

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)
- Alphamager® (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 (NH₄)₂SO₄ (Fisher Scientific, Pittsburgh, PA)
 - 22.5g KH₂PO₄ (Fisher Scientific, Pittsburgh, PA)
 - 52.5g K₂HPO₄ (Fisher Scientific, Pittsburgh, PA)
 - 2.5g sodium citrate•2H₂O (Fisher Scientific, Pittsburgh, PA)

- 1 M MgSO₄ (sterilized by filtration, Fisher Scientific, Pittsburgh, PA)
- 0.1 M CaCl₂ (sterilized by filtration, Fisher Scientific, Pittsburgh, PA)
- 1M ZnSO₄ (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 MgSO₄
2.5 mL 0.1 M CaCl₂
0.1 mL 1 M ZnSO₄
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 MgSO₄
2.5 mL 0.1 M CaCl₂
0.1 mL 1 M ZnSO₄
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

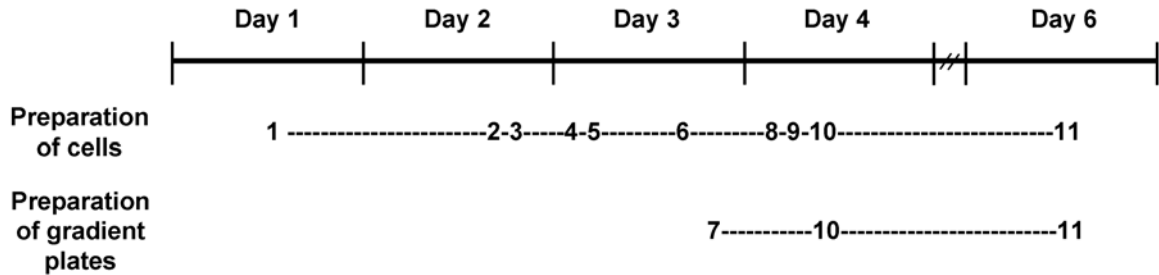
5 mL of minAaa media
5 µL of 34 mg/mL chloramphenicol

■ Preparation of culture with OD₆₀₀ = 0.2 (the measured OD₆₀₀ = 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 $OD_{600} = 3$.

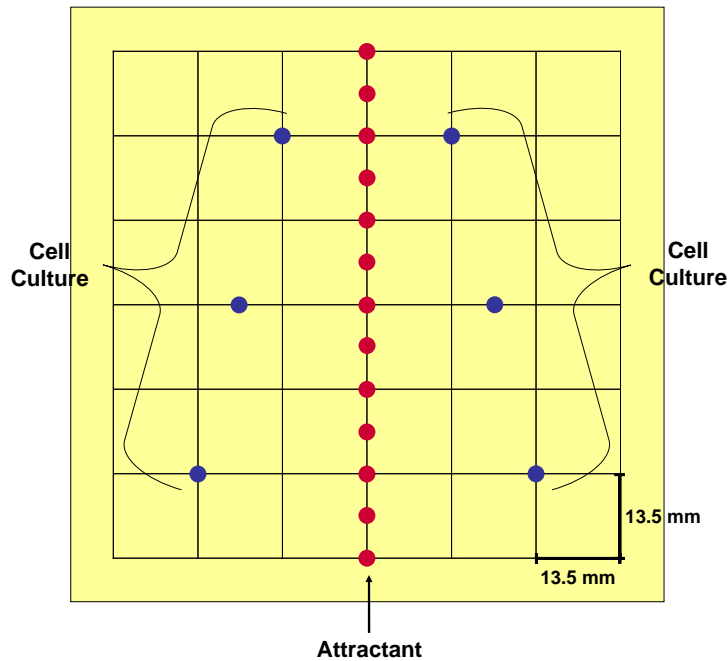
Day 3

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 media 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 media supplemented with 34 µg/mL chloramphenicol (washing solution), and measure OD₆₀₀ of the resuspended cells. Dilute cells to an OD₆₀₀ 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 mm²
 - 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

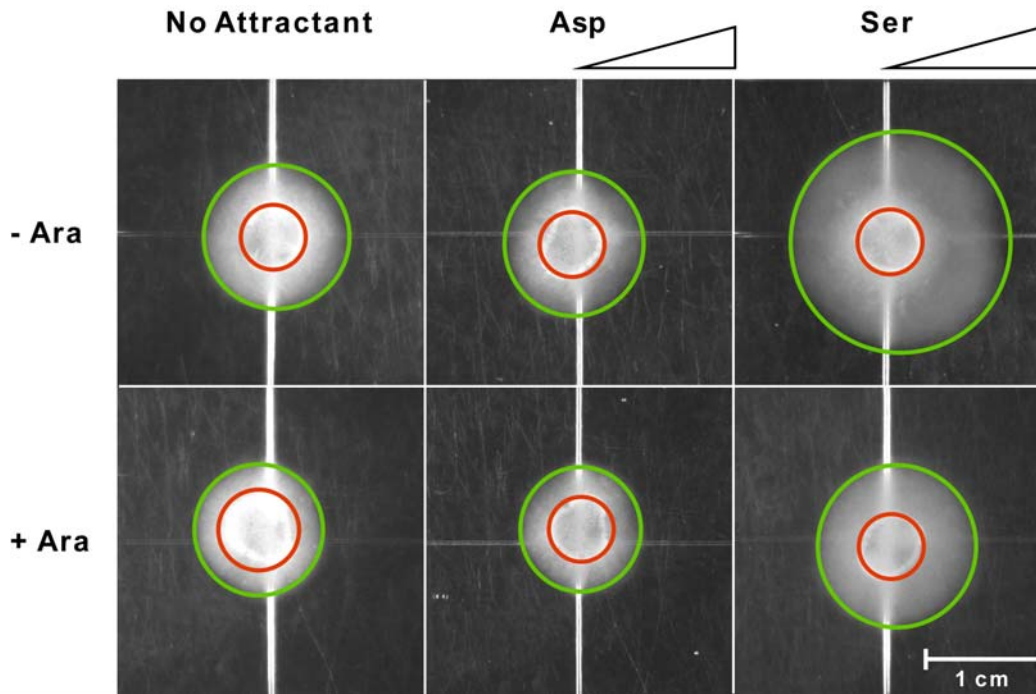


Figure S1A. Chemotaxis assay using the *cheW* deletion mutant. Images shown are representative of experiments that were performed at least three times (see Figure 4A).

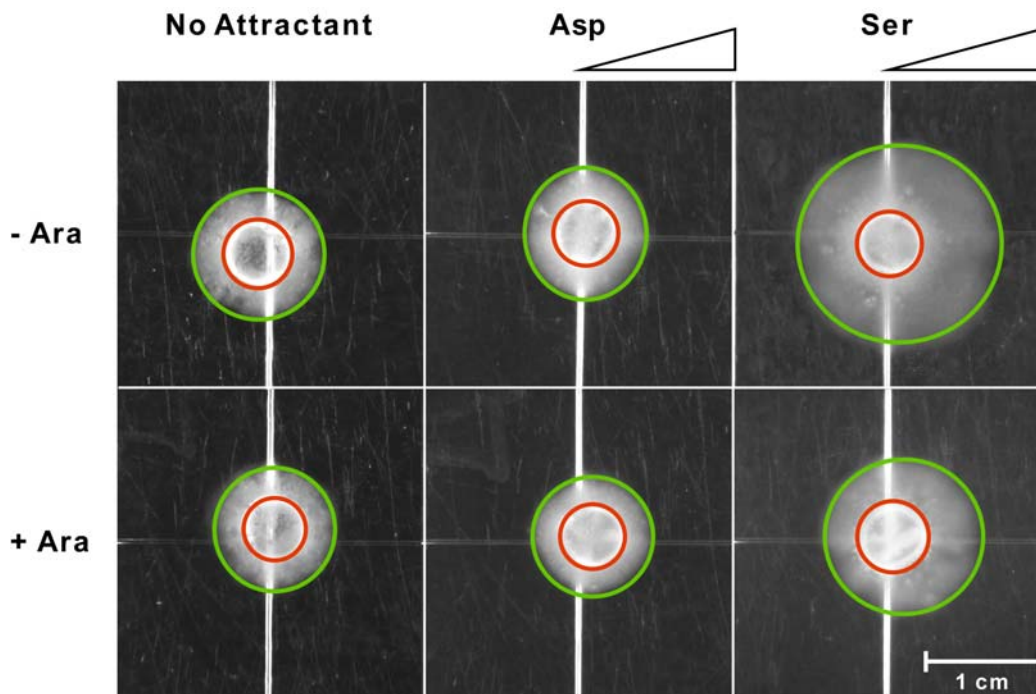


Figure S1B. Chemotaxis assay using the *tar* deletion mutant. Images shown are representative of experiments that were performed at least three times (see Figure 4B).

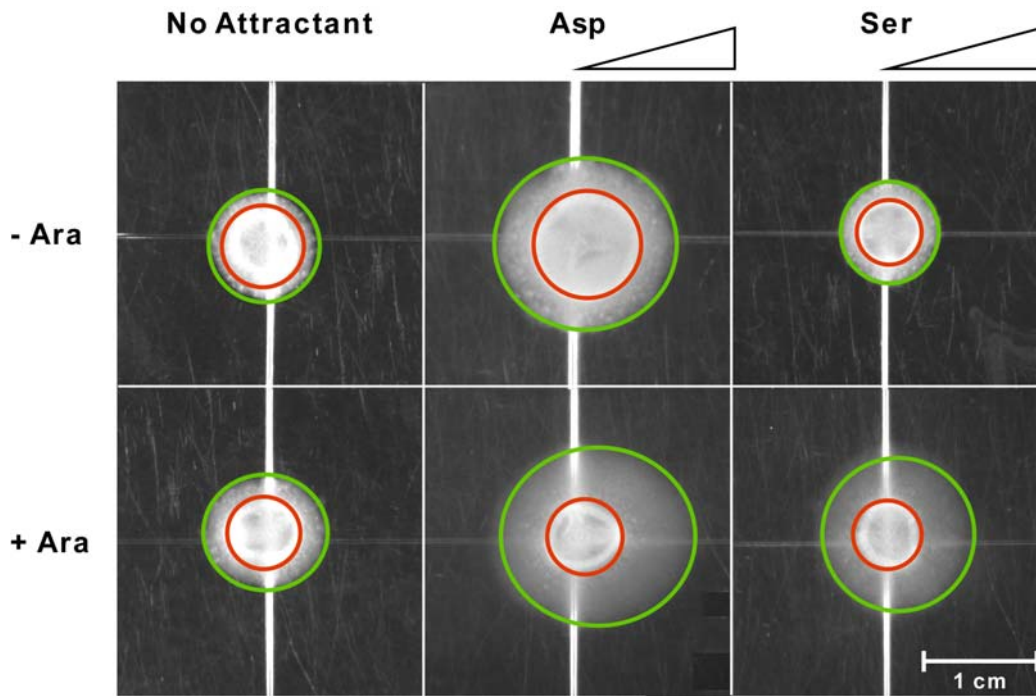


Figure S1C. Chemotaxis assay using the *cheW** deletion mutant. Images shown are representative of experiments that were performed at least three times (see Figure 4C).

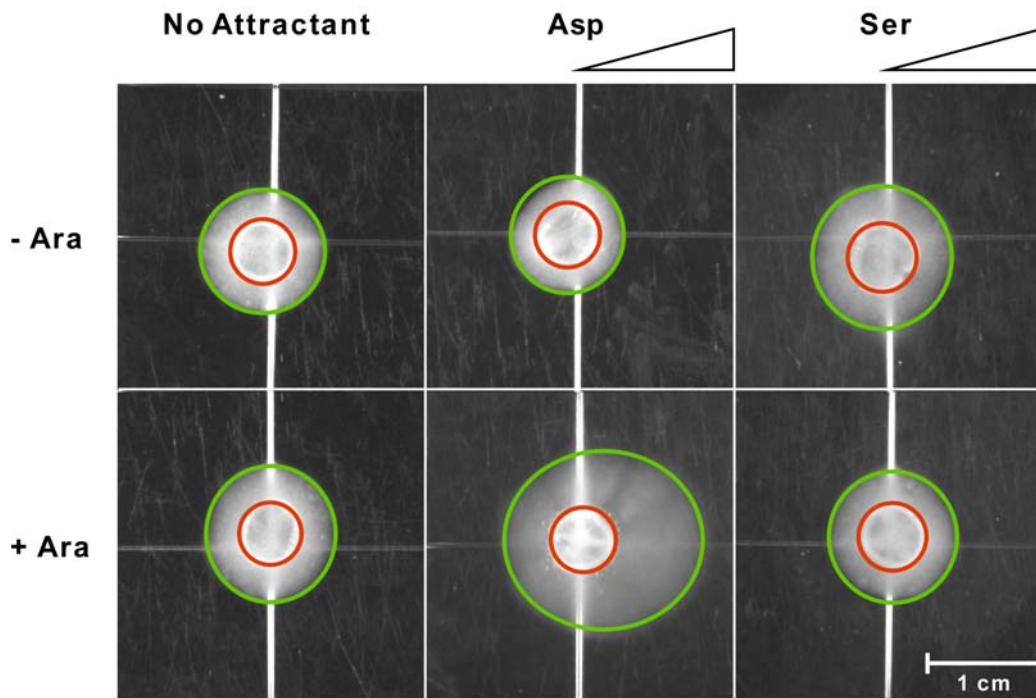


Figure S1D. Chemotaxis assay using the *tsr** deletion mutant. Images shown are representative of experiments that were performed at least three times (see Figure 4D).

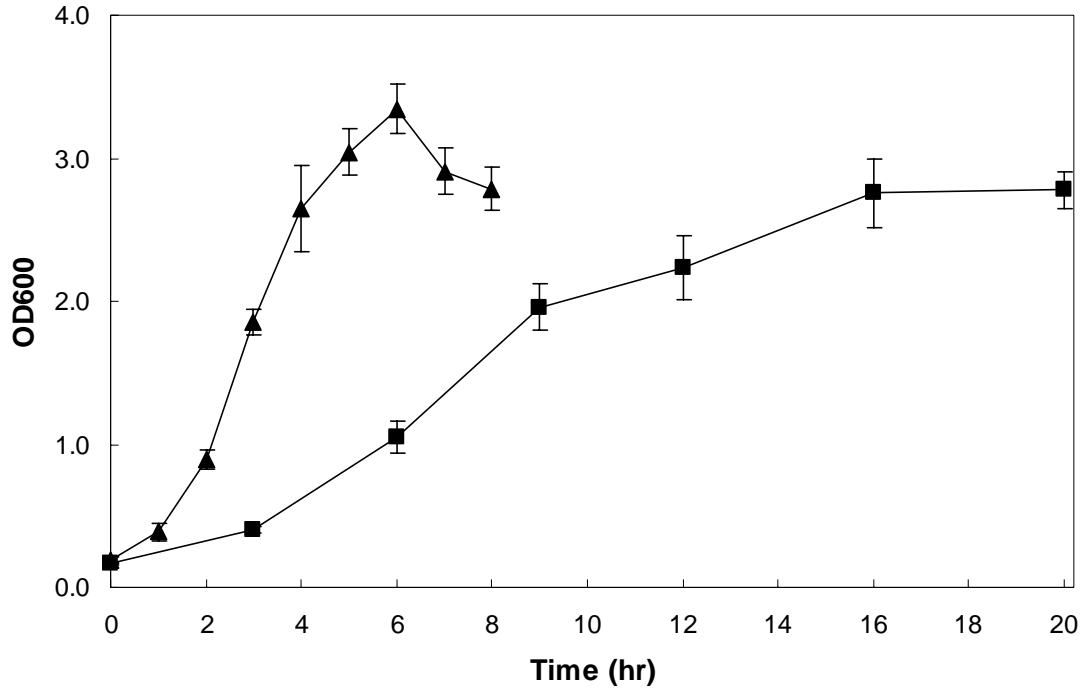


Figure S2. Growth curves for data presented in Figure 2C (\blacktriangle = LB, 5mM Ara) and 2F (\blacksquare = minAaa, 5mM Ara).

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  $\pm 0.01$ ) 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
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
%-----

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4. Sequence Alignment

tar WT (1) ATGATTAAACCGTATCCGCGTAGTCACGCTGTTGGTAATGGTGCTGGGGGTATTTCGCACTGTTACAGCTTATTTCCGGCAG
tar opt (1) ATGATCAATTCGTATTTCGTGTGGTTACTCTGCTGGTTCATGGTGTGGGGTGTTTTCGCGCTGCTGCAACTGATTAGCGGCAG
tar WT (81) TCTGTTTTTTTCTTCCCTTCACCATAGCCAGAAAGAGCTTTGTGGTTTCCAATCAATTACGGGAACAGCAGGGCGAGCTGA
tar opt (81) CCTGTTTTTTTCTTCCCTTCACCACAGCCAGAAATCTTTTCTGTTGTTAGCAATCAACTGCGTGAACAACAGGGTGAGCTGA
tar WT (161) CGTCAACCTGGGATTTAATGCTGCAAACGCGCATTAACTGAGTCGTTACGCGGTACGGATGATGATGGATTCTCCAAT
tar opt (161) CCAGCACCTGGGACCTGATGCTGCAAACGCGTATCAATCTGAGCCGTTCTGCCGTGCGTATGATGATGGACAGCAGCAAC
tar WT (241) CAACAAAGTAACGCCAAAGTTGAATTGCTCGATAGCGCCAGGAAAACATTGGCGCAGGCAGCGACGCATTATAAAAAATT
tar opt (241) CAACAAAGCAACGCCAAGGTTGAATTGCTGGACAGCGCTCGTAAGACGCTGGCCAGGCCGCAACCCACTACAAAAAGTT
tar WT (321) CAAAAGCATGGCACCGTTACCTGAAATGGTTCGCTACCAGTCGTAATATTGATGAAAAATATAAAAACTATTACACAGCGT
tar opt (321) TAAGAGCATGGCGCCTCTGCCGGAGATGGTTGCCACCAGCCGTAACATTGATGAAAAATACAAGAATTATTACACGGCAC
tar WT (401) TAACTGAACTGATTGATTATCTAGATTATGGCAATACTGGAGCTTATTTTCGCTCAGCCAACCCAGGGAATGCAAAATGCA
tar opt (401) TGACCGAGCTGATTGACTACTGGATTACGGTAATACGGGCGCCTACTTTCGCGCAGCCGACGCAAGGTATGCAGAACGCG
tar WT (481) ATGGGCGAAGCGTTTGTCTCAGTACGCCCTCAGCAGTGAAAACTGTATCGCGATATCGTCACTGACAACGCAGATGATTA
tar opt (481) ATGGGTGAGGCTTTTCGCGCAGTATGCGCTGAGCAGCGAGAAATTTGTACCGTGACATTGTTACCGATAACCGCGATGATTA
tar WT (561) CCGATTTGCCCAGTGGCAAACCTGGCGTTATCGCGCTGGTGGTATTGATTCTGCTGGTGGCGTGGTACGGCATTCCGC
tar opt (561) CCGTTTTGCGCAGTGGCAGCTGGCGGTTATCGCGCTGGTGGTGGTCTGATCTTGTGGTGGCCTGGTACGGTATTCCGTC
tar WT (641) GTATGTTGCTTACTCCGCTGGCAAAAATTATTGCTCACATTTCGCGAAATCGCCGGTGGTAACCTGGCGAATACCCTGACC
tar opt (641) GTATGCTGCTGACCCCGCTGGCAAAAGATTATTGCACACATCCGTGAGATCGCGGGTGGTAATCTGGCAAAACACCCTGACC
tar WT (721) ATTGACGGGCGCAGTGAAATGGGCGACCTGGCGCAGAGCGTTTTCACATATGCAACGCTCTTTGACTGACACCGTCACTCA
tar opt (721) ATTGACGGCCGTAGCGAGATGGGTGACCTGGCGCAAAGCGTCAGCCACATGCAACGTAGCCTGACCAGACACCGTCACGCA
tar WT (801) TGTCCGCGAAGGTTTCAGATGCATCTATGCCGGTACCCGTGAAATTTGCGGCAGGCAACACCGATCTTTCTCCCGTACTG
tar opt (801) CGTCCGCGAGGGTAGCGATGCAATCTACGCCGGCACCCGTGAAATTTGCGGCAGGTAACACCGACCTGAGCAGCCGTACTG
tar WT (881) AACAGCAGGCATCCGCGCTGGAAGAAACTGCGCCAGCATGGAGCAGCTCACCGCGACAGTGAAGCAAAACGCGGATAAC
tar opt (881) AGCAGCAGGCTTCCGCGCTGGAGGAAACCGCTGCCTCTATGGAACAACCTGACCGCGACGGTTAAACAAAACGCGAGACAAC
tar WT (961) GCCCGCCAGGCCTCGCAACTGGCGCAAAGTGCCTCCGACACCGCCAGCAGCGCGGCAAAAGTGGTGGATGGCGTAGTGAA
tar opt (961) GCACGTCAGGCATCCAGCTGGCGCAGAGCGCGTCTGACACCGCACAGCAGCGGTGGCAAGGTGGTTGACGGTGTGGTTAA
tar WT (1041) AACGATGCATGAGATCGCCGATAGTTTCGAAGAAAATTGCCGACATTATCAGCGTTATCGACGGTATTGCCTTTCCAGACTA
tar opt (1041) GACCATGCACGAGATTGCTGATAGCAGCAAAAAGATCGCCGACATCATTAGCGTGATTGACGGTATTGCCTTTCCAGACCA
tar WT (1121) ATATCCTCGCGCTGAATGCGCGGTTGAAGCCGCGCGTGCAGGTTGAACAGGGCCGTTGGTTTTGCGCGTGGTGGCGGGTGAA
tar opt (1121) ATATCCTGGCGTTGAATGCAAGCGGTGGAAGCGCACGTGCTGGCGAACAGGGCCGTTGGTTTTGCGAGTTGTGCGAGGCGAG
tar WT (1201) GTGCGTAATCTTGCCAGTTCGACGCGCCAGGCGGCAAAAGAGATCAAAGCCCTCATTGAAGACTCCGTCTCACCGTGTGA
tar opt (1201) GTTCGTAACCTGGCGAGCCGACGCGCAGGCGAGCAAGGAGATCAAAGCCCTCATTGAAGACTAGCGTTAGCCGTGTTGA
tar WT (1281) TACCGTTTCGGTGTCTGGTTCGAAAGCGCCGGGAAACAATGAAACAATATCGTCAATGCGGTGACCCGCGTGACTGACATTA
tar opt (1281) CACTGGCTCCGTGCTGGTGGAGTCCGCTGGTGGAGACTATGAATAACATCGTCAATGCGGTGACCCGCGTGACTGACATTA
tar WT (1361) TGGGCGAGATTGCATCGGCATCGGATGAAACAGAGCCGTTGGCATCGATCAAGTCGCATTGGCGGTTTTCGGAAATGGATCGC
tar opt (1361) TGGGCGAAATCGCGTCTGCTCTGATGAGCAAAGCCGTTGGCATCGATCAGGTCGCGCTGGCTGTCTCTGAGATGGACCGT
tar WT (1441) GTCACGCAACAGAACGCATCGCTGGTGCAGGAATCAGCTGCGCGCCGCTGCGCTGGAAGAACAGGCGAGTCGTTTAAAC
tar opt (1441) GTTACGCAGCAAAATGCGTCCCTGGTGCAGAAAGCGCGGCAAGCTGCGCGGCACTGGAGGAGCAAGCAAGCCGTCTGAC
tar WT (1521) GCAAGCAGTTTCCGCGTTCCGTCTGGCAGCCAGCCACTCACCAATAAACCGCAAAACCCATCCCGTCCCTGCCAGTGGAGC
tar opt (1521) CCAAGCAGTGTCCGCGTTCCGCTGGCCGCAAGCCCGTTGACCAATAAGCCGCGAGCAGCAAGCCGCTGCGAGCGAGC
tar WT (1601) AACCACCGGCTCAGCCAGACTGCGAATTGCTGAACAAGATCCAAACTGGGAAACATTTTGA
tar opt (1601) AGCCGCCAGCCAACTCGTCTGCGTATTGCGGAGCAAGATCCGAACTGGGAAACCTTCTAA

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

tsr WT (1) ATGTTAAAACGTATCAAAATTGTGACCAGCTTACTGCTGGTTTGGCCGTTTTGGCCTTTTCAAACTGACATCAGGCGG
*tsr** opt (1) ATGCTGAAGCGTATCAAAATTGTTACTTCCCTGCTGCTGGTTCTGGCCGTTTGGCCTGCTGCAAACTGACTTCCGGTGG
tsr WT (81) TCTGTTCTTTAATGCTTAAAGAATGACAAAGAAAATTTCACTGTTTTACAAACCATTCGCCAGCAGCAATCCACGCTGA
*tsr** opt (81) TCTGTTCTTTAATGCTTCTGAAAATGATAAAGAAAATTTACTGTACTGCAAACGATCCGCCAGCAGCAGTCTACTTCTGA
tsr WT (161) ATGGCAGCTGGGTGCGCTTGTGTCAGACGCGTAAACCCTCAACCAGCGGGTATCCGCTACATGATGGATCAGAATAAT
*tsr** opt (161) ACGGTTCTGGGTGCGCTGCTGCAAACCCGTAATACCCTGAACCAGCGCAGGCATTTCGTTACATGATGGACCAGAACAAC
tsr WT (241) ATTGGTAGCGGTTCAACCGTTGCTGAGCTGATGGAGAGTGCAGTATTTTCGCTGAAACAGGCGGAAAAAACTGGGCGGA
*tsr** opt (241) ATCGGCTCCGGCTCTACCGTCTGCTGAACTGATGGAATCCGCGTCCATCTCTCTGAAACAAGCTGAGAAGAACTGGGCGGA
tsr WT (321) TTACGAAGCGTTGCCGCGTGACCCGCGTCAGAGCACCCGCCAGCGGCAGAGATCAAACGTAATTACGATATTTATCACA
*tsr** opt (321) TTACGAGGCTCTGCCGCGTGATCCTCGCCAGTCTACTGCCGCTGCTGCCGAAATCAAACGTAATACGACATCTACCATA
tsr WT (401) ATGCGCTGGCGGAGCTGATCCAACGTTAGGTGCAGGCAAAATCAACGAGTTCTTTGATCAGCCGACCCAGGGATATCAG
*tsr** opt (401) ACGCGCTGGCCGAACTGATTCAACTGCTGGGTGCTGGCAAAATTAACGAGTTCTTTGACCAGCCGACCCAGGGCTATCAG
tsr WT (481) GACGGTTTCGAGAAGCAGTATGTGGCTTACATGGAGCAAACGATCGGCTCCATGATATCGCCGTCAGCGATAACAATGC
*tsr** opt (481) GACGGTTTCGAAAAACAGTACGTTGCGTATATGGAGCAGAACGACCGTCTGCACGACATCGCTGTCTCCGATAACAACGC
tsr WT (561) CTCCTACAGCCAGGCGATGTGGATTCTGGTGGCGTGATGATCGTCTGTAAGCGGTCATCTTCGCCGCTGTGGTTCCGTA
*tsr** opt (561) TTCCTACTCTCAGGCAATGTGGATTCTGGTGGTGTATGATTGTTGTTCTGGCGGTCATCTTCGCTGTGTTGGTTTGGTA
tsr WT (641) TTAAAGCCTCGCTGGTAGCGCCAAATGAATCGCCTGATTGACAGCATTTCGTCAATATTGCAGGCGGCGATCTGGTGAACCG
*tsr** opt (641) TTAAAGCCAGCCTGGTAGCTCCGATGAATCGTCTGATCGATAGCATCCGTCACATTGCGGGCGGTGATCTGGTAAAGCCG
tsr WT (721) ATTGAGGTGGATGGCTCTAATGAGATGGGGCAACTGGCAGAGAGTTTGGCGCATATGCAGGGAGAGCTGATGCGTACCCT
*tsr** opt (721) ATCGAAGTGGACGGCTCCAACGAAATGGGTCAACTGGCAGAAATCTCTGCGTCAATGCAGGGCGAGCTGATGCGCACTGT
tsr WT (801) CGGTGATGTGCGCAACGGGGCAATGCCATCTATAGCGGTGCAGCGAAATCGCCACCAGCAATAACGATCTCTCTTCGC
*tsr** opt (801) TGGTGATGTTGTAACGGTGCAAATGCTATTTACTCCGGTGCATCCGAGATTGCAACCGGTAAACAACGACCTGTCTCTC
tsr WT (881) GCACCGAGCAACAGGCCGCTTCGCTGGAAGAGACGGCAGCCAGCATGGAGCAACTGACCGCAACGGTGAAGCAGAACGCC
*tsr** opt (881) GTACTGAGCAGCAGGCTGCCTCCCTGGAAGAGACTGCGGCAAGCATGGAGCAACTGACTGCTACCCTTAAACAGAATGCG
tsr WT (961) GAAAATGCGCGCCAGGCCAGCCATCTGGCGTTAAGTGCTTCTGAAACGGCGCAACCGCGCGGTAAAGTGGTAGATAACGT
*tsr** opt (961) GAAAACGCACGTACAGGCATCCACCTGGCGCTGTCCGCTTCTGAAACCGCACAGCGTGGTGGCAAAGTTGTAGATAACGT
tsr WT (1041) GGTGCAGACTATGCGCGATATCTCCACCAGTTCGCAGAAAATCGCCGATATTATCAGCGTAATTGACGGCATTGCCTTCC
*tsr** opt (1041) AGTGCAGACGATGCGCGATATTTCTACCTCTAGCCAGAAATCGCAGACATCATCAGCGTCATTGACGGTATCGCGTTTC
tsr WT (1121) AGACCAATATTCTGGCTTTGAACGCGGGCGTTGAGGCTGCGCGTGCAGGGTGAAGCAAGGGCGCGGTTTTGCGGTGGTTCGG
*tsr** opt (1121) AGACCAACATCTGGCGCTGAACGCAGCCGTTGAGGCAAGTTCGTCGCGGGCGAAACAAGGCCGTGGCTTCGCGGTGGTTCGCT
tsr WT (1201) GGAGAAGTGCCTAATCTGGCCAGCAGCAGCGCCAGGCGGCTCGTGAATTTAAAAGCCTGATTGAAGACTCGGTGGGGAA
*tsr** opt (1201) GGCAGTGCCTAACCTGGCACAGCCTTCTGCCAGGCGGCAAGTGCAGTCCCTGATTGAAGACTCTGTGGGCAA
tsr WT (1281) AGTGGATGTTGGCTCTACGCTGGTGAAGAGCGCGGGGAAACAAATGGCGGAGATTGTGAGCGCGCTGACCCGCTGACCG
*tsr** opt (1281) AGTTGACGTGGGTTCTACCTGGTAGAGAGCGCGGTTGAAACGATGGCTGAAATTGTTCCGCGGTAACCCGTGTGACCG
tsr WT (1361) ACATTATGGGCGAAATGCTTCTGCTTCTGATGAGCAGAGCCGTTGATCAGATCAGGTTGGCTTAGCGGTTGCTGAGATG
*tsr** opt (1361) ACATCATGGGTGAGATCGCATCCGCTTCCGACGAGCAGTCCCGTGGTATCAGATCAGGTTGGCCTGGCGGTAGCTGAAATG
tsr WT (1441) GACCGGGTAACCTCAACAGAACGCCGCGCTGGTGAAGAGTCTGCGCTGCGCGCGCTGGAAGAGCAGGCCAGTTCG
*tsr** opt (1441) GACCGTGTACCAGCAAAACGCCGCCCTGGTGAAGAGTCTGCTGCGGCAAGCGGCTGCACTGGAAGAACAGGCCAAGCCG
tsr WT (1521) CCTGACCGAAGCAGTGGCAGTGTTCGGATTTCAGCAACAGCAGCGTGAACATCGGCTGTGGTAAAAACCGTGACGCCAG
*tsr** opt (1521) TCTGACCGAAGCGGTAGCAGTGTTCGGTATTTCAGCAGCAGCAGCGTGAACATCTGCGTGTGGTAAAAACCGTGACCCGG
tsr WT (1601) CTGCGCCGCGTAAAATGGCCGTGGCAGATAGCGAGGAGAAGTGGGAAACATTTTAA
*tsr** opt (1601) CAGCTCCGCGCAAAATGGCAGTTGCAGATTCGGAAGAGAAGTGGGAAACCTTCTAA

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) ATGACC**CGGT**TATGACGAATGT**AACA**AAAGCTGGC**CAGCGAGCCGTC**AGGCCAGGAATTTCTGGT**ATTTAC**CCTTGGT**GATGA**
cheW opt (1) ATGACTGG**C**ATGACGAATGT**TACG**AAATTGGC**AAGCGAACCA**TC**TGGT**CAGGAATTTCTGGT**TTT**CACGTTGGG**C**GACGA
cheW WT (81) **AGAGT**ACGGTATT**TGAT**TATCCTGAAAGT**GCAGGAGAT**CCGTGG**CTACGAT**CAGGT**AACACGGATT**GCGAACACGCC**AGCGT**
cheW opt (81) **GGAAT**ACGGTAT**CGAC**ATCCTGAAAGT**TCAGGAAAT**CCGTGG**TTATGACC**AGGT**TACGCGC**ATTGCGAATACGCC**GGCTT**
cheW WT (161) TTATCAA**AGGCGT****CACGA**ATCTGCGCGGC**GT**TATTGTGCCGATTGTTGACT**TACGA**ATTAAGTT**CAGCC**AGGTGGAT**GTG**
cheW opt (161) TTATCA**AGGGT**GT**TACCA**ATCTGCGCGGC**GT**CATTGTGCCGATTGTTGACT**TGCGC**ATTAAGTT**TTCT**CAAGTT**GAC**GTG
cheW WT (241) GACTATAACGACA**ACACGGT****AGTT**TATCGT**CCTGAATCTCGG**ACAGCGGGTGGT**CGGC**ATCGTGGTTGACGG**CGTCTC**AGA
cheW opt (241) GACTATAACGACA**ATACGGT****CGTT**ATTGT**CCTGAATTTGGG**CCAGCGCGTGGT**TGGC**ATCGT**TGTT**GATGGT**GT**CAGCGA
cheW WT (321) CGTGCT**TT**CATTGACGGCGGAGCAA**ATTCGT**CCGGCACC**GGAAATTTGCC**GTGAC**GC**TTT**CAAC**AGAATATCT**CACT**GGAC
cheW opt (321) CGTGCT**GAGCC**TGAC**CGCGG**AGCAA**ATTCGT**CCGGCACC**GGAAATTTGCC**GT**TAC**CCT**GAGC**AC**GGA**ATATCT**GAC**GGGT**C**
cheW WT (401) TGGG**CGC**ACTGGG**CGACCG**GATG**TTG**ATTCTGGTGAACATCGA**AAAACT**GCTGAACAGCGAAGAGATGGCG**CTGTT**AGAT
cheW opt (401) TGGG**TGC**CCTGGG**TGACCG**TATG**CTG**ATTCTGGTGAACATCGA**GAAACT**GCTGAACAGCGAAGAGATGGCG**TTGCT**GGAT
cheW WT (481) AGCGCGG**CGT**CAGAAGTGGCGTAA
cheW opt (481) AGCGCAGC**CAGC**GAGGTTGCATAA

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) ATGACC**GGT**TATGAC**GAAT**GTAA**CA**AAAGCTGGC**CAGCGAGCCGTC**AGGCCAGGAATTTCTGGT**ATTTAC**CCTTGGT**GATGA**
*cheW** opt (1) ATGACC**GGT**TATGAC**TAAC**GTAA**CT**GGC**TTCT**GAGCCGT**CCGG**CCAGGAGTT**CCT**GGT**TTT**CACCCTGGG**C**GATGA
cheW WT (81) **AGAGT**ACGGTATT**TGAT**TAT**CCT**GAAAGT**GCAGGAGAT**CCGTGG**CTACGAT**CAGGT**AACACGGATT**GCGAACACGCC**AGCGT**
*cheW** opt (81) **AGAAT**ACGGTATT**TGAC**AT**TCT**GAAAGT**ACAGGAAAT**TCGTGG**TTACGACC**AGGT**CACT**CGTAT**CGT**AAAC**CCCG**GGCTT
cheW WT (161) **T**TATCAA**AGGCGT****CACGA**ATCTGCGCGGC**GT**TATT**TGT**GCCGATTGTTGACT**TACGA**ATTAAGTT**CAGCC**AGGTGGAT**GTG**
*cheW** opt (161) **T**CATCAA**AGGT**GT**TAAC**CA**ACT**GCGCGGT**GT**TATCGT**TCCA**ATTGT**AGACCT**GCGTATCAA**ATT**AGCCAGGTGGAC**GT**
cheW WT (241) GACTATA**ACG**ACA**ACACGGT****AGTT**TATCGT**CCTGAATCTCGG**ACAGCGGGTGGT**CGGC**ATCGTGGTTGACGG**CGTCTC**AGA
*cheW** opt (241) G**ATT**ACA**ATG**ATA**AACT**GT**GGT**GATCGT**TT**TGA**ACT**GGG**TCA**ACGT**TT**GT**GGC**ATCGTGGTTGACGGT**GT**GAGCGA
cheW WT (321) CGTGCT**TT**CATTGACGGCGGAGCAA**ATTCGT**CCGGCACC**GGAAATTTGCC**GTGAC**GC**TTT**CAAC**AGAATATCT**CACT**GGAC
*cheW** opt (321) **C**ATGCT**GT**CCTGAC**CGCC**GAGCAGAT**CCGT**CCGGCACC**GGAGTT**CGCAGT**CACGCT**GAGCACC**GAA**TACCTGACTGGCC
cheW WT (401) TGGG**CGC**ACTGGG**CGACCG**GATG**TTG**ATTCTGGTGAACATCGA**AAAACT**GCTGAACAGCGAAGAGATGGCG**CTGTT**AGAT
*cheW** opt (401) TGGG**TGC**TCTGGG**TGACCG**TATG**CTG**AT**CCT**GGT**AA**ACATCGA**GAAACT**GCTGAATAGCGAAG**AAAT**GGC**ACT**GCTGGAC
cheW WT (481) **AGCGCGGCGT**CAGAAGTGGCGTAA
*cheW** opt (481) **TCT**G**CAGC**CT**T**G**AA**GT**AG**CGTAA

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