ISOLATION OF UNCULTIVABLE ENDO- OR ECTOSYMBIOTIC BACTERIA OUT OF FLAGELLATED PROTISTS IN TERMITE GUT BY MEANS OF LASER NANODISSECTION.

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INTRODUCTION
Termites harbor a symbiotic gut microbial community that is responsible for their ability to thrive on recalcitrant plant matter. The community comprises diverse microorganisms, most of which are as yet uncultivable [1]. In order to acquire complete genome sequence with minimal genomic variations material from a single protist cell is required [2, 3]. Here we report the successful removal of bacteria out of flagellated protists combining laser assisted excision and extraction of excised cells using a laser nanodissection system and a micromanipulator setup.

MATERIALS AND METHODS
We used CellSurgeon laser nanodissection system (ROWIAK) in combination with a DC3-KS micromanipulator (Märzhäuser) and IM-9B microinjector (Narishige) with 10 µm microcapillaries. The analyzed protist Trichonympha agilis was supplied by the group of Y. Hongoh. After DAPI staining the excision and collection of endosymbiotic bacteria localized just beneath the cell membrane of the protist was performed.

RESULTS
Flagellated protists were imaged in multi photon laser scanning mode to determine their x-, y- and z-dimensions. The parameters established were then used to neatly excise the bacterial clusters. Excised clusters were then imaged in epifluorescence mode and aspirated by means of microinjector.

CONCLUSION
It was possible to precisely excise uncultivable bacteria. So they were then further processed for whole genome amplification and circular chromosome reconstruction.

REFERENCES