Supplementary Figure 5. Determination of sensitivity of the PASS assay by serial dilution of target molecules

WEAU.wt molecules (100,000, 10,000, 1,000, 100 and 10) were embedded in each gel (a). The actual genome numbers detected in the PASS assay are shown in parenthesis. After PCR amplification, the polonies were sequenced with E44D primer using Cy5-dATP. When 10,000 or more molecules were used for analysis, many spots tended to fuse to each other and were difficult to count, while the spots were easily and accurately countable when less than 10,000 molecules were used. When one or no copy was used in the assay, we did not detect any polonies (data not shown). The arrows indicate two polonies detected in the 10-molecule image. The experiments were repeated six times. The linear regression analysis were performed and plotted (b). The error bars stand for mean ± SD. The spot numbers could not be reliably counted when 100,000 or more molecules were analyzed. Therefore, they were not included for estimation of assay sensitivity. Since the acrydite-modified primers were immobilized in the acrylamide gel and the PCR was carried out on a solid phase condition within the acrylamid gel, it was not surprising that not every expected molecule was detected in the PASS assay.