Potential pitfalls with directional RRBS libraries

MspI site MspI site

```
5'-.......CCGGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCCGG..........-3'
3'-.......GGCCNNNNNNNNNNNNNNNNNNNNNNNNNNNNNGGCC..........-5'
```

↓ MspI digest

```
5'          CGGNNNNNNNNNNNNNNNNNNNNNNNNNNNNC          -3'
3'-           CNNNNNNNNNNNNNNNNNNNNNNNNNNNNGGC          -5'
```

↓ end repair + A-tailing

```
5'-         CGGNNNNNNNNNNNNNNNNNNNNNNNNNNNNCCGA          -3'
3'-       AGCCNNNNNNNNNNNNNNNNNNNNNNNNNNNNGGC          -5'
```

↓ adapter ligation (X = adapter sequence)

```
5'-XXXXXXXTCGGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCCGAXXXXXXXXXX-3'
3'-XXXXXXXAGCCNNNNNNNNNNNNNNNNNNNNNNNNNNNNGGCTXXXXXXXXXX-5'
```

Blue cytosines retain the original genomic methylation state, whereas **red cytosines** are introduced experimentally during fragment end-repair (which can be accomplished with either unmethylated or methylated cytosines).

read length shorter than fragment length

read from original top strand

CGGNNNNNNNNNNNNNNNNNNNN

NNNNNNNNNNNNNNNNNNNNGGC

read from original bottom strand

read length longer than fragment length

read from original top strand

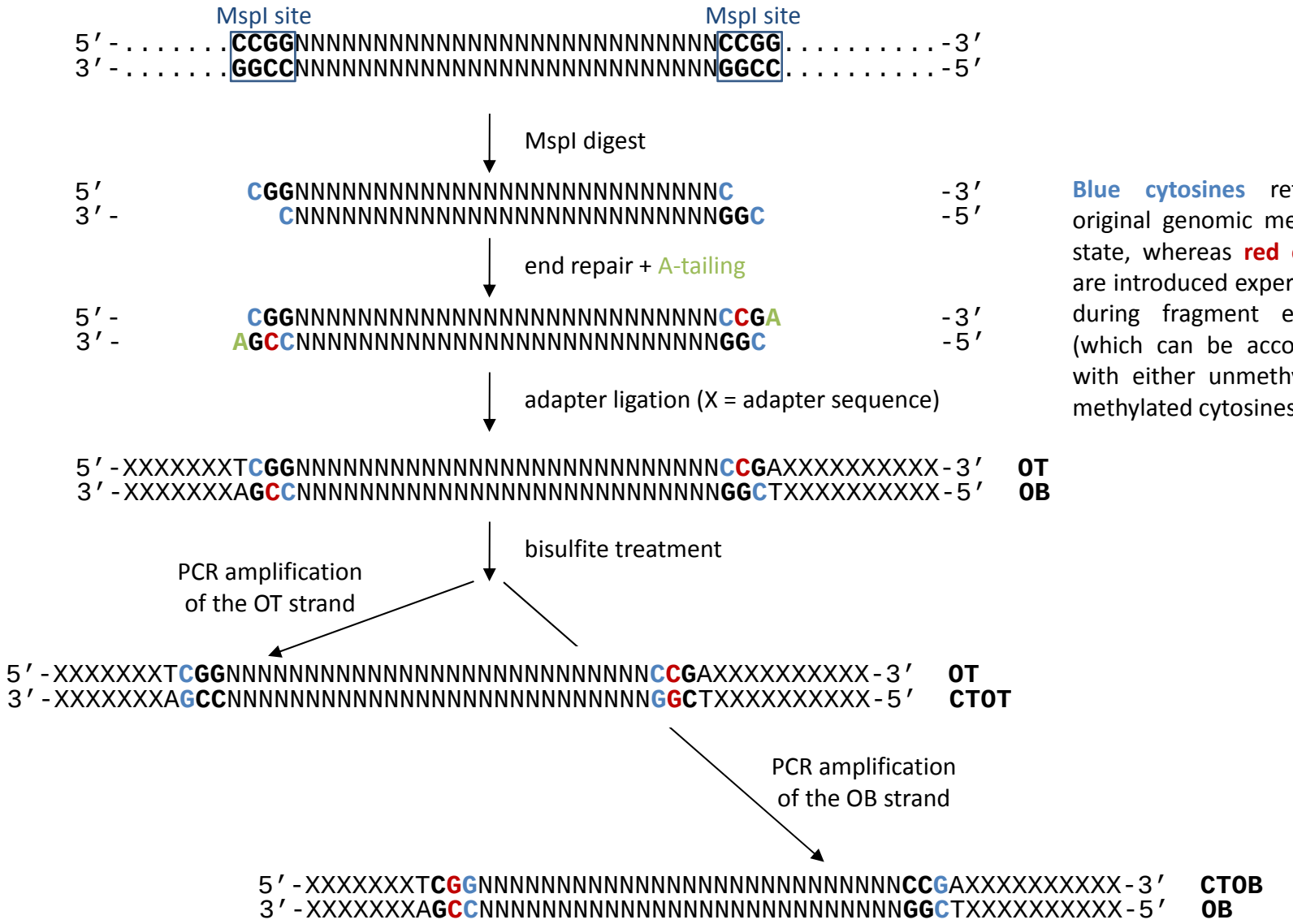
CGGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCCGAXXXXXXX

- reads may infer methylation state from experimentally introduced cytosines (**RED**)
- reads may (in addition) be contaminated with adapter sequence (**ORANGE**)

XXXXXXXAGCCNNNNNNNNNNNNNNNNNNNNNNNNNNNNGGC

read from original bottom strand

Potential pitfalls with non-directional RRBS libraries (1)



Blue cytosines retain the original genomic methylation state, whereas **red cytosines** are introduced experimentally during fragment end-repair (which can be accomplished with either unmethylated or methylated cytosines).

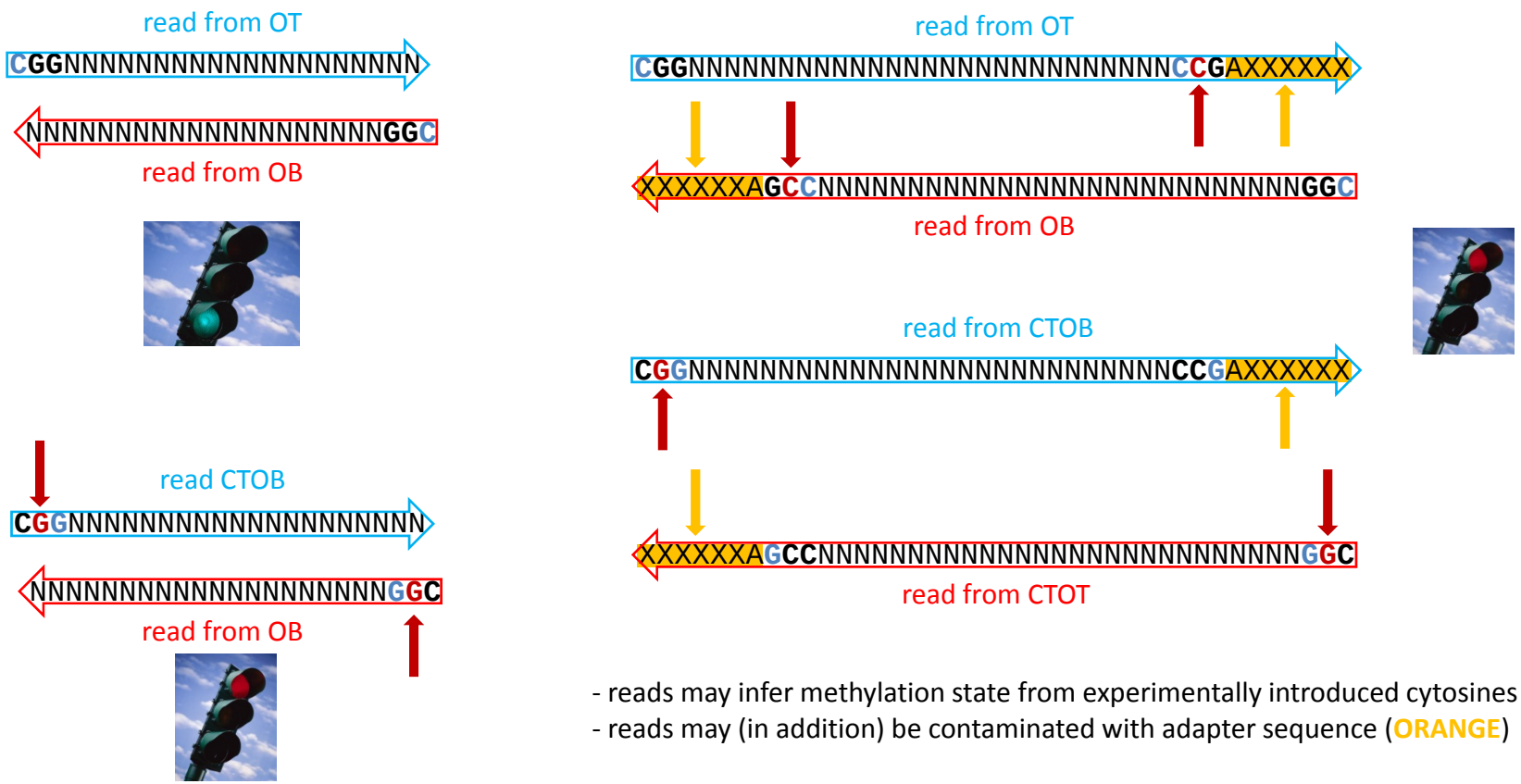
Potential pitfalls with non-directional RRBS libraries (2)

```
5′-XXXXXXXTCGGNNNNNNNNNNNNNNNNNNNNNNNNNNNNCCGAXXXXXXXXXX-3′   OT
3′-XXXXXXXAGCCNNNNNNNNNNNNNNNNNNNNNNNNNNNNGGCTXXXXXXXXXX-5′   CTOT

5′-XXXXXXXTCGGNNNNNNNNNNNNNNNNNNNNNNNNNNNNCCGAXXXXXXXXXX-3′   CTOB
3′-XXXXXXXAGCCNNNNNNNNNNNNNNNNNNNNNNNNNNNNGGCTXXXXXXXXXX-5′   OB
```

read length shorter than fragment length

read length longer than fragment length



- reads may infer methylation state from experimentally introduced cytosines (**RED**)
- reads may (in addition) be contaminated with adapter sequence (**ORANGE**)

Common initial bases:

Directional libraries, with either methylated or non-methylated cytosines:

CGG (OT,OB)
TGG (OT,OB)

Non-directional libraries, end-repair with **non-methylated** cytosines:

CGG (OT,OB)
TGG (OT,OB)
C**A**A (CTOT,CTOB)

Non-directional libraries, end-repair with **methylated** cytosines:

CGG (OT,OB)
TGG (OT,OB)
C**G**A (CTOT,CTOB)