



Assessing the permissiveness of complex bacterial communities towards conjugal plasmids – Development of a novel method

Klümper, Uli; Riber, Leise; Sannazzaro, Analia; Dechesne, Arnaud; Musovic, Sanin; Hansen, Lars H.; Sørensen, Søren J.; Smets, Barth F.

Publication date:
2013

Document Version
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

Citation (APA):
Klümper, U., Riber, L., Sannazzaro, A., Dechesne, A., Musovic, S., Hansen, L. H., Sørensen, S. J., & Smets, B. F. (2013). Assessing the permissiveness of complex bacterial communities towards conjugal plasmids – Development of a novel method. Poster session presented at 12th Symposium on Bacterial Genetics and Ecology, Ljubljana, Slovenia.

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Assessing the permissiveness of a bacterial community towards conjugal plasmids

Uli Klümper^a, Leise Riber^b, Analia Sannazzaro^b, Arnaud Dechesne^a, Sanin Musovic^a, Lars H. Hansen^b, Søren J. Sørensen^b, Barth F. Smets^a

^aTechnical University of Denmark, DTU Miljø, Kgs. Lyngby

^bUniversity of Copenhagen, Section of Microbiology, Copenhagen



Introduction

A crucial parameter governing horizontal gene transfer (HGT) in complex bacterial communities is community permissiveness. The permissive fraction of a microbial community is defined as that fraction able to receive a given plasmid¹.

Permissiveness in complex, natural communities has not been extensively studied at the quantitative or at the phylogenetic level because no suitable methods have been available.

Objective

To develop a new method for assessing the permissiveness of a soil microbial community towards an introduced conjugal plasmid which allows one to:

1. Estimate transfer frequency
2. Isolate and taxonomically identify the transconjugants

Materials and Methods

3 green fluorescent tagged plasmids:

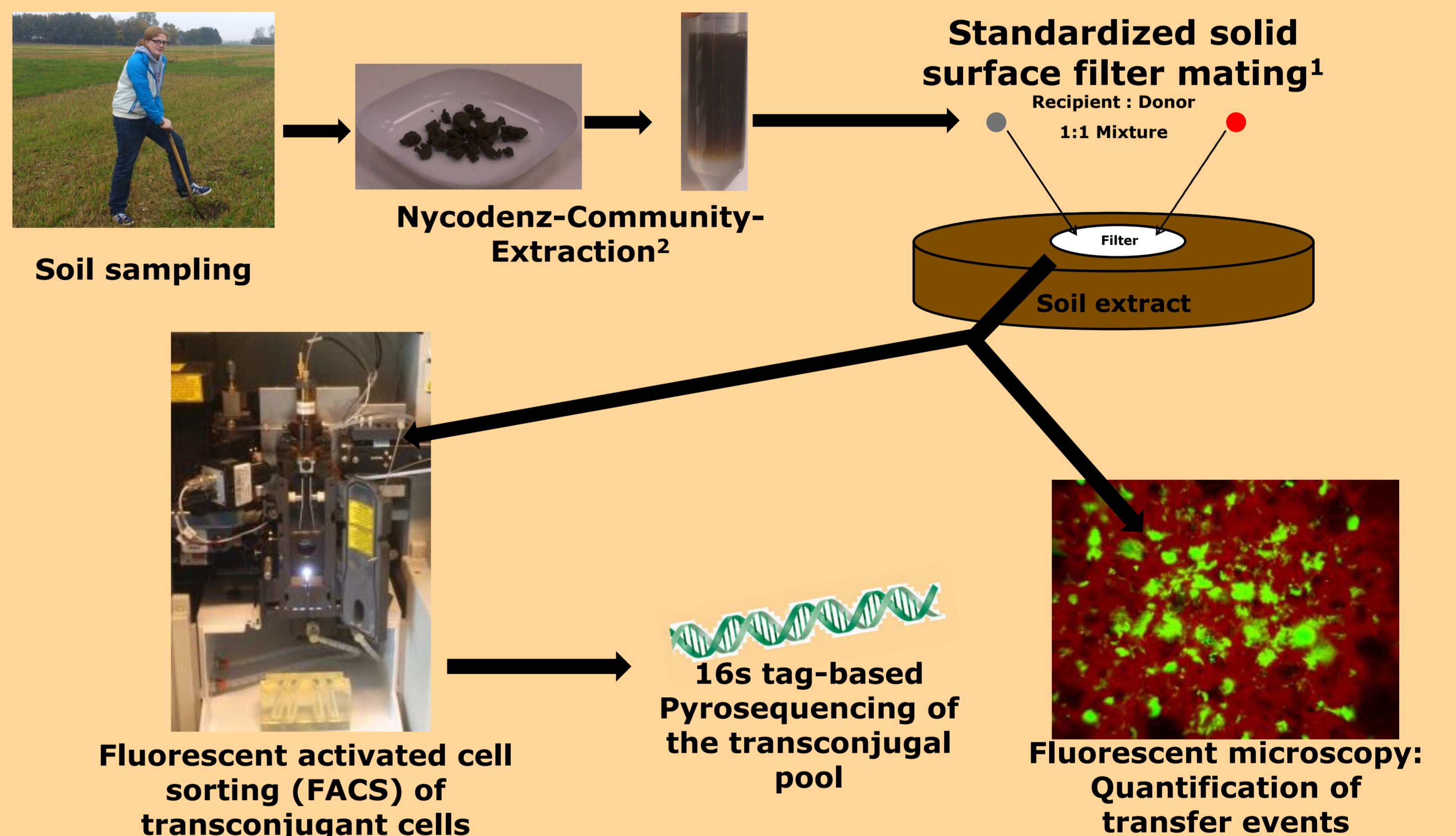
Plasmids	Inc-group	Phenotype	host range
RP4::Plac::gfp	IncP-1	resistance	broad
pIPO2T::Plac::gfp	IncQ-mobilizer	cryptic	broad
pKJK5::Plac::gfp	IncP-1	catabolic	broad

3 gfp-repressing red fluorescent donor strains:

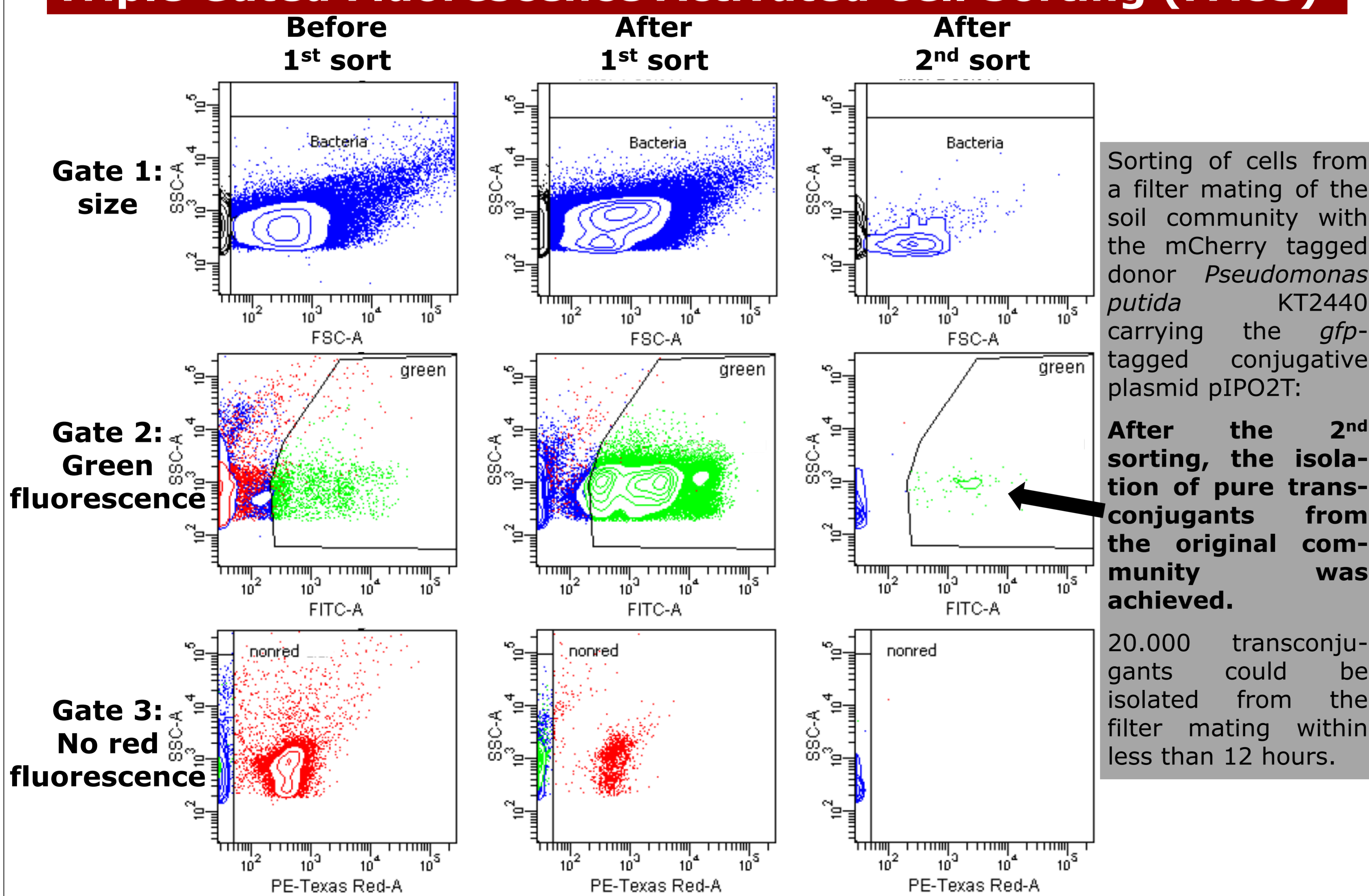
- Pseudomonas putida* KT2440::lacIq-pLpp-mCherry
- Pseudomonas putida* KT2440::lacIq-pLpp-DsRed
- Kluyvera* spp. KT2440::lacIq-pLpp-mCherry
- Escherichia coli* KT2440::lacIq-pLpp-mCherry

1 recipient community:

Soil origin	Soil type	Soil treatment
CRUCIAL, Taastrup, Denmark	agricultural	untreated



Triple Gated Fluorescence Activated Cell Sorting (FACS)



The transconjugants are first enriched and then isolated from the filter matings in two consecutive sorts:
1. raw sorting at high speed to enrich the transconjugants from the whole community
2. fine sorting at low speed to purify the sorted transconjugant fraction

Plating of transconjugal pool

Filter mating		Colonies analyzed		
mCherry Donor	gfp Plasmid	Green Transconjugants	Red Donors	Colorless
<i>P. putida</i>	RP4	41	0	1
<i>P. putida</i>	pIPO2T	50	0	1
<i>P. putida</i>	pKJK	20	0	0
<i>E. coli</i>	pKJK	52	0	0
<i>Kluyvera</i>	pKJK	47	0	0

After 2nd sort, cells were plated on R2A medium and checked for green fluorescence by stereo microscopy: All donor cells have been eliminated by FACS sorting. The transconjugal pool has been enriched from as little as 0.2% of the total community to 97-100%.

Conclusions

- High-throughput isolation of transconjugants from a complex microbial community is possible.
- The number of isolated transconjugants is sufficient for subsequent pyrosequencing.
- The new method allows one to assess the permissiveness of a soil microbial community simultaneously at the quantitative and at the taxonomic levels.