTTACCCTTGGTGATGA
 cheW opt    (1) ATGACTGGCATGACGAATGTTACGAAATTGGCAAGCGAACCATCTGGTCAGGAATTTCTGGTTTTCACGTTGGGCGACGA
 cheW  WT   (81) AGAGTACGGTATTGATATCCTGAAAGTGCAGGAGATCCGTGGCTACGATCAGGTAACACGGATTGCGAACACGCCAGCGT
 cheW opt   (81) GGAATACGGTATCGACATCCTGAAAGTTCAGGAAATCCGTGGTTATGACCAGGTTACGCGCATTGCGAATACGCCGGCTT
 cheW  WT  (161) TTATCAAAGGCGTCACGAATCTGCGCGGCGTTATTGTGCCGATTGTTGACTTACGAATTAAGTTCAGCCAGGTGGATGTG
 cheW opt  (161) TTATCAAGGGTGTTACCAATCTGCGCGGCGTCATTGTGCCGATTGTTGACTTGCGCATTAAGTTTCTCAAGTTGACGTG
 cheW  WT  (241) GACTATAACGACAACACGGTAGTTATCGTCCTGAATCTCGGACAGCGGGTGGTCGGCATCGTGGTTGACGGCGTCTCAGA
 cheW opt  (241) GACTATAACGACAATACGGTCGTTATTGTCCTGAATTTGGGCCAGCGCGTGGTTGGCATCGTTGTTGATGGTGTCAGCGA
 cheW  WT  (321) CGTGCTTTCATTGACGGCGGAGCAAATTCGTCCGGCACCGGAATTTGCCGTGACGCTTTCAACAGAATATCTCACTGGAC
 cheW opt  (321) CGTGCTGAGCCTGACCGCGGAGCAAATTCGTCCGGCACCGGAATTTGCGGTTACCCTGAGCACGGAATATCTGACGGGTC
 cheW  WT  (401) TGGGCGCACTGGGCGACCGGATGTTGATTCTGGTGAACATCGAAAAACTGCTGAACAGCGAAGAGATGGCGCTGTTAGAT
 cheW opt  (401) TGGGTGCCCTGGGTGACCGTATGCTGATTCTGGTGAACATCGAGAAACTGCTGAACAGCGAAGAGATGGCGTTGCTGGAT
 cheW  WT  (481) AGCGCGGCGTCAGAAGTGGCGTAA
 cheW opt  (481) AGCGCAGCCAGCGAGGTTGCATAA
```

Figure S2C. Sequences of wild type *cheW* (WT) and codon-optimized *cheW* (opt). The codon-optimized gene was synthesized and used in this work.

```
 cheW  WT    (1) ATGACCGGTATGACGAATGTAACAAAGCTGGCCAGCGAGCCGTCAGGCCAGGAATTTCTGGTATTTACCCTTGGTGATGA
cheW* opt    (1) ATGACCGGTATGACTAACGTAACTAAACTGGCTTCTGAGCCGTCCGGCCAGGAGTTCCTGGTTTTCACCCTGGGCGATGA
 cheW  WT   (81) AGAGTACGGTATTGATATCCTGAAAGTGCAGGAGATCCGTGGCTACGATCAGGTAACACGGATTGCGAACACGCCAGCGT
cheW* opt   (81) AGAATACGGTATTGACATTCTGAAAGTACAGGAAATTCGTGGTTACGACCAGGTCACTCGTATCGCTAACACCCGGCTT
 cheW  WT  (161) TTATCAAAGGCGTCACGAATCTGCGCGGCGTTATTGTGCCGATTGTTGACTTACGAATTAAGTTCAGCCAGGTGGATGTG
cheW* opt  (161) TCATCAAAGGTGTAACCAACCTGCGCGGTGTTATCGTTCCAATTGTAGACCTGCGTATCAAATTTAGCCAGGTGGACGTT
 cheW  WT  (241) GACTATAACGACAACACGGTAGTTATCGTCCTGAATCTCGGACAGCGGGTGGTCGGCATCGTGGTTGACGGCGTCTCAGA
cheW* opt  (241) GATTACAATGATAACACTGTGGTGATCGTTCTGAACCTGGGTCAACGTGTTGTGGGCATCGTGGTTGACGGTGTGAGCGA
 cheW  WT  (321) CGTGCTTTCATTGACGGCGGAGCAAATTCGTCCGGCACCGGAATTTGCCGTGACGCTTTCAACAGAATATCTCACTGGAC
cheW* opt  (321) CATGCTGTCCCTGACCGCCGAGCAGATCCGTCCGGCACCGGAGTTCGCAGTCACGCTGAGCACCGAATACCTGACTGGCC
 cheW  WT  (401) TGGGCGCACTGGGCGACCGGATGTTGATTCTGGTGAACATCGAAAAACTGCTGAACAGCGAAGAGATGGCGCTGTTAGAT
cheW* opt  (401) TGGGTGCTCTGGGTGACCGTATGCTGATCCTGGTAAACATCGAGAAACTGCTGAATAGCGAAGAATGGCACTGCTGGAC
 cheW  WT  (481) AGCGCGGCGTCAGAAGTGGCGTAA
cheW* opt  (481) TCTGCAGCCTCTGAAGTAGCGTAA
```

Figure S2D. Sequences of wild type *cheW* (WT) and codon-optimized *cheW** (opt). The codon-optimized gene was synthesized and used in this work.