RE: Editorial comments:

NOTE: The manuscript has been modified to include minor grammar and formatting changes. The updated manuscript 57812\_R1.docx is located in your Editorial Manager account. In the revised PDF submission, there is a hyperlink to download the .docx file. Please download the .docx file and use this updated version for future revisions. The file is also attached.

Thank you very much for your handling for our manuscript. We carefully rewrote the manuscript based on the comments from the editor and reviewers. We added Table 1 to show bacterial strain and plasmids for this study, and former Table 1 was renamed as Table 2 in the revised manuscript.

• The manuscript will benefit from thorough language revision as there are a number of grammatical errors throughout. Please thoroughly review the manuscript and edit any errors.

The revised manuscript was edited by professional English editing services (editage.com).

• Protocol Detail:

1) 4.3: Please describe all button clicks and menu selections.

Because we replaced the commercial name of FACS into generic one (L303-307), the procedures with Summit software were also removed in the revised manuscript.

2) 3.8: By naked eye?

This might be for 4.8, and yes, by naked eye (L324).

• Protocol Highlight:

1) Please ensure that the manuscript title best reflects the filmable content (i.e. the portions you highlight).

We slightly change the title in the revised manuscript.

2) The highlighted steps should form a cohesive narrative, that is, there must be a logical flow from one highlighted step to the next.

3) Notes cannot be filmed and should be excluded from highlighting.

4) Please bear in mind that software steps without a graphical user interface/calculations/ command line scripting cannot be filmed.

5) We cannot film in 2 different locations, so I suggest excluding the FACS steps (which would need to be filmed in Riken from highlighting), while ensuring that the above conditions are met.

We excluded notes from highlighting and the FACS steps in the revised manuscript.

• References: Please edit your references to comply with JoVE instructions for authors. Citation formatting should appear as follows: (For 6 authors or less list all authors. For more than 6 authors, list only the first author then et al.): [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. Source. Volume (Issue), FirstPage – LastPage, (YEAR).]

We edited our reference lists according to the JoVE instructions.

• Commercial Language: Please replace the commercial names [MoFlo XDP IntelliSort II instrument (Beckman Coulter), CyClone, Summit software (Beckman Coulter), with generic alternatives.

We replaced the instrument name into generic one (L303-307).

• If your figures and tables are original and not published previously or you have already obtained figure permissions, please ignore this comment. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."

The figure in the present manuscript was originally made.

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RE:Reviewer #1:

Manuscript Summary:

The revised manuscript now breaks down the protocol into a number of very clear sections that describe the tagging of plasmids, checking that the plasmids still transfer and then estimating the transfer rate parameters.

Thank you very much for your reviewing and comments.

Major Concerns:

The language of the manuscript still needs improvement.

The revised manuscript was edited by professional English editing services (editage.com).

Minor Concerns:

The title for sections 3 and 4 are identical but the methods are different. The titles needs to be revised.

The title of section 3 was changed into “Preparation for estimation of the probability of donor-initiated conjugation”.

RE:Reviewer #2:

The clarity of the paper has significantly improved compare with the first version. Few grammatical errors and typos need to be fixed.

Thank you very much for your reviewing and comments. The revised manuscript was edited by professional English editing services (editage.com).

RE:Reviewer #3:

I appreciate the authors' effort to include additional step-by-step details of the protocol in the revised manuscript. The added details improved the clarity of the core protocols (Protocol 2, 3, and 4) in the manuscript.

Thank you very much for your reviewing and comments.

However, the description of protocol 1 was poorly written and difficult to follow. I had to go back to the original submission to figure out the objectives of these steps. I recommend re-write this section combining the big picture/goal-orientated description writing style from the original submission and the step-by-step style in this revised version. A table listing all the strains used and their purpose would be greatly helpful. So is adding a summary statement to clearly describe the purpose of each experiment. For example, the goal for protocol 1.1 could be something like 'generating a donor strain P. putita SMDBS carrying the pPB136::gfp plasmid'.

Currently it reads the goal is to generate a plasmid, which indicates the end product of this protocol is a plasmid DNA. This is clearly not the case.

Thank you for your important comments. Steps 1.1 and 1.2 were written for construction of tagged plasmids, and 1.3 was for preparing donors in next steps. We added brief sentences to show goal of each step (L92-93, 158, 189-190) and Table 1 to show lists of strains and plasmids used in the present study.

The revised manuscript still needs significant editing to improve its English. Professional editing is highly recommended.

The revised manuscript was edited by professional English editing services (editage.com).