

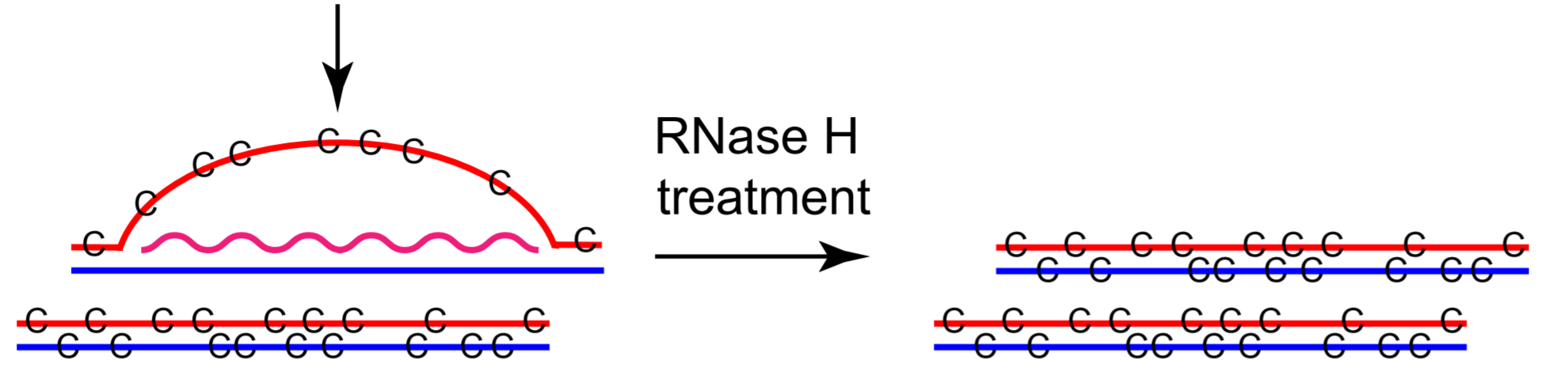
Figure S1

A

Gentle DNA isolation and restriction enzyme treatment

R-loop:

positive
negative

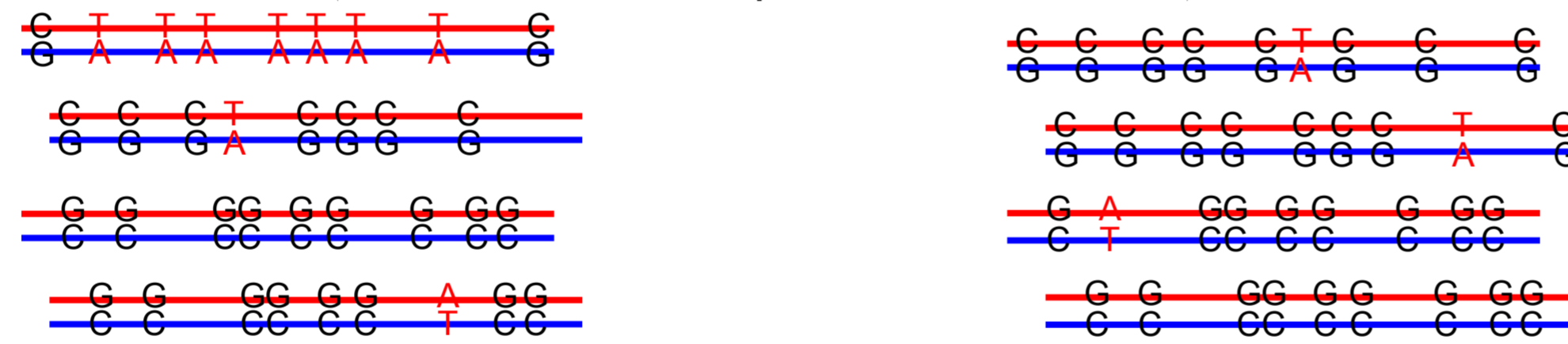


Non-denaturing bisulfite treatment

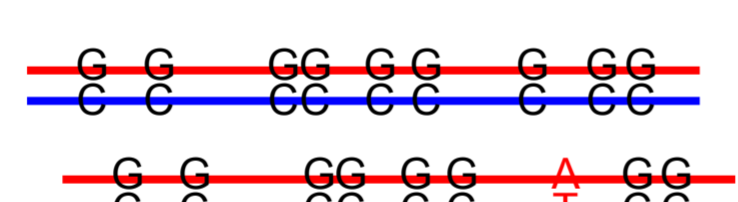
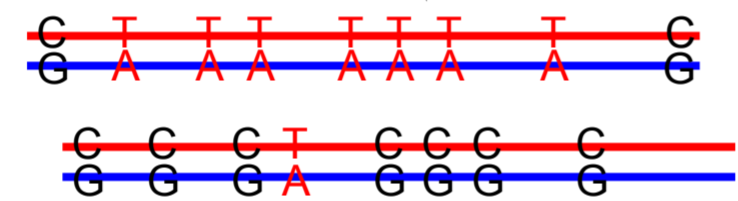


Amplified from:

Site specific PCR amplification

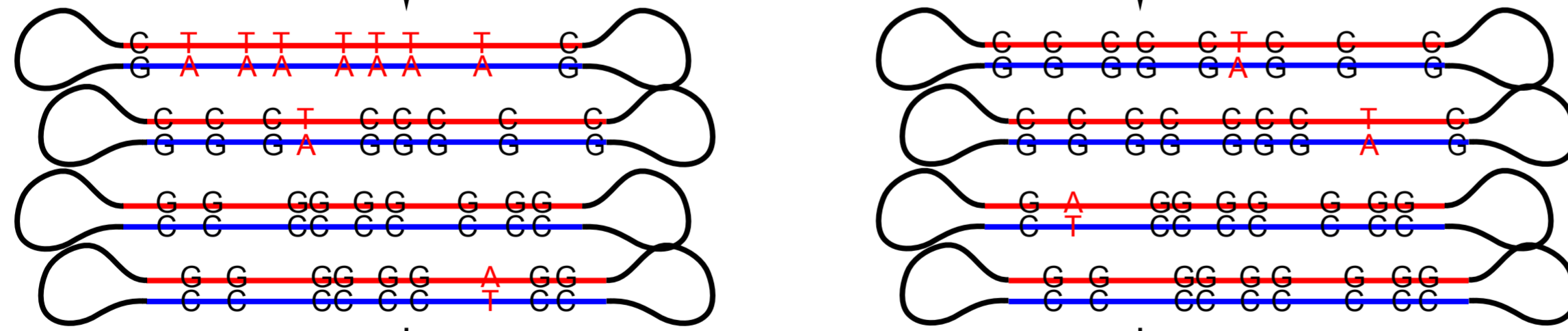


Top strand



Bottom strand

SMRTbell library construction



SMRT sequencing & Computational processing

B

CCS read generation (ConsensusTools)

Remove PCR duplicates (Dedupe)

Map reads & assign strand (footLoop (Bismark))

Call conversion tracks (footPeak)

Cluster peak calls (footClust)

Visualization (footPeak_graph)

Gargamel pipeline overview for SMRF-seq analysis

| | | Strand? | Peak? | | |
|---------------|--|---------|-------|--------|----|
| Molecule 1 | | Top | Yes | Top | No |
| Molecule 2 | | Top | No | Top | No |
| Molecule 3 or | | ? | No | Bottom | No |
| Molecule 4 | | Bottom | No | ? | No |