Blue/White Selection

Materials

Materials needed for DNA origami (see preparation)
E. coli MG1655 strain was obtained from DiBo biotechnology® (Shanghai, China).
100 mM IPTG and 20 mg/ml X-gal solutions were obtained from Solarbio® (Beijing, China).

Procedures

1. Loading Helper Strand
   a. Prepare the M13, S-x, S-G4-x, Apt-cap-x, and S-PAM-cap-x mix in a single microcentrifuge tube on ice by adding the components indicated below. The molar ratio of M13 (5nM) and helper strands (50 nM) is 1:10.

DNA origami (DO G) (with Apt-cap-x, and S-PAM-cap-x)

Reagents	Volume
TAE/Mg^{2+}/K^{+} buffer	48 μl
S-G4-x	0.25ul × 136 = 34μl
S-x	0.25ul × 34 = 8.5μl
Apt-cap-x	0.25ul × 12 = 3μl
S-PAM-cap-x	0.25ul × 6 = 1.5μl
M13	5μl

S-G4-15	**S-G4-64**	**S-G4-124**	**S-G4-174**	**S-108**
S-G4-16	**S-G4-65**	**S-G4-129**	**S-G4-175**	**S-109**
S-G4-17	**S-G4-66**	**S-G4-130**	**S-G4-176**	**S-116**
S-G4-20	**S-G4-67**	**S-G4-131**	**S-G4-177**	**S-125**
S-G4-21	**S-G4-68**	**S-G4-134**	**S-G4-178**	**S-126**
S-G4-22	**S-G4-69**	**S-G4-135**	**S-G4-179**	**S-127**
S-G4-26	**S-G4-70**	**S-G4-136**	**S-G4-182**	**S-128**
S-G4-27	**S-G4-71**	**S-G4-137**	**S-G4-183**	**S-132**
S-G4-28	**S-G4-74**	**S-G4-138**	**S-G4-184**	**S-133**
S-G4-29	**S-G4-75**	**S-G4-139**	**S-G4-185**	**S-156**
S-G4-30	**S-G4-76**	**S-G4-140**	**S-G4-186**	**S-157**
S-G4-31	**S-G4-77**	**S-G4-141**	**S-G4-187**	**S-180**
S-G4-32	**S-G4-78**	**S-G4-142**	**S-G4-188**	**S-181**
S-G4-33	**S-G4-79**	**S-G4-143**	**S-G4-189**	**S-194**
S-G4-34	**S-G4-80**	**S-G4-146**	**S-G4-190**	**S-198**
S-G4-35	**S-G4-81**	**S-G4-147**	**S-G4-191**	**S-199**
S-G4-38	**S-G4-82**	**S-G4-148**	**S-G4-195**	**S-203**
S-G4-39	**S-G4-83**	**S-G4-149**	**S-G4-196**	**S-204**

S-G4-40	S-G4-86	S-G4-150	S-G4-197	S-PAM-Cap102
S-G4-41	S-G4-87	S-G4-151	S-G4-200	S-PAM-Cap103
S-G4-42	S-G4-88	S-G4-152	S-G4-201	S-PAM-Cap104
S-G4-43	S-G4-93	S-G4-153	S-G4-202	S-PAM-Cap113
S-G4-44	S-G4-94	S-G4-154	S-13	S-PAM-Cap114
S-G4-45	S-G4-95	S-G4-155	S-14	S-PAM-Cap115
S-G4-46	S-G4-98	S-G4-158	S-18	Apt-Cap1
S-G4-47	S-G4-99	S-G4-159	S-19	Apt-Cap2
S-G4-50	S-G4-100	S-G4-160	S-23	Apt-Cap6
S-G4-51	S-G4-105	S-G4-161	S-36	Apt-Cap7
S-G4-52	S-G4-106	S-G4-162	S-37	Apt-Cap11
S-G4-53	S-G4-107	S-G4-163	S-60	Apt-Cap12
S-G4-54	S-G4-110	S-G4-164	S-61	Apt-Cap205
S-G4-55	S-G4-111	S-G4-165	S-84	Apt-Cap206
S-G4-56	S-G4-112	S-G4-166	S-85	Apt-Cap210
S-G4-57	S-G4-117	S-G4-167	S-89	Apt-Cap211
S-G4-58	S-G4-118	S-G4-170	S-90	Apt-Cap215
S-G4-59	S-G4-119	S-G4-171	S-91	Apt-Cap216
S-G4-62	S-G4-122	S-G4-172	S-92	
S-G4-63	S-G4-123	S-G4-173	S-101	

b. Mix the above reagents and briefly centrifuge them. The mix is first heated at 95 °C for 10 minutes. Subsequently, annealing is performed by slowly cooling the mixture from 95 °C to 20 °C at a rate of 1 °C/min.
c. The Amicon® Ultra-0.5 Centrifugal Filter Devices (100kDa) are used to remove redundant helper strands three times. The purified DO G solution is obtained.

2. Loading functional strands
   a. Add 1.5 μL PAM-rich and 1.5μl aptamer stock solution to the purified DO G solution. Then, restore the volume to 100ul with TAE/Mg^{2+}/K^{+}buffer.
   b. Mix the above reagents and briefly centrifuge them. The mix is annealed from 45 °C to 25 °C at a rate of 5 min/°C for six cycles.
   c. The Amicon® Ultra-0.5 Centrifugal Filter Devices (100kDa) are used to remove redundant PAM-rich and aptamer three times. The purified $DO^{A}_{PAM}G$ solution is obtained.

3. Loading $sgRNA_L$/Cas9
   a. Prepare the $sgRNA_L$ and Cas9 mix in a single microcentrifuge tube on ice by adding the components indicated below. The molar ratio of $sgRNA_L$ and Cas9 is 1:1.

Reagents	Volume

PBS (PH=7.4, free of RNase H)	94 μl
sgRNA-L (5 μM)	3μl
Cas9 (5 μM)	3μl

b. Mix the above reagents and briefly centrifuge them. Incubate at 37 °C for 10 minutes to get $sgRNA_L$/Cas9 complex.
c. Add 10 μl of $sgRNA_L$/Cas9 mixture to the purified $DO^A{}_{PAM}G$ solution, and make up to 100 μl with TAE/Mg^{2+}/K^+ Buffer. The molar ratio of $sgRNA_L$/Cas9 complex and $DO^A{}_{PAM}G$ is 3:1.
d. Mix the above reagents and briefly centrifuge them. The mix is annealed from 37 °C to 4 °C at a rate of 1°C/min.
e. The Amicon® Ultra-0.5 Centrifugal Filter Devices (100 kDa) are used to remove the redundant $sgRNA_L$/Cas9. The purified $DO^A{}_{PAM}RCG$ is obtained.

4. Loading Hemin
   a. Add 1 μL of hemin stock solution to the concentrated $DO^A{}_{PAM}RCG$ solution obtained in the last step. Then, restore the volume to 100ul with TAE/Mg^{2+}/K^+ buffer.
   b. Gently resuspend the solution by pipetting three times using a wide-bore pipette tip.
   c. Briefly centrifuge at 500 × g for 3 seconds to sediment droplets.
   d. Incubate at room temperature for 1 hour.
   e. The Amicon® Ultra-0.5 Centrifugal Filter Devices (100 kDa) are used to remove redundant hemin. The purified $DO^A{}_{PAM}RCGH$ is obtained.

5. Verifying the loading of Hemin
   a. Measure the absorbance of $DO^A{}_{PAM}RCGH$ solution at 385nm using Thermo Fisher Scientific NanoDrop One 2.12.0 and Python 3.13.
   b. Plot the absorption spectrum of the $DO^A{}_{PAM}RCGH$ solution.

6. Blue/White Selection
   a. Revitalize the *E. coli* MG1655 strain and make a 5ml LB liquid overnight culture.
   b. Dilute the overnight culture to $1x10^6$ CFU/ml.
   c. Co-culture 20 μl $1x10^6$ CFU/ml bacterial suspension with the $DO^A{}_{PAM}RCGH$ solution with appropriate concentration (sgRNA=35 nM) xon a 96-well plate under 37 °C for 12 hours. Use an equal volume of TAE/Mg^{2+}/K^+ buffer as a control. Avoid light exposure as much as possible.
   d. Evenly spread 40 μl 100 mM IPTG and 120 μl 20 mg/ml X-gal solutions on each LB agar plate. Place the plates at 37 °C for at least half an hour to allow complete absorption of the mixed solution. Avoid light exposure

as much as possible.

e. Evenly spread the liquid in each well of the 96-well plate on LB plates supplemented with IPTG and X-gal plates. Place the plates at 37 °C for 16 hours. Avoid light exposure as much as possible.
f. Observe the plates.