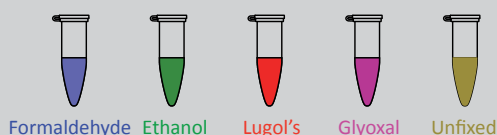


1 Influence of cell fixation on signal intensity and sequencing quality

a



Maribacter forsetii - 34.3%
Gramella forsetii - 36.6%
Escherichia coli - 50.6%
Micrococcus sp. - 73.0%



HCR-FISH (Yamaguchi et al.)
Signal intensity measurement (flow cytometry)
and cell sorting (100 and 500 cells)

b



Maribacter forsetii - 34.3%
Gramella forsetii - 36.6%
Micrococcus sp. - 73.0%

X

MDA (100 and 500 cells)

X

c



Maribacter forsetii - 34.3%
Gramella forsetii - 36.6%

★

Sequencing (100 and 500 cells)

➡ Ethanol best cell fixative and 500 cells superior to 100 cells.

2 HCR-FISH optimization



Maribacter forsetii - 34.3%
Gramella forsetii - 36.6%
Escherichia coli - 50.6%
Micrococcus sp. - 73.0%

Tested parameters:

Denaturation (55-85°C)
Hybridization buffer
Amplification times



Signal intensity measurement (microscopy)

➡ Optimized HCR-FISH protocol

3 Validation of optimized protocol on isolates



Gramella forsetii - 36.6%
Shewanella oneidensis - 46.0%
Pseudomonas putida - 62.2%

Fixation



HCR-FISH (optimized)



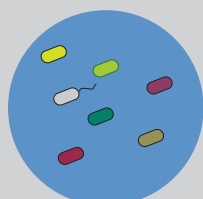
Sorting 500 cells



MDA and sequencing

➡ High completeness genome sequencing

4 Validation of optimized protocol on seawater sample



Seawater

Fixation



HCR-FISH (optimized)



Sorting 500 cells



MDA and sequencing

➡ Metagenome assembled genomes of targeted population