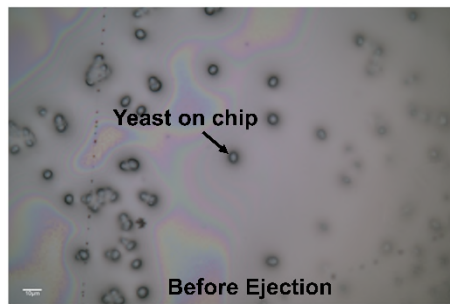


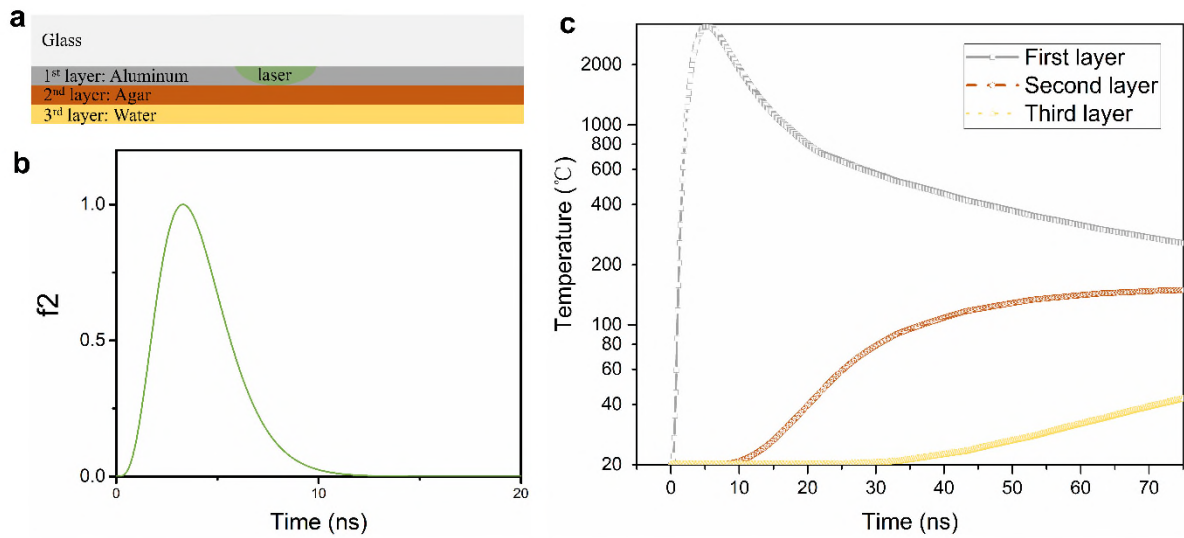
Supplementary information

Isolating and culturing of single cells by laser ejection sorting technology

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Huang^{c,e,*}, Bei Li^{a,f,*}



Supplementary Figure S1. Simple ejection of the cell cannot get cultivable colony.

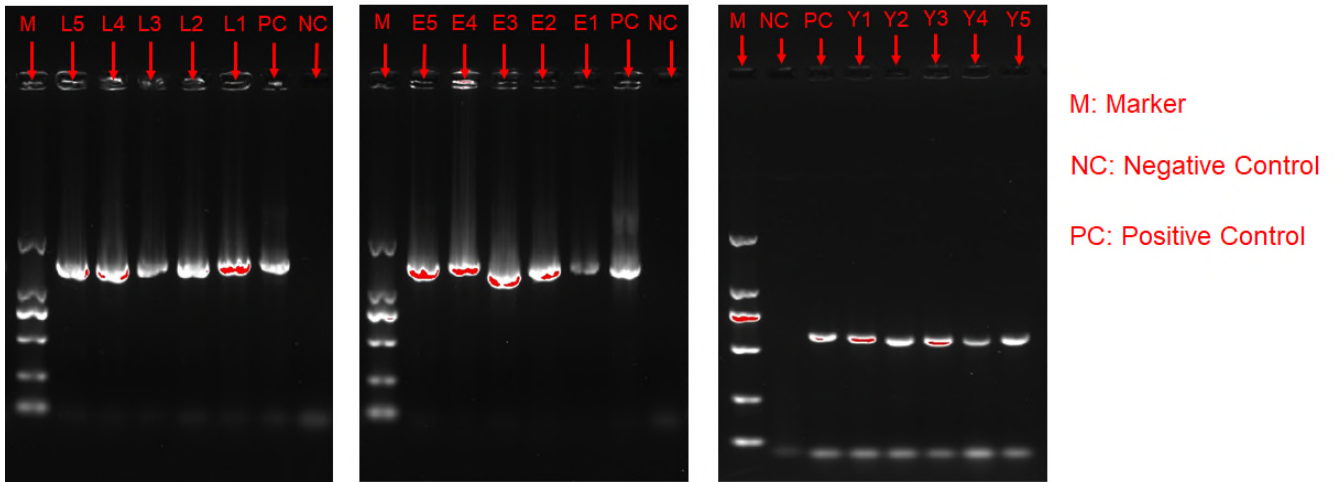


Supplementary Figure S2. Numerical simulation of the temperature on the surface of each layer with Comsol®. (a): Schematic of the FE model. (b): Laser pulse's energy variation along time. The single laser pulse ends at about 10 ns. (c): Temperature variation of each layer along time.

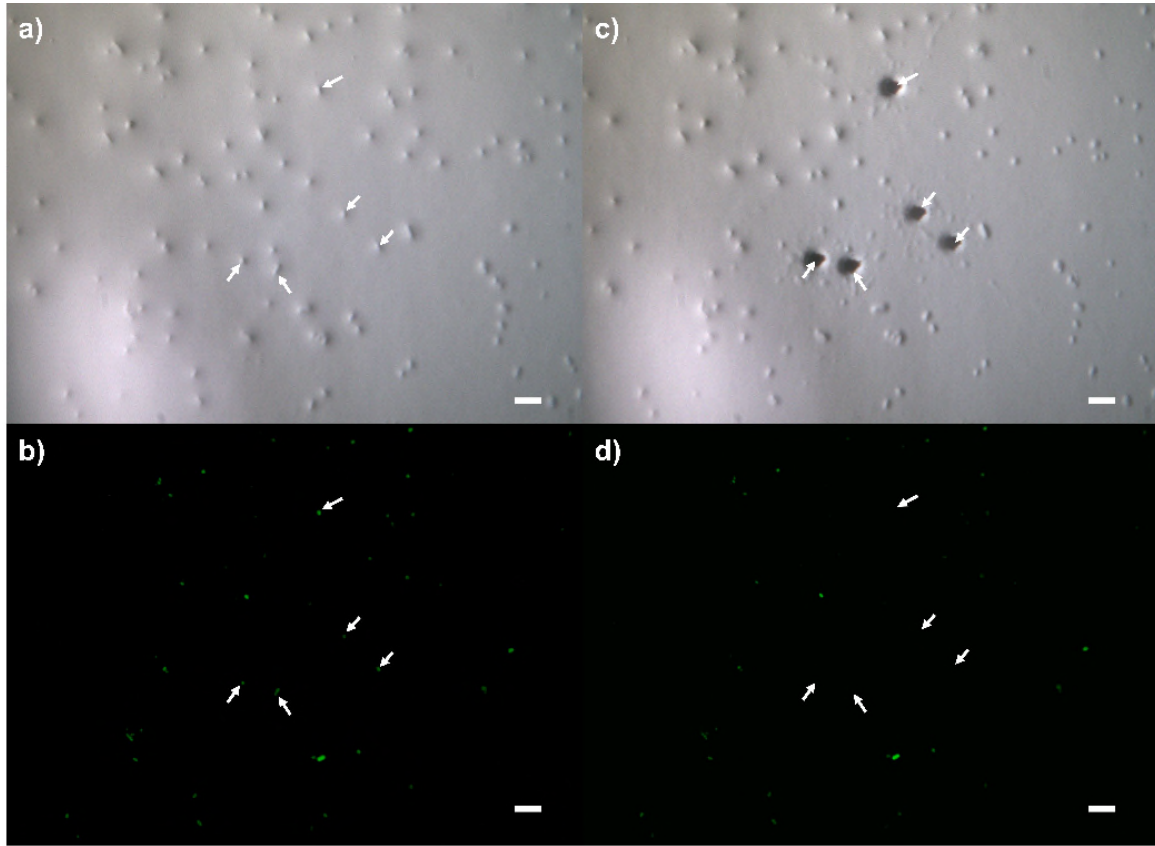
Lactobacillus rhamnosus GG (L)

E. coli (E)

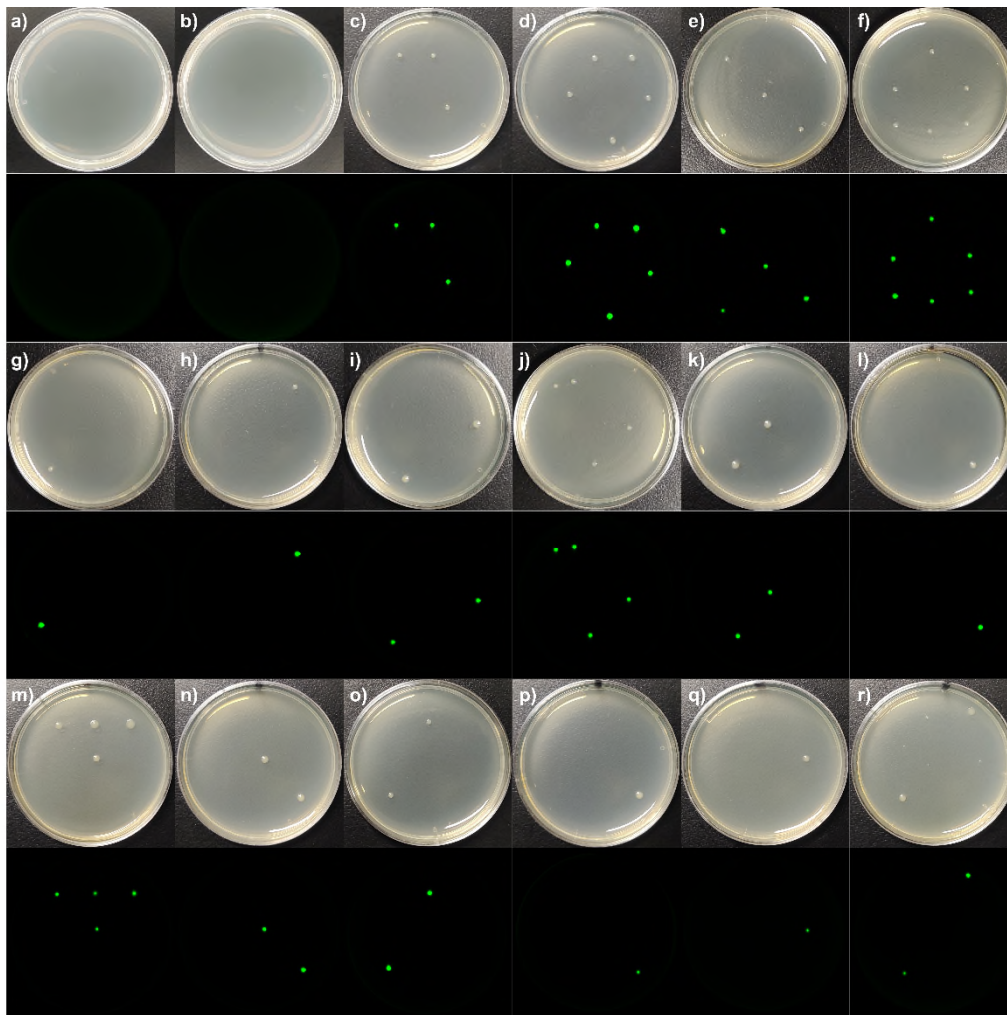
Saccharomyces cerevisiae (Y)



Supplementary Figure S3. PCR results from culturable colonies after single cell ejection. The picture was taken by ChemiDoc™ MP Imaging System (Bio-Rad)



Supplementary Figure S4. Fluorescence isolating single *E. coli* JM109 cells with GFP plasmid from *E. coli* DH5 α by LIFT. a) Image before ejecting. b) Fluorescence image before ejecting. c) Image after ejecting. d) Fluorescence image after ejecting. The bar represents 10 μ m.



Supplementary Figure S5. Culturing results of isolated single Jm109 cells from DH5 α , cultured after 24h, the black picture below each panel is the corresponding fluorescence picture, taken by ChemiDoc MP Imaging System (BIO-RAD) operating on DyLight 488 mode (at 488 and at 532/28).

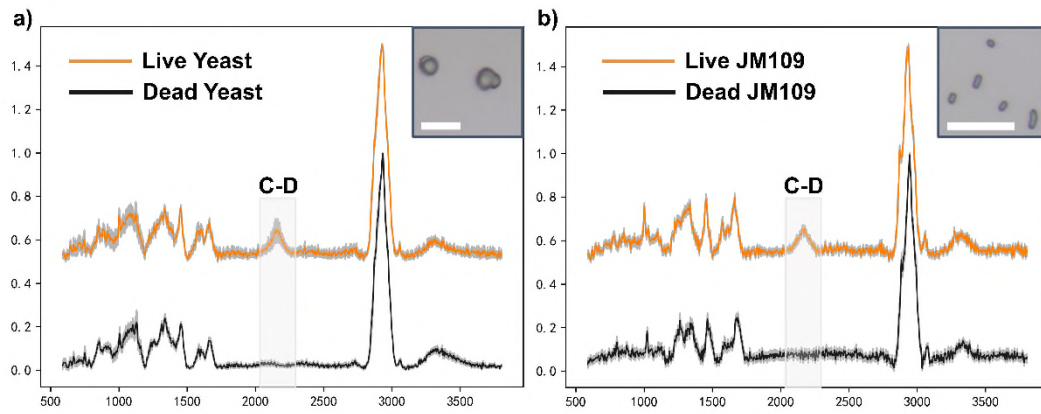
Panel a: control group which was placed in the air. Panel b: control group which ejecting the blank place around the cell and receiving. Panels c-i show the first experiment, we ejected 9 single cells in each petri dish and 81 single cells on 9 petri dishes totally, about 22 colonies grow on 7 petri dishes. Panels j-r show the second experiment, we ejected 9 single cells in each petri dish and 81 single cells on 9 petri dishes totally, about 19 colonies grow on 9 petri dishes. The single cell's recultivation ratio is about 25.3% (41/162).

Score	Expect	Identity	Gap	Strand	Score	Expect	Identity	Gap	Strand	Score	Expect	Identity	Gap	Strand
1365	0.0	72.67(199%)		PlusMinus	1306	0.0	71.67(199%)		PlusMinus	1306	0.0	71.67(199%)		PlusMinus
Query 1	CTCAAGGATTA	CTCAAGGATTA	CTCAAGGATTA	CTCAAGGATTA	Query 4	CTCCAGGATTA	CTCCAGGATTA	CTCCAGGATTA	CTCCAGGATTA	Query 12	CTCAAGGATTA	CTCAAGGATTA	CTCAAGGATTA	CTCAAGGATTA
Subject 164	CTCAAGGATTA	CTCAAGGATTA	CTCAAGGATTA	CTCAAGGATTA	Subject 740	CTCCAGGATTA	CTCCAGGATTA	CTCCAGGATTA	CTCCAGGATTA	Subject 758	CTCAAGGATTA	CTCAAGGATTA	CTCAAGGATTA	CTCAAGGATTA
Query 50	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Query 64	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Query 71	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA
Subject 280	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Subject 690	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Subject 678	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA
Query 120	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Query 134	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Query 131	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA
Subject 462	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Subject 620	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Subject 618	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA
Query 180	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Query 154	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Query 191	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA
Subject 374	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Subject 592	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Subject 608	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA
Query 240	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Query 246	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Query 251	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA
Subject 458	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Subject 592	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Query 498	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA
Query 300	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Query 304	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Query 511	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA
Subject 536	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Subject 612	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Subject 430	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA
Query 360	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Query 366	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Query 371	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA
Subject 624	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Subject 632	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Query 451	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA
Query 420	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Query 426	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Subject 518	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA
Subject 710	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Subject 638	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Query 491	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA
Query 480	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Query 486	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Subject 518	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA
Subject 800	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Subject 644	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Query 519	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA
Query 540	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Subject 650	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Query 520	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA
Subject 886	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Subject 656	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Subject 528	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA
Query 600	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Query 606	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Query 551	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA
Subject 974	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Subject 612	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Subject 499	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA
Query 660	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Query 666	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Query 551	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA
Subject 1062	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Subject 622	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Subject 499	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA
Query 720	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Query 726	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Query 571	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA
Subject 1150	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Subject 628	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Query 578	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA
Query 780	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Query 786	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Subject 578	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA
Subject 1238	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Subject 634	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	Subject 578	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA	TTTCAGGATTA

Supplementary Figure S6. Plasmid sequencing results of the recultivated *E. coli* JM109 (pGFP).s

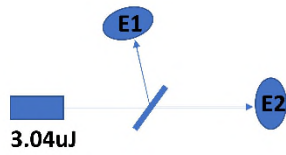
SPARKeasy Superpure Mini Plasmid Kit (SparkJade, AD0102-B) was used for plasmid extraction.

- (1) Pick and transfer 5 colonies on the sorting culture plate to a 3ml culture tube, and culture overnight at 37°C and 200rpm shaking;
- (2) Centrifuge the 3ml bacterial solution cultured overnight, centrifuge at 13000g for 1min, and collect the bacterial sediment;
- (3) Add 250µl of solution P1 to resuspend the bacterial pellet, shake and mix well;
- (4) Add 250µl of solution P2, gently turn up and down and mix 3-5 times, and leave it at room temperature for 4 minutes;
- (5) Add 350µl of solution P3, immediately and gently mix up and down for 7-8 times to produce a white flocculent precipitate;
- (6) Centrifuge at 13000g for 10 min, and collect the supernatant;
- (7) Add the supernatant to the adsorption column AC, put the adsorption column into the collection tube, centrifuge at 13000g for 1 min, and discard the waste liquid.
- (8) Add 500µl of deproteinized liquid PE, centrifuge at 13000g for 1min, discard the waste liquid;
- (9) Add 600µl rinsing solution WB, centrifuge at 13000g for 1min, discard the waste liquid;
- (10) Repeat the previous step;
- (11) Put the AC column into the empty collection tube, centrifuge at 13000g for 2 minutes to remove the WB residual liquid;
- (12) Remove the AC column and put it in a new centrifuge tube, add 30-70µl of eluent EB in the middle of the adsorption membrane, leave it at room temperature for 2 minutes, and centrifuge at 13000g for 1 minute;



Supplementary Figure S7. a) Raman spectroscopy of recultivated yeast. b) Raman spectroscopy of recultivated JM109. The bar represents 10 μm .

The two experiment groups were handled for Raman spectroscopy, as shown in figure, C-D band ($2040\text{--}2300\text{ cm}^{-1}$) exists could prove that the cell's active metabolism.

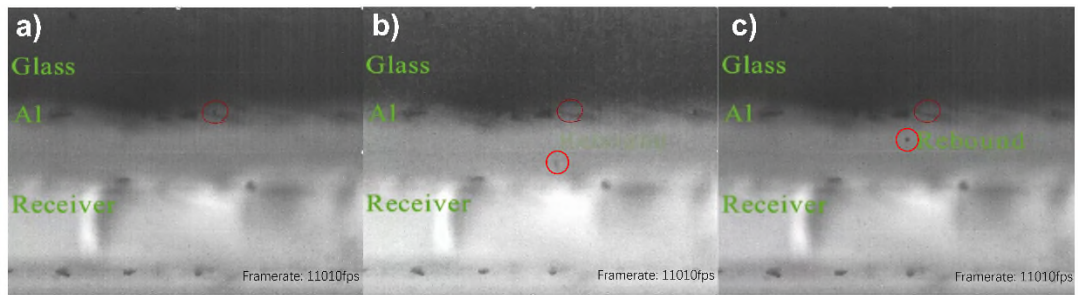
a

Thickness of AL	E1	E2	Absorption (%)
25nm(Domestic)	2.33uJ	124.6nJ	19.26
25nm(Imported)	2.50uJ	51.4nJ	16.07
35nm	2.53uJ	23.0nJ	16.02
45nm(Imported)	2.65uJ	7.8nJ	12.57
45nm(Domestic)	2.53uJ	8.0nJ	16.51
60nm(Imported)	2.60uJ	0	14.47
60nm(Domestic)	2.60uJ	0	14.47
80nm	2.67uJ	0	12.17
100nm(Imported)	2.70uJ	0	11.18
100nm(Domestic)	2.58uJ	0	15.13
150nm	2.71uJ	0	10.86
200nm	2.68uJ	0	11.84

b

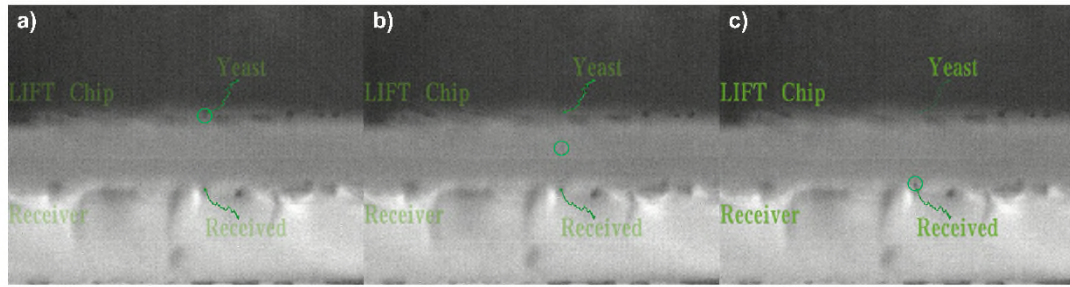
Supplementary Figure S8 (a): The absorption of different thickness of aluminum film;

(b): ejection of three-layer LIFT. I: ejection with inadequate energy, the agar film didn't break and the cell were not ejected successfully, II: two cells were close, III: with a suitable ejecting energy, only one cell was ejected with no broken of agar film.

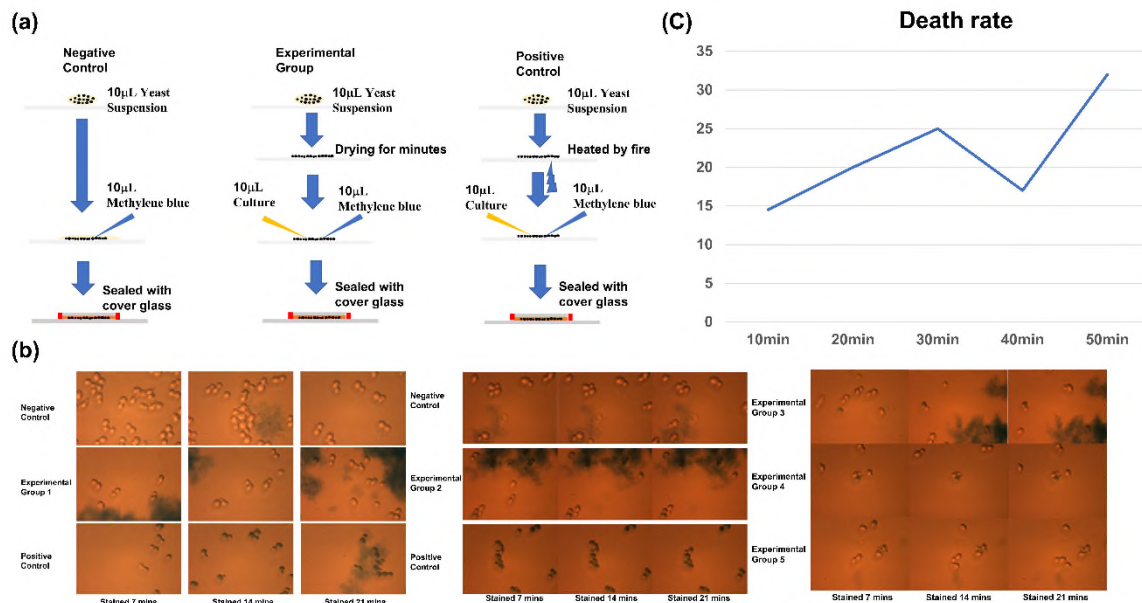


Supplementary Figure S9. a) The yeast cell on the LIFT chip; b) The ejected yeast cell hit the receiver (cover glass); c) The yeast cell rebound to highest position and fall slowly again.

Detail could be seen in supplementary video 1



Supplementary Figure S10. Flying and landing processes of single yeast cell. a): the yeast cell on the LIFT chip; b): the yeast cell is flying in the air between the LIFT chip and receiver; c): the yeast cell lands on the receiver. The velocity could be calculated about 0.35m/s, and the whole process was shown in supplementary video 2.



Supplementary Figure S11. The death rate of yeast cell drying with time on LIFT chip.

a): diagram of the experiment setup: three groups (Negative group: 10 μ L yeast suspension stained with 10 μ L 200mg/L Methylene blue; Experimental group: 10 μ L yeast suspension drying for 10, 20, 30, 40, 50 minutes respectively, stained with 10 μ L culture and 10 μ L 200mg/L Methylene blue; Positive group: 10 μ L yeast suspension heated with fire for 20 seconds, stained with 10 μ L culture and 10 μ L 200mg/L Methylene blue;) of yeast cells were stained on LIFT chip, sealed with cover glass and recorded under microscope.

b): picture of each group of cells after stained 7, 14, 21 minutes.

c): death rate curve of yeast cell with drying time (count according to video recorded)

Supplementary Table S1. Physical parameters of materials

Physical parameters of materials	Aluminum	Agar ($w = 0.1$)	Water
ρ: density (kg/m^3)	2700	1065	1000
k: thermal conductivity ($W/m.K$)	238	0.566	0.599
C_p: heat capacity ($J/kg.K$)	900	4200	4200

Supplementary Table S2. Statistics of *S. cerevisiae*, *E. coli* and LGG's culturing results

Number of ejected cells in each microwell	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Recovery rate	
<i>S. cerevisiae</i>	1	3	5	5	7	6	
	1	5	6	6	7	7	63%
<i>E. coli</i>	1	4	1	1	2	1	
	1	5	1	2	2	1	22%
<i>L. rhamnosus</i> GG (LGG)	1	6	5	2	5	5	
	1	9	9	8	9	9	74%