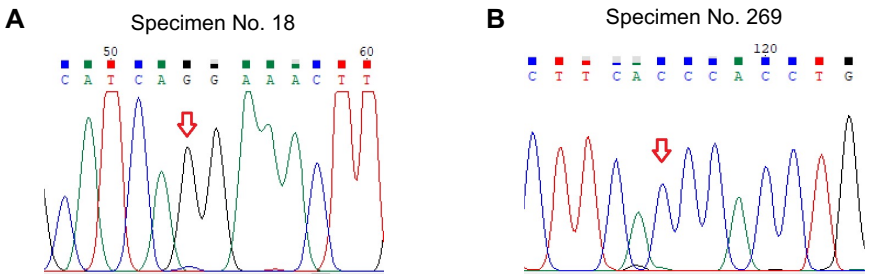
Supplemental Digital Content, containing 1 figure and 5 tables. The Supplemental Digital Content was not copyedited by *Archives of Pathology & Laboratory Medicine*.



Supplemental Figure 1. POLE mutations consistency between Dalton-MIT and next-generation sequencing were confirmed by sanger sequencing in two specimens. A. c.857C>G mutation in POLE was confirmed by sanger sequencing in specimen No.18; B. c.1231G>T mutation in POLE was confirmed by sanger sequencing in specimen No.269. Red arrows show the mutation respectively.

Supplemental Table 1. POLE mutations detected in Dalton-MIT

Name	Nucleotide Substitution	Amino acid change	Exon	Cosmic ID
857C-G	c.857C>G	P286R	9	937332
890C-T	c.890C>T	S297F	9	937330
1100T-C	c.1100T>C	F367S	11	204095
1231G-T	c.1231G>T	V411L	13	204094
1231G-C	c.1231G>C	V411L	13	4716439
1270C-G	c.1270C>G	L424V	13	937328
1270C-A	c.1270C>A	L424I	13	937326
1307C-G	c.1307C>G	P436R	13	284130
1331T-A	c.1331T>A	M444K	13	937322
1366G-C	c.1366G>C	A456P	14	937318
1376C-T	c.1376C>T	S459F	14	170809

Supplemental Table 2. Primers used in Dalton-MIT

Number	Name	Sequence
1	857-M	5'-ACTGCCCCTCAAGTTACG-3'
2	890-M	5'-CAGACCAGATTATGATGATTAT-3'
3	1100-M	5'-CACAGACTCACCAGTCCG-3'
4	1231C-M	5'-ATTCTCCTTCCAGGTCGC-3'
5	1231T-M	5'-ATTCTCCTTCCAGGTGAT-3'
6	1270A-M	5'-CCTGTGGGCAGTCATAAAA-3'
7	1270G-M	5'-CCTGTGGGCAGTCATATTG-3'
8	1307-M	5'-CAAGCTAGGCTATGAACG-3'
9	1331-M	5'-GCTAGACCCGGAGGATAA-3'
10	1366-M	5'-TCTCTCCTCAGACTCTCC-3'
11	1376-M	5'-GACTCTGGCCACGTAATT-3'
12	EX9-P	5'-FAM-ACATGATCGATGGCCAGGTGAG-BHQ1-3'
13	EX11-P	5'-FAM-TCCCCGTTGTAGGTGACCATGA-BHQ1-3'
14	EX13-P1	5'-FAM-CCAAGGCCAAGCTAGGCTATGATC-BHQ1-3'
15	EX13-P2	5'-CY5-TGGAGCTAGACCCGGAGGAC-BHQ2-3'
16	EX13-P3	5'-CY5-CGGATGGCCACGGAGCAG-BHQ2-3'
17	EX14-P	5'-CY5-TGTCAGATGCTGTCGCCACTT-BHQ2-3'
18	EX9-R	5'-AGGAGCTTACTTCCCAGA-3'
19	EX11-F	5'-TTTGAACACGTCCAGGAG-3'
20	EX13-R1	5'-CATGTCCTCCGGGTCTAG-3'
21	EX13-R2	5'-GGGATGTGGCTTACGTG-3'
22	EX14-R	5'-ACGTACTTCATGTACAGGTAG-3'
23	Globin-F	5'-ACCCTTAGGCTGCTGGTGG-3'
24	Globin-R	5'-GGAGTGGACAGATCCCCAAA-3'
25	Globin-P	5'-VIC-CTACCCTTGGACCCAGAGGTTCTTTGAGTC-BHQ1-3'

Supplemental Table 3. Standards for identifying POLE-mut in Dalton-MIT kit

Tube	A		B		C	D		E	
Channel	FAM	CY5	FAM	CY5	FAM	FAM	CY5	FAM	CY5
AA change	P286R	P436R	S297F	M444K	V411L	L424V/I	S459F	F367S	A456P
Mutation	857	1307	890	1331	1231	1270	1376	1100	1366
Positive ΔCt	≤7	≤9	≤9	≤8	≤10	≤9	≤9	≤9	≤10
Negative ΔCt	>7	>9	>9	>8	>10	>9	>9	>9	>10

AA: Animo Acid

Supplemental Table 4. POLE mutation (POLE-mut) detected by Dalton-MIT in 613 patients

Tube	Mutation	Nucleotide Substitution	Amino acid change	Number	Average ΔCt	Frequency[a] (%)	Percentage[b] (%)
A	857C-G	c.857C>G	P286R	33	5.41	5.38	47.14
	1307C-G	c.1307C>G	P436R	1	5.48	0.16	1.43
B	890C-T	c.890C>T	S297F	2	3.76	0.33	2.86
	1331T-A	c.1331T>A	M444K	1	3.36	0.16	1.43
C	1231G-T/C	c.1231G>T/G	V411L	20	6.77	3.26	28.57
D	1270C-G/A	c.1270C>G/A	L424V/I	1	7.83	0.16	1.43
	1376C-T	c.1376C>T	S459F	3	6.18	0.49	4.29
E	1100T-C	c.1100T>C	F367S	2	2.73	0.33	2.86
	1366G-C	c.1366G>C	A456P	7	8.49	1.14	10.00

[a] Frequency (%) was the frequency of each nucleotide mutation in all detected specimens.

[b] Percentage (%) was the percentage of each mutation in POLE-mut specimens.

Supplemental Table 5. POLE pathogenic mutations detected by NGS in 365 patients

Nucleotide Substitution	Amino Acid Change	Number	Percentage (%)	Average of Mutation Frequency (%)
c.857C>G	P286R	29	45.3	16
c.1231G>T/C	V411L	19	29.7	15.2
c.1366G>C	A456P	6	9.4	15.3
c.1376C>T	S459F	3	4.7	16
c.1100T>C	F367S	2	3.1	27
c.890C>T	S297F	2	3.1	33.4
c.1270C>G	L424V	1	1.6	5.6
c.1331T>A	M444K	1	1.6	24.5
c.1307C>G	P436R	1	1.6	23.5