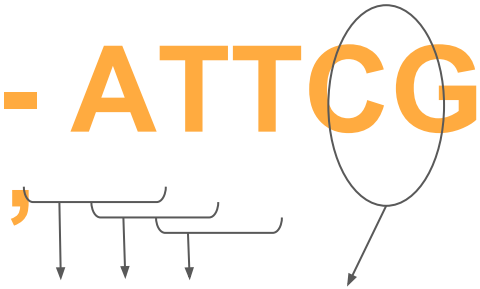
5’ - ATTCGA

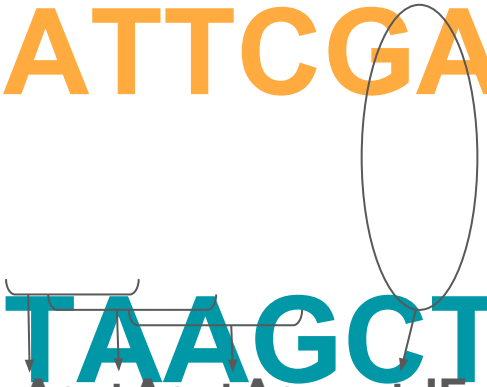
- 3’



$\Delta G = \Delta g_1 + \Delta g_2 + \Delta g_3 \ldots$

3’ + IF - TAAGCT

5’ - ATTCGA -
3’



3’ - TAAGCT -
5’

$\Delta G = \Delta g_1 + \Delta g_2 + \Delta g_3 \ldots + IF$

0.1
0.9
...ATGCTTTTCCGACGTA...
...TACGAAAAGGCTGCAT...
0.1
0.9

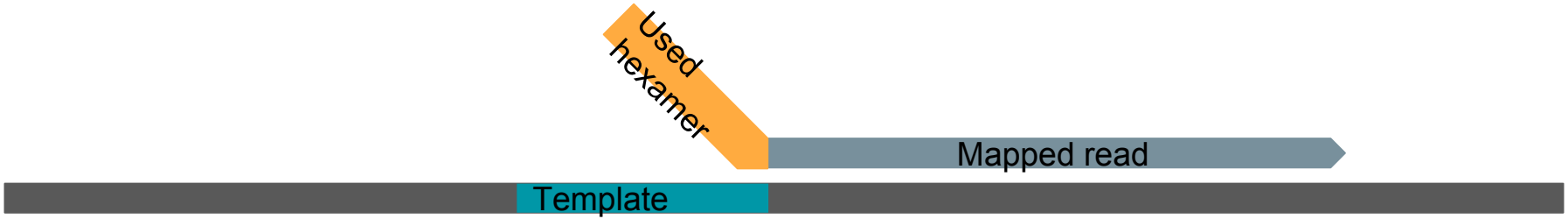
OT

CGATGT = 0.1 x 0.1
CGACGT = 0.1 x 0.9
TGATGT = 0.9 x 0.1
TGACGT = 0.9 x 0.9

OB

ATGTCG = 0.1 x 0.1
ACGTCG = 0.1 x 0.9
ATGTTG = 0.9 x 0.1
ACGTTG = 0.9 x 0.9

OT
CGATGT = 0.1 x 0.1
CGACGT = 0.1 x 0.9
TGATGT = 0.9 x 0.1
TGACGT = 0.9 x 0.9
ACATCG = 0.1 x 0.1
ACGTCG = 0.1 x 0.9
ACATCA= 0.9 x 0.1
ACGTCA = 0.9 x 0.9
CTOT
0.1
0.9
...ATGCTTTTCCGACGTA...
...TACGAAAAGGCTGCAT...
0.1
0.9
OB
ATGTCG = 0.1 x 0.1
ACGTCG = 0.1 x 0.9
ATGTTG = 0.9 x 0.1
ACGTTG = 0.9 x 0.9
CGACAT = 0.1 x 0.1
CGACGT = 0.1 x 0.9
CAACAT = 0.9 x 0.1
CAACGT = 0.9 x 0.9
CTOB

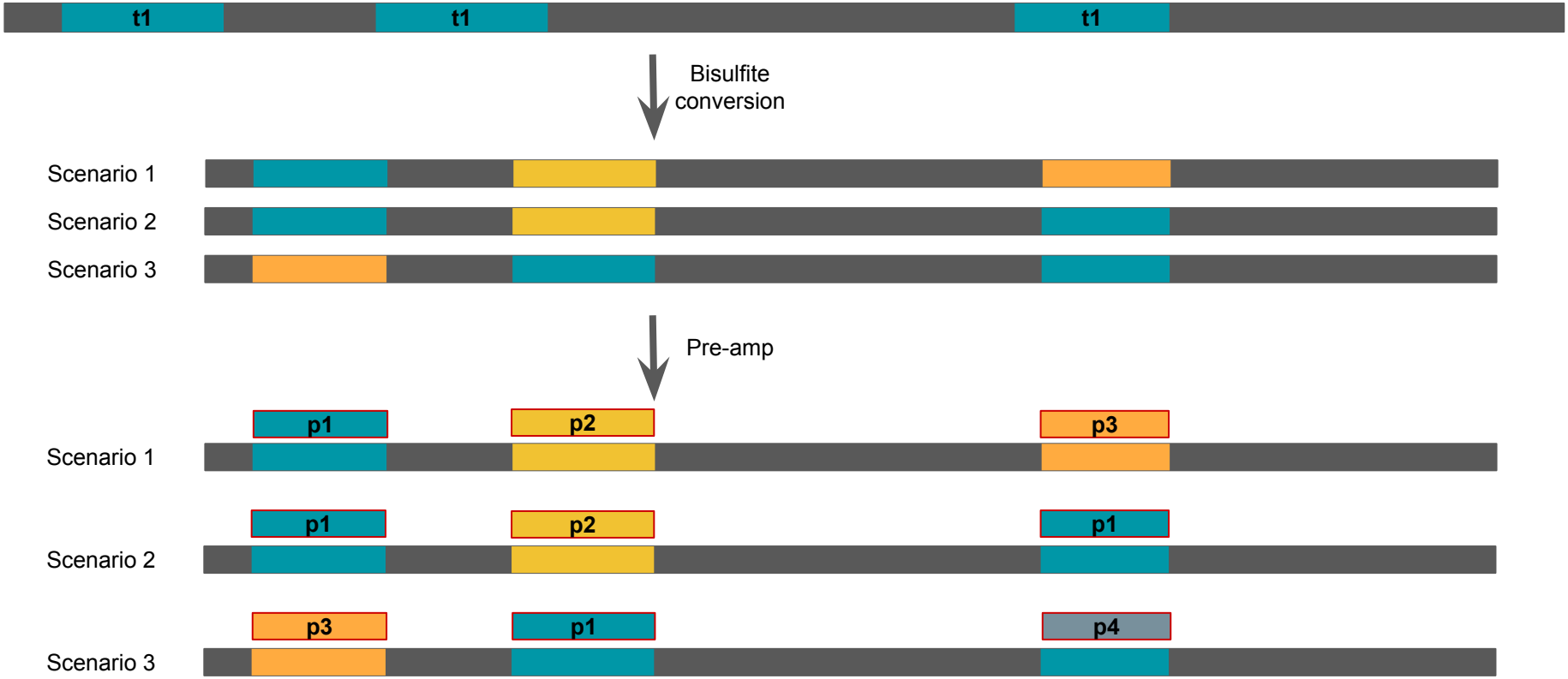

Used hexamer
Mapped read
Template

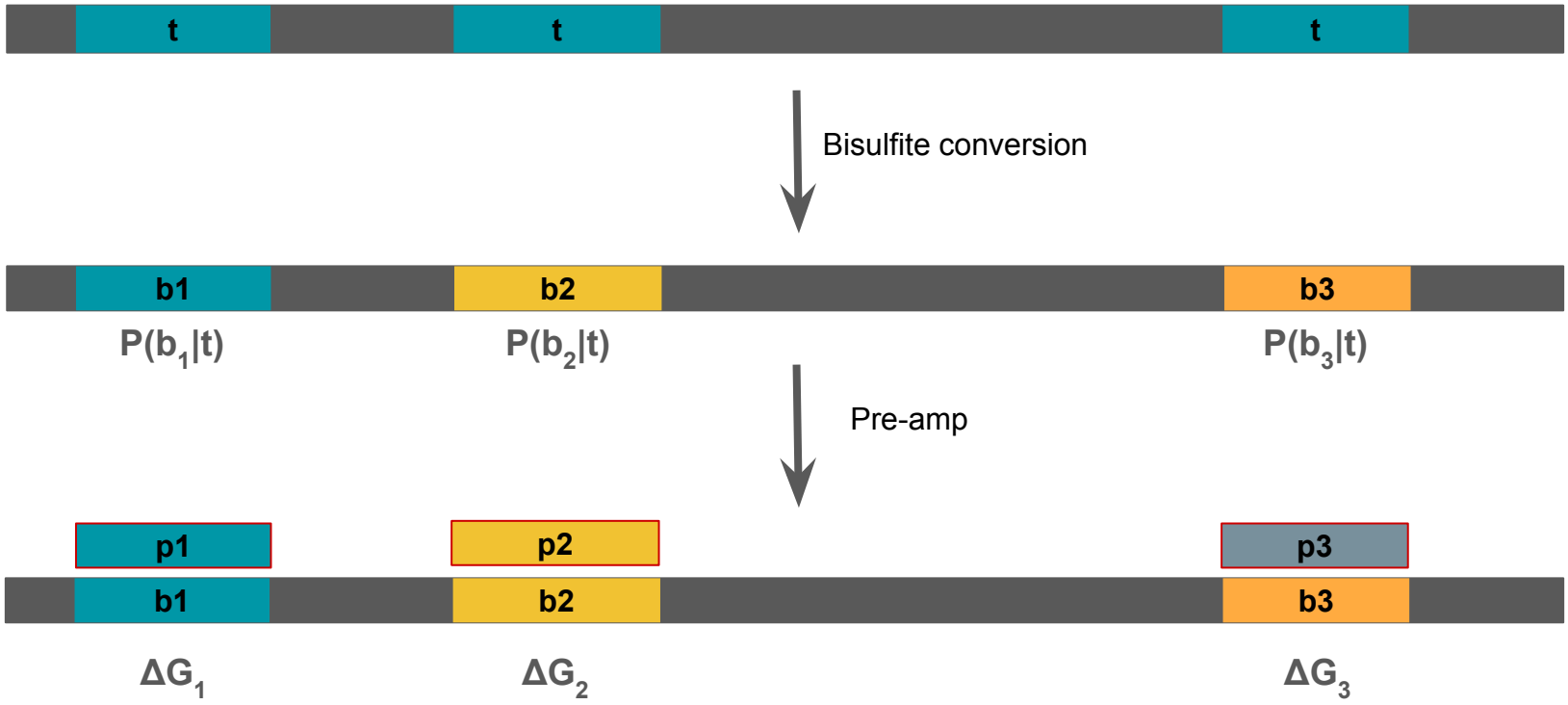
CGTTGT

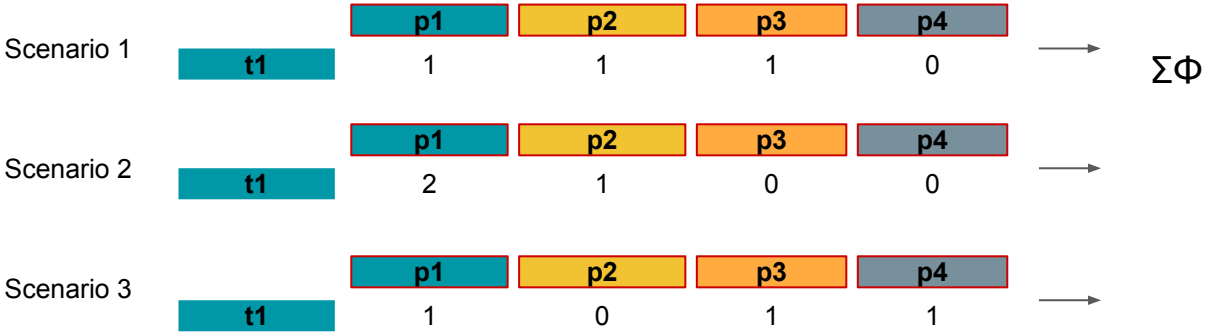
Mapped read

ATGTCG
ACGTCG
ATGTTG
ACGTTG
CGATGT *Alignment score*
CGACGT
TGATGT
TGACGT

Primer
Mapped read
Template


t1
t1
t1
Bisulfite conversion
Scenario 1
Scenario 2
Scenario 3
Pre-amp
p1
p2
p3
Scenario 1
p1
p2
p1
Scenario 2
p3
p1
p4
Scenario 3


t
t
t
Bisulfite conversion
b1
b2
b3
P(b1|t)
P(b2|t)
P(b3|t)
Pre-amp
p1
p2
p3
b1
b2
b3
ΔG1
ΔG2
ΔG3



p-t table reflects the real scenario!

Reference genome

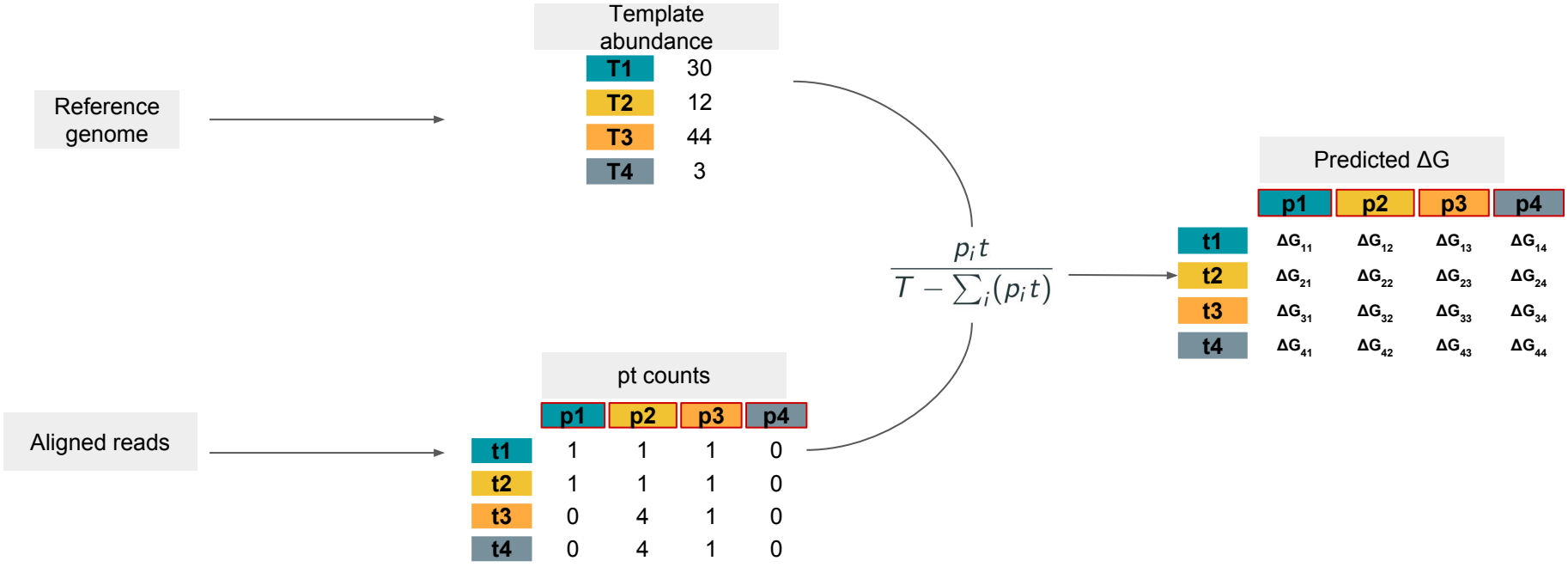
Aligned reads

Reference genome

Template abundance	
T1	30
T2	12
T3	44
T4	3

Aligned reads

	pt counts			
	p1	p2	p3	p4
t1	1	1	1	0
t2	1	1	1	0
t3	0	4	1	0
t4	0	4	1	0


Reference genome
Template abundance
T1 30
T2 12
T3 44
T4 3
Aligned reads
pt counts
p1 p2 p3 p4
t1 1 1 1 0
t2 1 1 1 0
t3 0 4 1 0
t4 0 4 1 0
$\frac{p_i t}{T - \sum_i (p_i t)}$
Predicted ΔG
p1 p2 p3 p4
t1 ΔG_{11} ΔG_{12} ΔG_{13} ΔG_{14}
t2 ΔG_{21} ΔG_{22} ΔG_{23} ΔG_{24}
t3 ΔG_{31} ΔG_{32} ΔG_{33} ΔG_{34}
t4 ΔG_{41} ΔG_{42} ΔG_{43} ΔG_{44}