Modified U- tube for Ruling out Naked DNA Transfer during Conjugation and application in Antibiotic Resistance Genes Transfer Research

Ning Zhang[a], Xiang Liu[a*], Bing Li[b], Limei Han[a], Xuejiao Ma[a,c], Fanbin Meng[a], Miao Li[a*]

Contents：

Text S1

Leakage tests of the modified U-tube were performed using water and *P. putida* vs sterile LB medium, respectively. 80 ml distilled water was poured into the donor flask of the U-tube, then standing for 12 hours to observe the liquid leakage from the flange and filter. The compared images were presented in figure S1-A. Compared to Figure S1-A1, there was no liquid leakage from the U-tube. The volume of distilled water in the donor flask was still 80 ml. After the initial verification test, *P. putida* and sterile LB medium were injected into the donor and recipient flask, respectively. Pump was used to assist the exchange of liquid in the system. After 4 hours samples from the donor and recipient flask of the U-tube were inoculated on selective medium containing 50 mg/L kanamycin to observe the cell transfer. AsFigure S1-B shows, there was no colony on the plate, which mean the *P. putida* cannot pass from the donor flask to the other.

Text S2

PCR assays were conducted in a Bio-Rad C1000 gradient thermal cycler (Bio-Rad, USA) to verify whether plasmid RP4 (the presence of *tra*F and *aph*A genes) had been transferred into recipient. The total volume of PCR mixture was 20μL, containing 10μl PCR mix (TIANGEN, China), 7μL ddH_2O, 1μLof each 10μM primer, and 1μl DNA template. The PCR procedure for *tra*F and *aph*A gene amplification was: initial denaturation at 95℃ for 3 min; 40 cycles of 30 s at 95℃, 30 s of annealing at a specified temperature for each gene, and extension for 1 min at 72℃; with a final

extension at 72℃ for 5 min. The nucleotide sequence of PCR primers and annealing temperatures are shown in Table S1 [1]. The PCR products were determined by comparison with DNA standards (Marker Ladder, TIANGEN, China) on 1.5% agarose gels run for 30 min at 120 V.

Text S3

FCM and CLSM were used to sort and identify donor, recipient and transconjugant (or transformant) by fluorescence. In preparation for FCM, 1 mL samples were centrifuged (10000 g for 1 min), and then resuspended in PBS. Flow cytometric detection of cells was carried out using a FACS Calibur system (BD, USA). The excitation wavelengths of GFP and RFP were 488 nm and 561 nm, respectively [2]. A flow rate of 1 μl/s was chosen, and 10000 events/s were counted. Bacterial populations were gated according to GFP vs. RFP plots. *P. putida* and *E. coli* were used as positive and negative controls, respectively. BD FACS Diva software was used for data acquisition, and subsequent analysis was performed on FlowJo v.10 software. For CLSM, samples (100 μl) were performed on Olympus FV3000, Japan, which were added to the confocal plate and excited with a 488 nm and 561 nm diode-pumped solid-state laser. Fluorescence was observed using an Olympus UApo N 100×1.49 numerical aperture oil-immersion objective lens with an extra 3.0× intermediate magnification lens with the same zoom and different Z dimensions. The images were processed using ImageJ software v.1.47 and Adobe Photoshop 7.0.

Figure S1

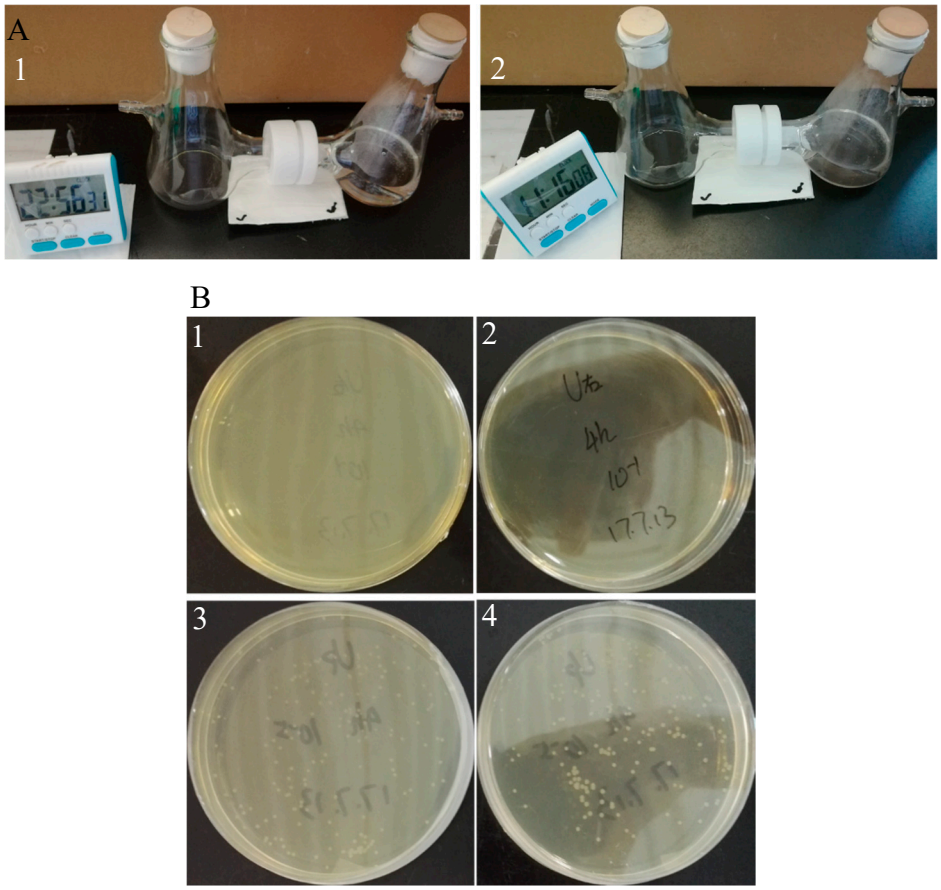


Figure S1. The filter sealing verification, A Liquid leakage, A1 original state after water poured, A2 state of the system after 12 hours; B plate counting of *P. putida*, B1-2 colony in recipient flask after 4 hours, B3-4 colonies in donor flask after 4 hours

Figure S2

Bands of gene *tra*F in samples from recipient flask of test 1# at 180 min, test 3# at 180 min and test 4# at 180 min were sent for sequence analysis. The sequences were corrected by blasting to Genbank. Sequence results were shown in Figure S2.

(A)

Gene sequence:

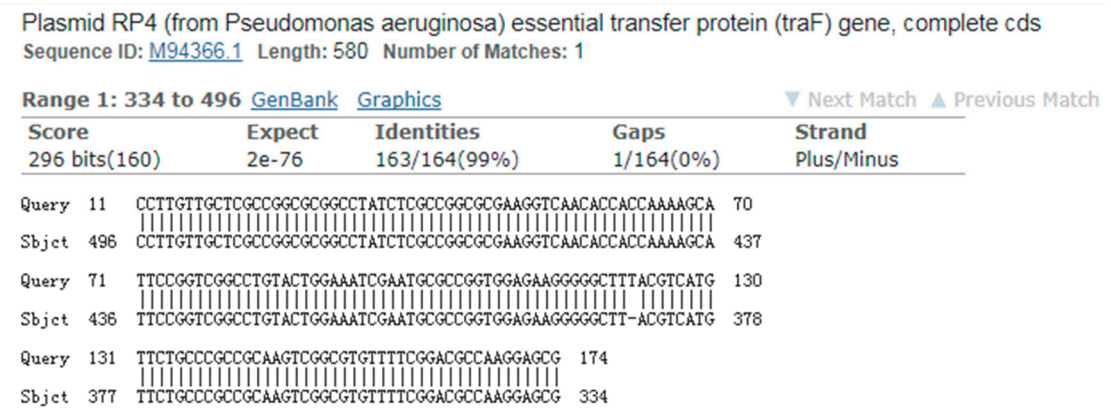
CGGGGTTTGTCCTTGTTGCTCGCCGGCGCGGCCTATCTCGCCGGCGCGAAG

GTCAACACCACCAAAAGCATTCCGGTCGGCCTGTACTGGAAATCGAATGC

GCCGGTGGAGAAGGGGGCTTTACGTCATGTTCTGCCCGCCGCAAGTCGGC

GTGTTTTCGGACGCCAAGGAGCGTATCTGGCGCCAGACGCAG

NCBI blasting:



(B)

Gene sequence:

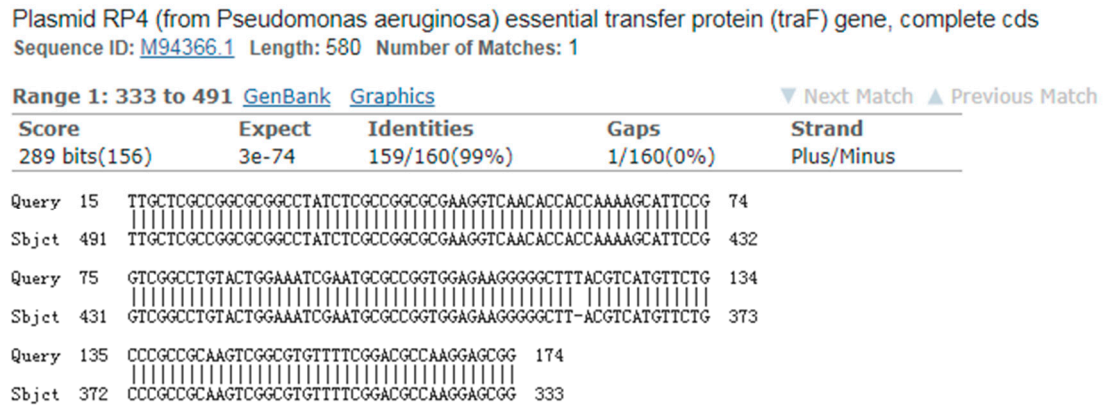
CTCCGATGGAGGCCTTGCTCGCCGGCGCGGCCTATCTCGCCGGCGCGAAGG

TCAACACCACCAAAAGCATTCCGGTCGGCCTGTACTGGAAATCGAATGCGC

CGGTGGAGAAGGGGGCTTTACGTCATGTTCTGCCCGCCGCAAGTCGGCGT

GTTTTCGGACGCCAAGGAGCGGAGGGGGCAGATGTGATCGA

NCBI blasting:



(C)

Gene sequence:

CGCATCGTCTCCTTGTTGCTCGCCGGCGCGGCCTATCTCGCCGGCGCGAAG

GTCAACACCACCAAAAGCATTCCGGTCGGCCTGTACTGGAAATCGAATGCG

CCGGTGGAGAAGGGGGCTTTACGTCATGTTCTGCCCGCCGCAAGTCGGCG

TGTTTTCGGACGCCAAGGAGCGGGGCTACATCGCCGGCGGTT

NCBI blasting:

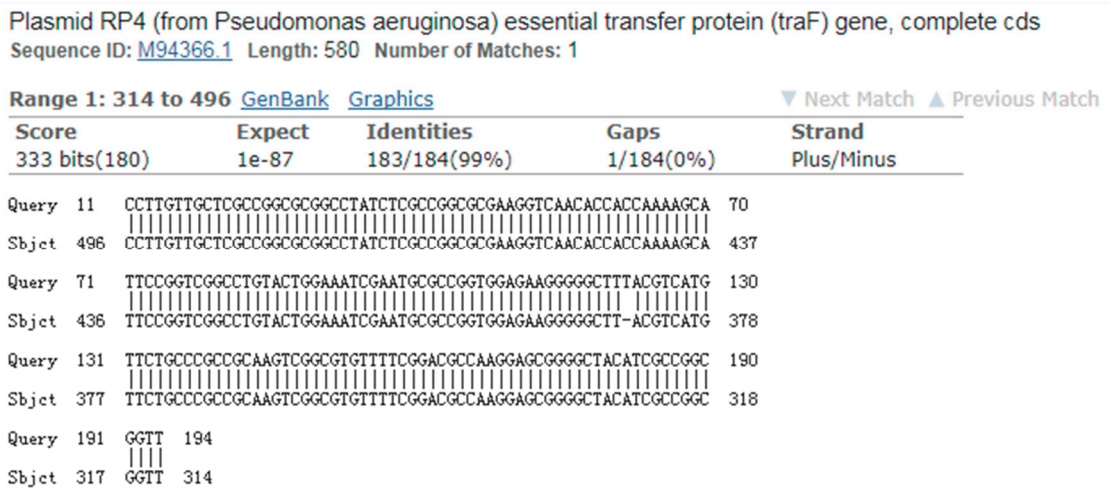


Figure S2. *tra*F gene sequence analyses of gel bands at 180min, (A) *tra*F sequence of test 1#; (B) *tra*F sequence of test 3#; (C) *tra*F sequence of test 4#

Table S1

Table S1 PCR primers and PCR conditions

Primer	Target	Sequence (5'-3')	PCR annealing temp (ºC)	Amplicon size (bp)
*aph*A-FW	*aph*A	GGCTTCGTGATGCCTGCTT	62	198
*aph*A-RV		CATTCCTGGCCGTGGTTCT		
traF-FW	*traF*	CTCCGATGGAGGCCGGTAT	54.1	196
traF-RV		GGGAATGCCATCTGCCTTGA		

Table S2

Table S2 Statistical test of donor and donor/receptor

Test	Donor			Donor/receptor		
	mean value	square deviation	P-value	mean value	square deviation	P-value
7#	5786.16	332265.3	7.95E-04	10.06	0.90	5.95E-05
8#	2306	102529		0.33	0.003	

References

[1] Wang, Q.; Lu, Q.; Mao, D.; Cui, Y.; Luo, Y. The horizontal transfer of antibiotic resistance

genes is enhanced by ionic liquid with different structure of varying alkyl chain length. *Front Microbiol*, 2015, 6, 864.

[2] Dunny, G.M.; Craig, R.A.; Carron, R.L.; Clewell, D.B. Plasmid transfer in Streptococcus faecalis: production of multiple sex pheromones by recipients. *Plasmid.* 1979, 2, 454-465.