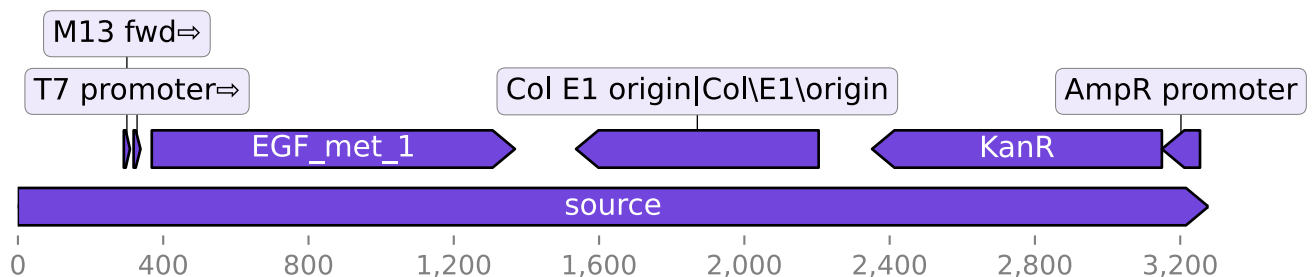


# Methylation analysis report

This document reports the sequencing results of the **Sequeduct Methyl Demo** project. There are **2** samples in this report. Each chapter details the results for one sample (plasmid), and the chapter sections report on each methylation subtype (modified nucleotide) × methylation pattern (methylase enzyme recognition site) analysis. The following methylases (patterns) were used in this analysis: **EcoKDcm BamHI**. Please see the Appendix on the last page for further explanation of the report.

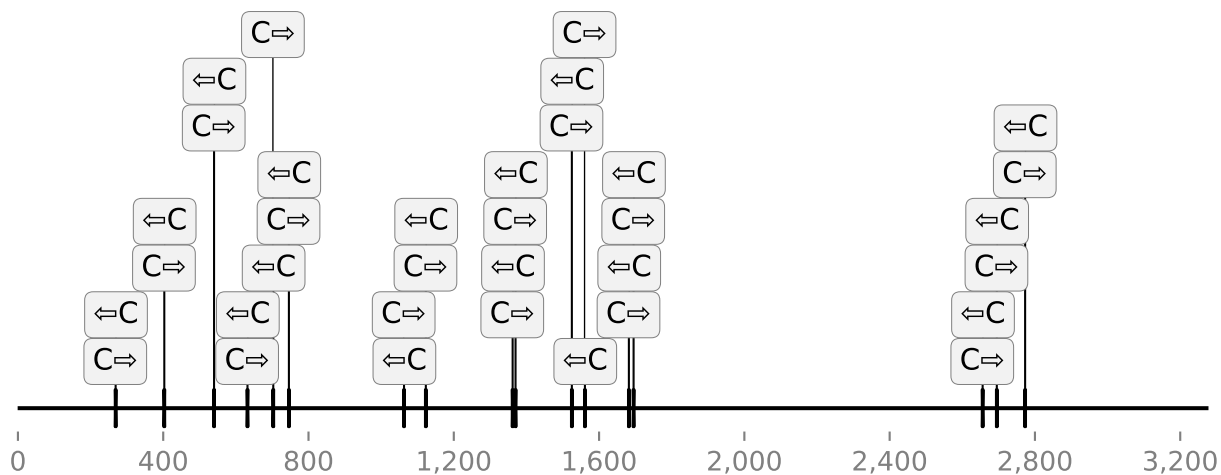
## barcode1 | EGF\_met\_1

Plot of the sequence:



## 5hmC × EcoKDcm

Modification code "h" × **CCWGG** sequence pattern.



Sample: barcode1 | EGF\_met\_1

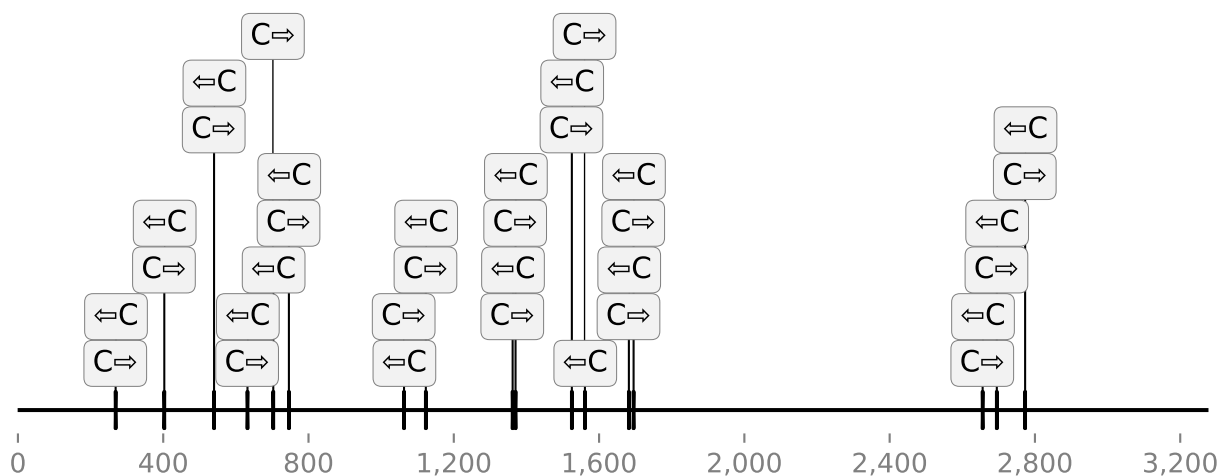
Bedmethyl table:

LOC	Strand	COV	% mod	MOD	STD	OTH	del	fail	diff	nocall	STATUS
268	+	193	2.07	4	181	8	5	52	4	0	0
270	-	170	1.18	2	163	5	4	95	3	0	0
402	+	217	1.38	3	212	2	2	50	1	0	0
404	-	207	1.45	3	204	0	16	50	8	0	0
539	+	121	0.00	0	119	2	27	121	19	0	0
541	-	245	0.41	1	244	0	26	13	9	0	0
631	+	253	2.77	7	240	6	2	42	1	0	0
633	-	283	1.41	4	274	5	5	12	2	0	0
702	+	225	0.44	1	223	1	3	70	5	0	0
704	-	252	0.79	2	249	1	25	34	1	0	0
745	+	228	0.44	1	222	5	12	62	2	0	0
747	-	295	1.02	3	292	0	3	14	2	0	0
1062	+	255	1.96	5	242	8	4	79	6	0	0
1064	-	226	0.44	1	225	0	18	57	41	0	0
1122	+	287	0.35	1	284	2	0	59	3	0	0
1124	-	323	0.93	3	313	7	4	26	0	0	0
1361	+	275	1.45	4	266	5	6	91	4	0	0
1363	-	316	1.27	4	311	1	41	28	5	0	0
1369	+	341	1.47	5	335	1	1	31	4	0	0
1371	-	343	0.29	1	341	1	11	36	1	0	0
1524	+	290	6.55	19	264	7	4	91	4	0	0

LOC	Strand	COV	% mod	MOD	STD	OTH	del	fail	diff	nocall	STATUS
1526	-	257	0.39	1	256	0	119	12	12	0	0
1560	+	180	0.00	0	175	5	5	205	3	0	0
1562	-	360	1.94	7	352	1	10	19	10	0	0
1681	+	313	0.96	3	308	2	19	61	3	0	0
1683	-	201	11.94	24	172	5	65	112	21	0	0
1694	+	334	2.10	7	327	0	0	57	6	0	0
1696	-	159	3.77	6	150	3	45	190	3	0	0
2655	+	210	0.95	2	206	2	2	91	4	0	0
2657	-	155	2.58	4	147	4	0	139	0	0	0
2694	+	105	1.90	2	100	3	12	185	1	0	0
2696	-	221	2.26	5	209	7	1	66	0	0	0
2772	+	185	3.24	6	176	3	2	104	1	0	0
2774	-	222	0.90	2	218	2	15	36	4	0	0

## 5mC × EcoKDcm

Modification code "m" × CCWGG sequence pattern.



Sample: barcode1 | EGF\_met\_1

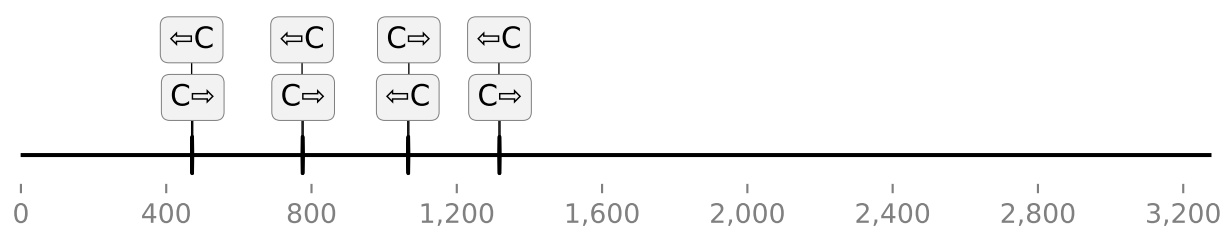
Bedmethyl table:

LOC	Strand	COV	% mod	MOD	STD	OTH	del	fail	diff	nocall	STATUS
268	+	193	4.15	8	181	4	5	52	4	0	0

LOC	Strand	COV	% mod	MOD	STD	OTH	del	fail	diff	nocall	STATUS
270	-	170	2.94	5	163	2	4	95	3	0	0
402	+	217	0.92	2	212	3	2	50	1	0	0
404	-	207	0.00	0	204	3	16	50	8	0	0
539	+	121	1.65	2	119	0	27	121	19	0	0
541	-	245	0.00	0	244	1	26	13	9	0	0
631	+	253	2.37	6	240	7	2	42	1	0	0
633	-	283	1.77	5	274	4	5	12	2	0	0
702	+	225	0.44	1	223	1	3	70	5	0	0
704	-	252	0.40	1	249	2	25	34	1	0	0
745	+	228	2.19	5	222	1	12	62	2	0	0
747	-	295	0.00	0	292	3	3	14	2	0	0
1062	+	255	3.14	8	242	5	4	79	6	0	0
1064	-	226	0.00	0	225	1	18	57	41	0	0
1122	+	287	0.70	2	284	1	0	59	3	0	0
1124	-	323	2.17	7	313	3	4	26	0	0	0
1361	+	275	1.82	5	266	4	6	91	4	0	0
1363	-	316	0.32	1	311	4	41	28	5	0	0
1369	+	341	0.29	1	335	5	1	31	4	0	0
1371	-	343	0.29	1	341	1	11	36	1	0	0
1524	+	290	2.41	7	264	19	4	91	4	0	0
1526	-	257	0.00	0	256	1	119	12	12	0	0
1560	+	180	2.78	5	175	0	5	205	3	0	0
1562	-	360	0.28	1	352	7	10	19	10	0	0
1681	+	313	0.64	2	308	3	19	61	3	0	0
1683	-	201	2.49	5	172	24	65	112	21	0	0
1694	+	334	0.00	0	327	7	0	57	6	0	0
1696	-	159	1.89	3	150	6	45	190	3	0	0
2655	+	210	0.95	2	206	2	2	91	4	0	0
2657	-	155	2.58	4	147	4	0	139	0	0	0
2694	+	105	2.86	3	100	2	12	185	1	0	0
2696	-	221	3.17	7	209	5	1	66	0	0	0
2772	+	185	1.62	3	176	6	2	104	1	0	0
2774	-	222	0.90	2	218	2	15	36	4	0	0

# 5hmC × BamHI

Modification code "h" × GGATCC sequence pattern.



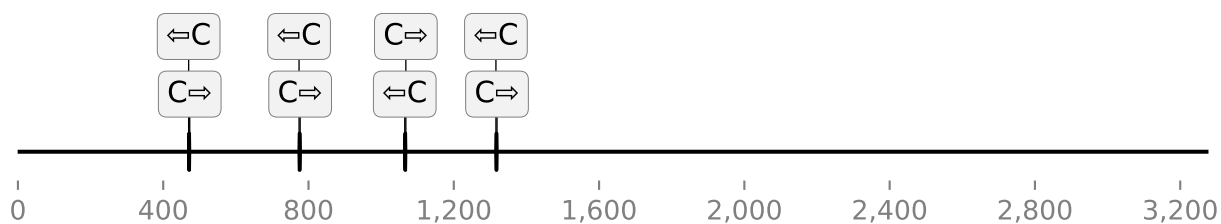
Sample: barcode1 | EGF\_met\_1

Bedmethyl table:

LOC	Strand	COV	% mod	MOD	STD	OTH	del	fail	diff	nocall	STATUS
470	-	102	0.98	1	101	0	37	90	60	0	0
473	+	158	0.00	0	158	0	17	28	73	0	0
774	-	77	1.30	1	76	0	12	17	213	0	0
777	+	124	0.81	1	123	0	25	23	133	0	0
1065	-	195	1.03	2	193	0	4	62	81	0	0
1068	+	214	1.87	4	209	1	23	26	81	0	0
1316	-	255	1.18	3	252	0	24	35	72	0	0
1319	+	284	1.41	4	280	0	24	12	54	0	0

# 5mC × BamHI

Modification code "m" × GGATCC sequence pattern.



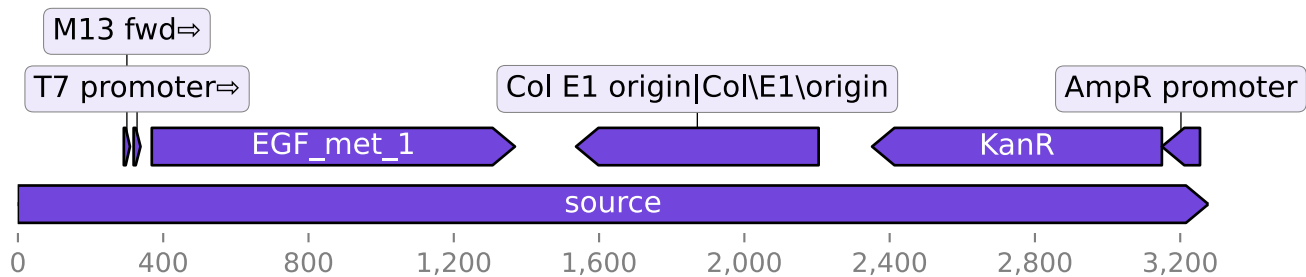
Sample: barcode1 | EGF\_met\_1

Bedmethyl table:

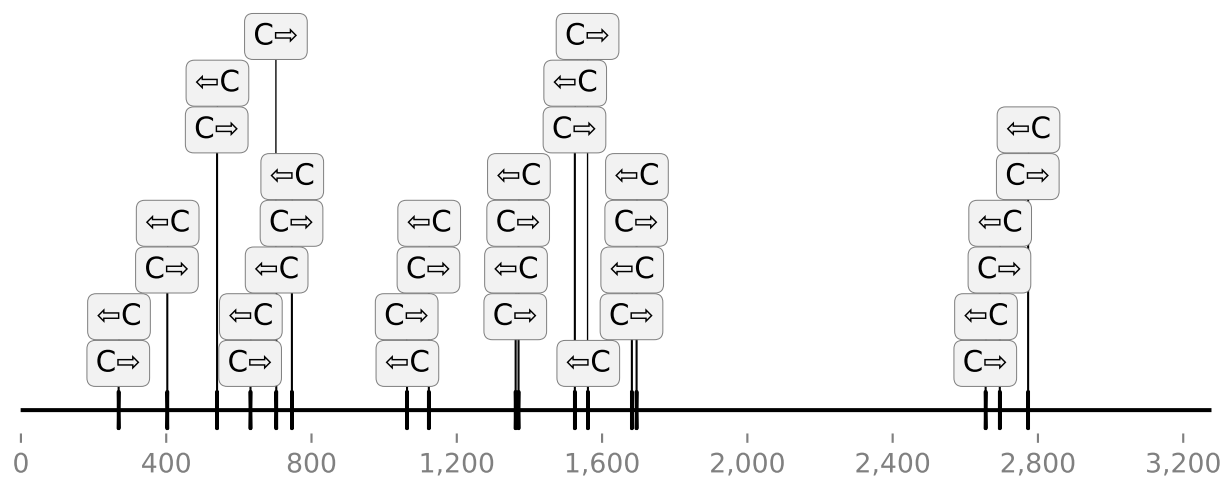
LOC	Strand	COV	% mod	MOD	STD	OTH	del	fail	diff	nocall	STATUS
470	-	102	0.00	0	101	1	37	90	60	0	0
473	+	158	0.00	0	158	0	17	28	73	0	0
774	-	77	0.00	0	76	1	12	17	213	0	0
777	+	124	0.00	0	123	1	25	23	133	0	0
1065	-	195	0.00	0	193	2	4	62	81	0	0
1068	+	214	0.47	1	209	4	23	26	81	0	0
1316	-	255	0.00	0	252	3	24	35	72	0	0
1319	+	284	0.00	0	280	4	24	12	54	0	0

# barcode2 | EGF\_met\_1

Plot of the sequence:



Modification code "h" × CCWGG sequence pattern.



Sample: barcode2 | EGF\_met\_1

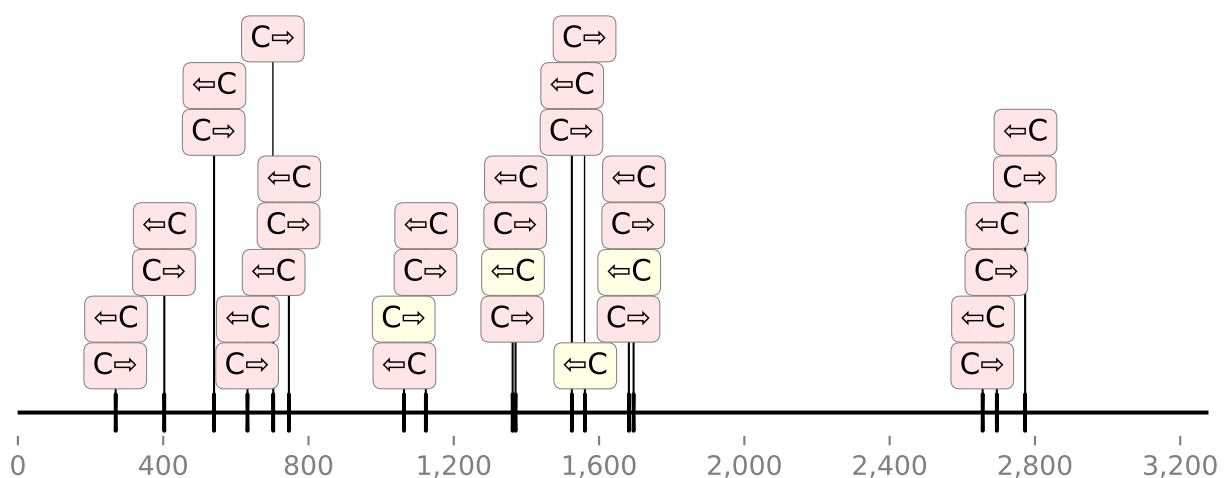
Bedmethyl table:

LOC	Strand	COV	% mod	MOD	STD	OTH	del	fail	diff	nocall	STATUS
268	+	174	2.87	5	23	146	8	60	13	0	0
270	-	177	1.13	2	22	153	7	42	8	0	0
402	+	209	5.74	12	10	187	3	53	2	0	0
404	-	184	2.17	4	21	159	7	56	2	0	0
539	+	99	5.05	5	18	76	25	127	28	0	0
541	-	161	4.35	7	33	121	31	64	0	0	0
631	+	157	6.37	10	35	112	5	134	2	0	0
633	-	206	3.88	8	11	187	6	65	0	0	0
702	+	173	5.20	9	11	153	13	82	40	0	0
704	-	201	9.45	19	14	168	8	80	2	0	0
745	+	185	1.62	3	10	172	12	110	5	0	0
747	-	249	0.40	1	26	222	5	37	4	0	0
1062	+	145	11.72	17	44	84	9	187	0	0	0
1064	-	223	0.00	0	22	201	3	111	12	0	0
1122	+	157	10.19	16	16	125	3	164	23	0	0
1124	-	301	1.99	6	5	290	4	48	0	0	0
1361	+	219	3.20	7	16	196	4	150	8	0	0
1363	-	129	4.65	6	61	62	29	220	18	0	0

LOC	Strand	COV	% mod	MOD	STD	OTH	del	fail	diff	nocall	STATUS
1369	+	199	1.01	2	36	161	12	165	7	0	0
1371	-	235	3.83	9	36	190	23	127	12	0	0
1524	+	294	5.44	16	25	253	6	67	11	0	0
1526	-	156	1.28	2	38	116	149	84	16	0	0
1560	+	257	1.95	5	4	248	25	91	9	0	0
1562	-	262	1.91	5	87	170	3	135	7	0	0
1681	+	201	2.99	6	46	149	12	170	10	0	0
1683	-	193	14.51	28	77	88	39	158	24	0	0
1694	+	195	2.05	4	21	170	3	192	2	0	0
1696	-	117	9.40	11	14	92	158	127	11	0	0
2655	+	183	1.09	2	17	164	0	91	4	0	0
2657	-	244	2.46	6	4	234	3	53	3	0	0
2694	+	100	3.00	3	13	84	6	161	7	0	0
2696	-	239	1.67	4	15	220	2	54	2	0	0
2772	+	193	3.63	7	5	181	2	62	4	0	0
2774	-	164	3.05	5	18	141	33	89	6	0	0

## 5mC × EcoKDcm

Modification code "m" × CCWGG sequence pattern.



Sample: barcode2 | EGF\_met\_1

Bedmethyl table:

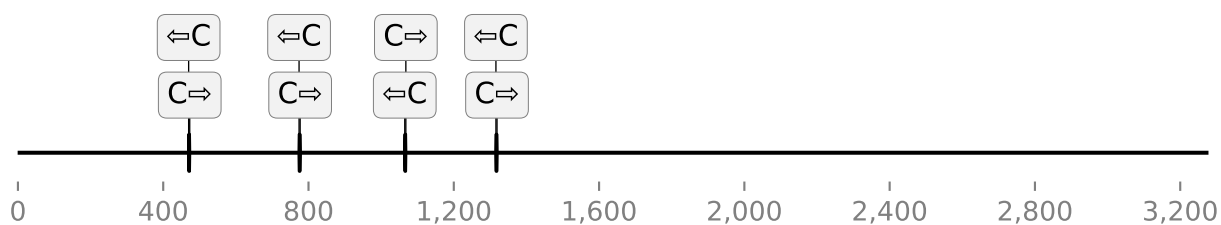


LOC	Strand	COV	% mod	MOD	STD	OTH	del	fail	diff	nocall	STATUS
268	+	174	83.91	146	23	5	8	60	13	0	1
270	-	177	86.44	153	22	2	7	42	8	0	1
402	+	209	89.47	187	10	12	3	53	2	0	1
404	-	184	86.41	159	21	4	7	56	2	0	1
539	+	99	76.77	76	18	5	25	127	28	0	1
541	-	161	75.16	121	33	7	31	64	0	0	1
631	+	157	71.34	112	35	10	5	134	2	0	1
633	-	206	90.78	187	11	8	6	65	0	0	1
702	+	173	88.44	153	11	9	13	82	40	0	1
704	-	201	83.58	168	14	19	8	80	2	0	1
745	+	185	92.97	172	10	3	12	110	5	0	1
747	-	249	89.16	222	26	1	5	37	4	0	1
1062	+	145	57.93	84	44	17	9	187	0	0	U
1064	-	223	90.13	201	22	0	3	111	12	0	1
1122	+	157	79.62	125	16	16	3	164	23	0	1
1124	-	301	96.35	290	5	6	4	48	0	0	1
1361	+	219	89.50	196	16	7	4	150	8	0	1
1363	-	129	48.06	62	61	6	29	220	18	0	U
1369	+	199	80.90	161	36	2	12	165	7	0	1
1371	-	235	80.85	190	36	9	23	127	12	0	1
1524	+	294	86.05	253	25	16	6	67	11	0	1
1526	-	156	74.36	116	38	2	149	84	16	0	1
1560	+	257	96.50	248	4	5	25	91	9	0	1
1562	-	262	64.89	170	87	5	3	135	7	0	U
1681	+	201	74.13	149	46	6	12	170	10	0	1
1683	-	193	45.60	88	77	28	39	158	24	0	U
1694	+	195	87.18	170	21	4	3	192	2	0	1
1696	-	117	78.63	92	14	11	158	127	11	0	1
2655	+	183	89.62	164	17	2	0	91	4	0	1
2657	-	244	95.90	234	4	6	3	53	3	0	1
2694	+	100	84.00	84	13	3	6	161	7	0	1
2696	-	239	92.05	220	15	4	2	54	2	0	1
2772	+	193	93.78	181	5	7	2	62	4	0	1

LOC	Strand	COV	% mod	MOD	STD	OTH	del	fail	diff	nocall	STATUS
2774	-	164	85.98	141	18	5	33	89	6	0	1

## 5hmC × BamHI

Modification code "h" × **GGATCC** sequence pattern.



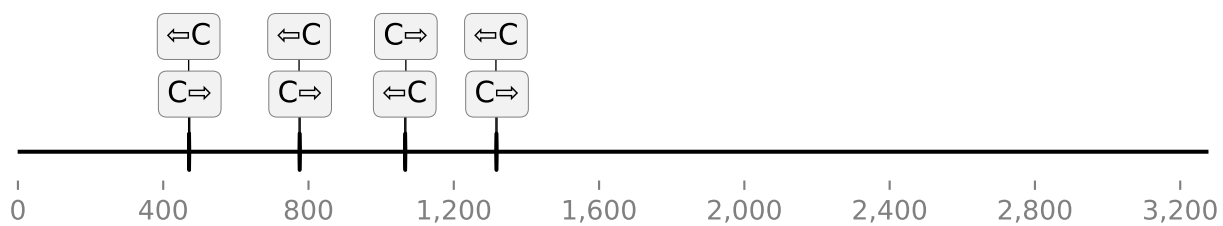
Sample: barcode2 | EGF\_met\_1

Bedmethyl table:

LOC	Strand	COV	% mod	MOD	STD	OTH	del	fail	diff	nocall	STATUS
470	-	132	1.52	2	130	0	0	112	10	0	0
473	+	229	0.44	1	228	0	1	27	16	0	0
774	-	236	0.85	2	234	0	0	3	62	0	0
777	+	226	0.44	1	225	0	10	22	59	0	0
1065	-	141	0.00	0	140	1	0	194	14	0	0
1068	+	283	0.00	0	282	1	9	41	8	0	0
1316	-	327	0.61	2	325	0	3	37	17	0	0
1319	+	327	0.31	1	326	0	9	25	14	0	0

## 5mC × BamHI

Modification code "m" × **GGATCC** sequence pattern.



Sample: barcode2 | EGF\_met\_1

Bedmethyl table:

LOC	Strand	COV	% mod	MOD	STD	OTH	del	fail	diff	nocall	STATUS
470	-	132	0.00	0	130	2	0	112	10	0	0
473	+	229	0.00	0	228	1	1	27	16	0	0
774	-	236	0.00	0	234	2	0	3	62	0	0
777	+	226	0.00	0	225	1	10	22	59	0	0
1065	-	141	0.71	1	140	0	0	194	14	0	0
1068	+	283	0.35	1	282	0	9	41	8	0	0
1316	-	327	0.00	0	325	2	3	37	17	0	0
1319	+	327	0.00	0	326	1	9	25	14	0	0

# Appendix

The report was generated by [EpiJinn](#), a software published by the Edinburgh Genome Foundry (EGF). EpiJinn is part of the [EGF Codons](#) engineering biology software suite for DNA design, manufacturing and validation.

Each chapter details the results for one plasmid, and the chapter sections report on each methylation subtype (modified nucleotide) ~ methylation pattern (methylase enzyme recognition site) analysis. The sections begin with a sequence plot which displays the locations of the pattern (modified nucleotide) in the sequence. If the pattern is present a large number of times, then this plot is not displayed. A simplified bedMethyl table is also displayed, which shows the position (using a zero-based index) and values for the modified nucleotides. A methylation status is also determined (`STATUS` column), based on user-specified thresholds. The table entries and the sequence plot annotations are coloured based on this status. The columns of the table are:

- LOC: Zero-based position of modified nucleotide
- Strand: DNA strand (+/-)
- COV: coverage = MOD + STD + OTH
- % mod: % = MOD / COV \* 100
- MOD: modified count
- STD: canonical base count
- OTH: count for other modifications
- del: number of reads with deletion at this position
- fail: number of calls below threshold
- diff: number of reads with base other than canonical at this position
- nocall: number of reads without modification call
- STATUS: methylated / undetermined / unmethylated (1/U/0)

Modified nucleotide summary table:

Code	Abbreviation	Name	ChEBI
m	5mC	5-Methylcytosine	27551
h	5hmC	5-Hydroxymethylcytosine	76792
21839	4mC	4-Methylcytosine	21839
a	6mA	6-Methyladenine	28871