

Supporting Information for

Addressing the requirements of high sensitivity single-molecule imaging of low-copy number proteins in bacteria

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SI Movie Captions

Movie S1. *B. subtilis* PY79 imaged under 561-nm laser excitation as described in Figure 1.

Movie S2. 3D localizations of fluorescent impurities in *B. subtilis* PY79 as described in Figure 2.

Movies S3-S8. Other Bacillus sp, *E. faecalis*, and *E. coli* MG1655 imaged under 561-nm laser excitation as described in Figure 3. **S3:** *B. megaterium*; **S4:** *B. thuringiensis*; **S5:** *B. cereus*; **S6:** *B. licheniformis*; **S7:** *E. faecalis*; **S8:** *E. coli* MG1655.

Table S1. Bacterial strains used in this study.

Strain Name	Genotype	Source
<i>B. subtilis</i> PY79	Wild type	BGSC [†]
<i>B. subtilis</i> HT01	<i>pamcherry-recO</i>	This work
<i>B. subtilis</i> ERB81	<i>amyE::P_{xyI}-recO-mCitrine(mls^R)</i>	This work
<i>B. subtilis</i> PG603	<i>recO-yfp</i>	Kidane et al. 2004
<i>B. megaterium</i> QM B1551	Wild type	BGSC [†]
<i>B. thuringiensis</i> HD735	Wild type	BGSC [†]
<i>B. cereus</i> ATCC 10987	Wild type	BGSC [†]
<i>B. licheniformis</i> ATCC 14580	Wild type	BGSC [†]
<i>E. faecalis</i> V583	Wild type	Kevin Wood
<i>E. coli</i> MG1655	Wild type	CGSC [‡]

[†]BGSC: Bacillus Genetic Stock Center

[‡]CGSC: Coli Genetic Stock Center