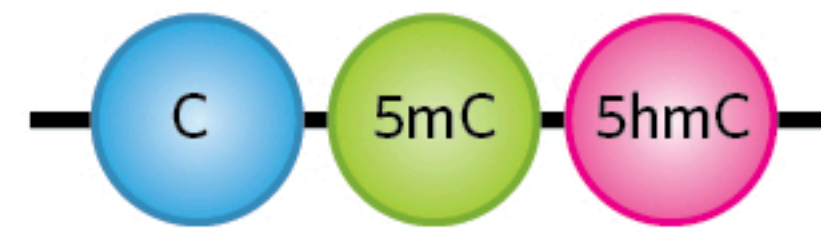


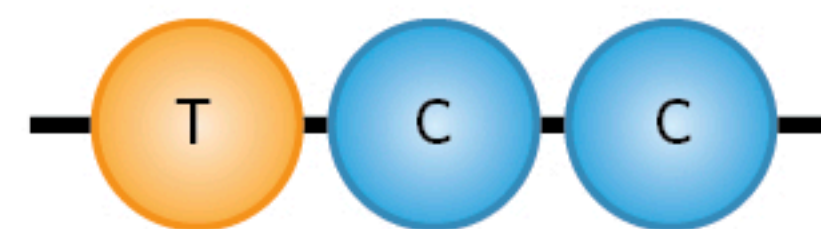
**TAB-SEQ METHOD:** Bisulfite sequencing alone (near right) cannot determine whether a base is 5mC or 5hmC. With TAB-Seq (far right), 5hmCs are protected from oxidation by glucosylation, while 5mCs are oxidized using a Tet enzyme, which converts 5mC to 5-carboxylcytosine (5caC). Bisulfite treatment then converts the 5caCs and unmodified cytosines to read as thymines following PCR. The 5hmCs continue to read as cytosines, revealing their locations.

### Traditional Bisulfite Sequencing



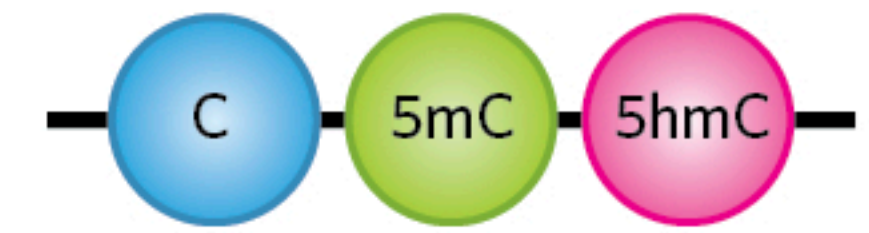
Bisulfite treatment

PCR amplification

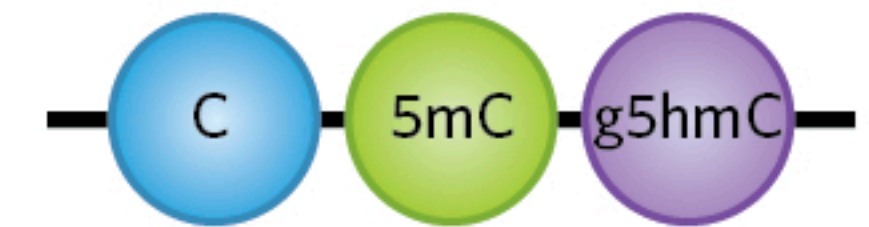


Cannot distinguish 5hmC from 5mC

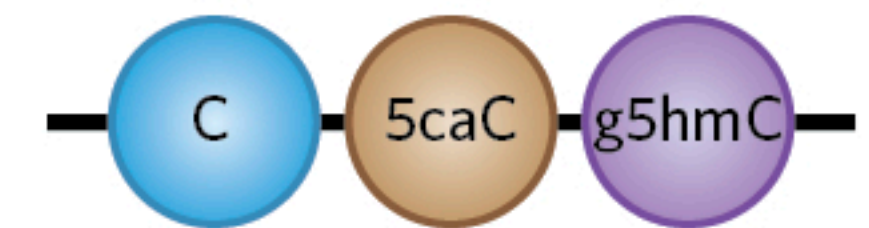
### Tet-Assisted Bisulfite Sequencing (TAB-Seq)



$\beta$ GT-catalyzed glucosylation

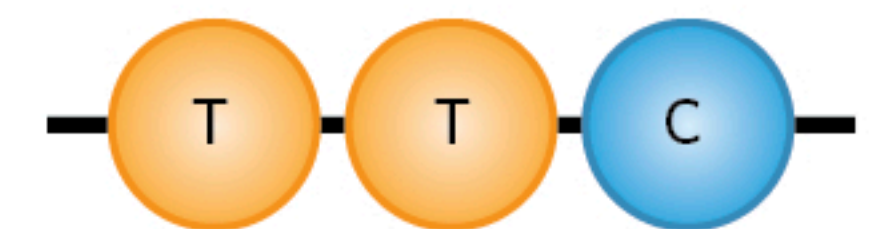


Tet oxidation



Bisulfite treatment

PCR amplification



Distinguishes 5hmC from 5mC when combined with traditional bisulfite sequencing