Cut and stretch assay reveals resistance genes

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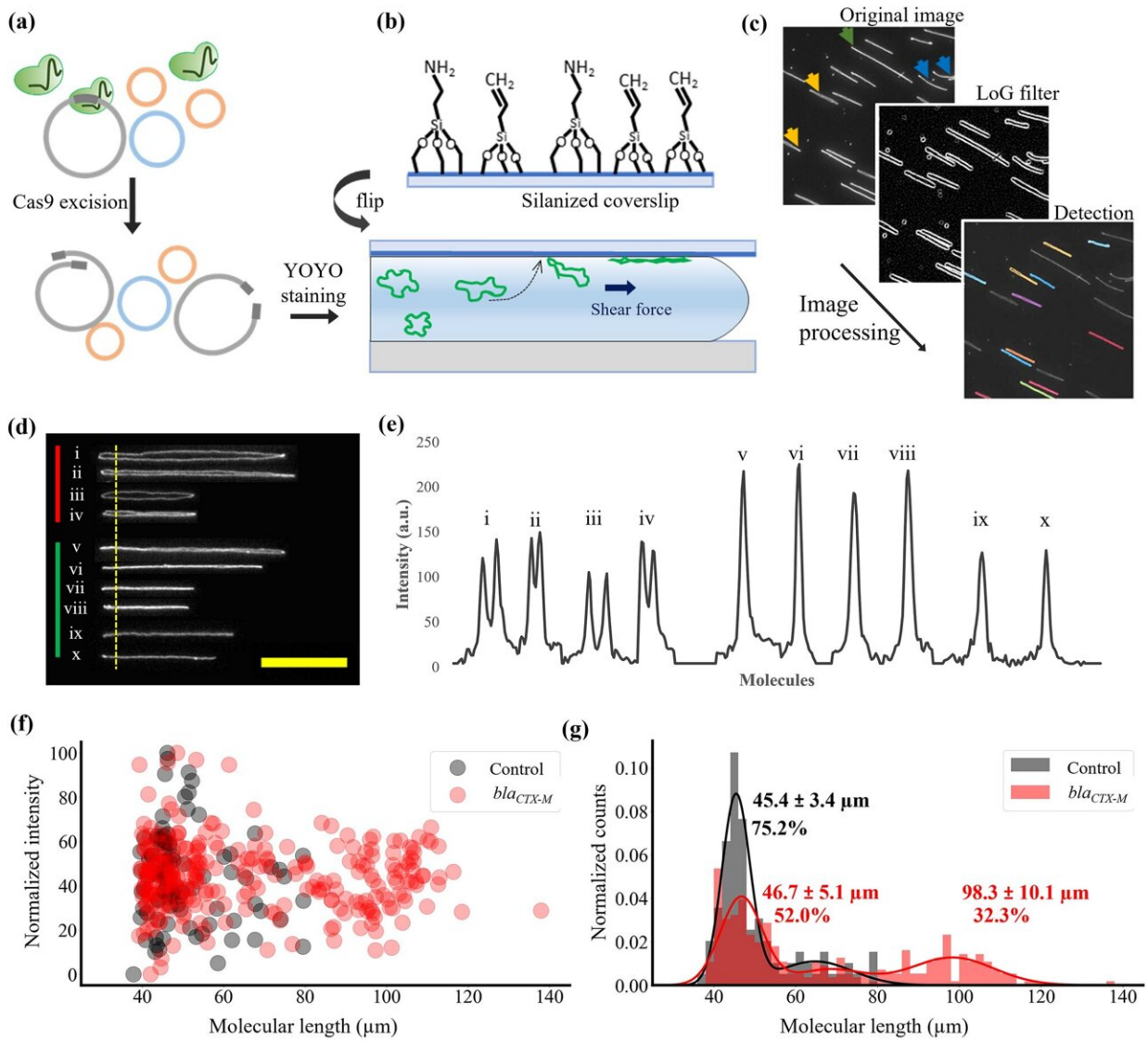

Experimental workflow. (a) Cas9-gRNA is added to the plasmid sample. If the AMR gene is present, Cas9 makes a double-stranded excision, resulting in plasmid linearization. (b) Glass coverslips are functionalized with a mixture of

silanes with amine and vinyl terminal groups. DNA molecules are stained with YOYO-1 and stretched by capillary force between the silanized coverslip and a glass slide. (c) Image processing to detect molecules. A Laplacian of Gaussian (LoG) filter is used for edge detection and molecular length, width, eccentricity, and straightness filters are used to accept or reject the detected molecules. Examples of molecules not accepted are marked with yellow (too wide), blue (too curved) or green arrows (overlapping molecules), respectively. (d) Representative images of molecules observed during imaging. Molecules i-iv (red bar) are rejected and molecules v-x (green bar) are accepted. (e) Intensity profile for molecules in (d) along the dotted yellow line. Intensity is higher where two strands of the circular plasmid are localized together. Scale bar 15 μm. (f) Length vs. intensity plot for a sample carrying a single plasmid. Data for Cas9 targeting the blaCTX-M gene is shown as red dots and control as black dots. (g) Length histograms of the data in (f). Credit: *Scientific Reports* (2022). DOI: 10.1038/s41598-022-13315-w

Which antimicrobial resistance genes are present in bacteria, for example in a hospital ward? For laboratories with limited financial resources characterizing bacterial DNA is difficult, as this often requires expensive equipment. Researchers at Chalmers have now developed a method that can detect specific bacterial genes that encode resistance using standard microscopes, which are already used to diagnose tuberculosis in low-income countries.

Antimicrobial resistance is one of the major health threats globally, as common infections no longer respond to antibiotics. This may result in severe illness and death, for example in neonatal sepsis, i.e., severe bacterial blood infections in new-born children.

The genes conferring resistance to bacteria, for example by breaking down antibiotics, are often found on plasmids, the circular DNA molecules that do not belong to the chromosomal bacterial DNA.

Plasmids can transfer between bacterial strains and species and can thus spread rapidly in a bacterial population.

Microscope already present in many labs

"Effective and simple methods are needed to characterize bacterial plasmids and detect resistance genes when an infection spreads in hospitals. This is a problem for laboratories with limited resources as existing methods require expensive equipment," says Fredrik Westerlund, Professor of Chemical Biology at Chalmers.

Thanks to a tuberculosis diagnosis program, many laboratories in low- and middle-income countries are already in possession of standard fluorescence microscopes. This was the starting point for Fredrik Westerlund's research group. They based their newly developed method on these microscopes, which are present in the hospital laboratory of their collaboration partners in Dar es Salam, Tanzania.

Linear DNA molecule can be detected

To find specific genes, the researchers use the so-called gene scissors, CRISPR-Cas9, which can recognize and cut DNA strands at any predetermined sequence, so unique that specific genes can be found.

"If a resistance gene is present on the plasmid, it will be cut by Cas9. The DNA is then stretched on a glass slide and imaged with fluorescence microscopy, and the linear molecule can be detected. The images for analysis, can be acquired by a regular smartphone, which you can easily attach to the microscope eyepiece," says Gaurav Goyal, a postdoc in the research group.

'Any microbiological lab can perform this plasmid

analysis'

Gaurav Goyal explains that the method is currently intended for epidemiological studies—to characterize bacterial plasmids and to understand the spread of antibiotic resistance. It might for example be relevant to examine how many new-borns in a hospital ward that carry bacteria with resistance genes. In the long run, it could also be used for diagnosis.

"We started to develop the method for laboratories with limited resources, but any microbiological lab can perform this plasmid analysis—and get relevant results. In addition to finding resistance genes on plasmids, the method can also be used to determine the size and the number of the plasmids in a sample. Our method is simple and faster than other methods, which can be useful in modern microbiology labs in high-income countries too," says Fredrik Westerlund.

The research was published in *Scientific Reports*.

More information: Gaurav Goyal et al, A simple cut and stretch assay to detect antimicrobial resistance genes on bacterial plasmids by single-molecule fluorescence microscopy, *Scientific Reports* (2022). DOI: 10.1038/s41598-022-13315-w

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