

Supporting Information

Rapid determination of the presence of EGFR mutations with DNA-based nanocalipers

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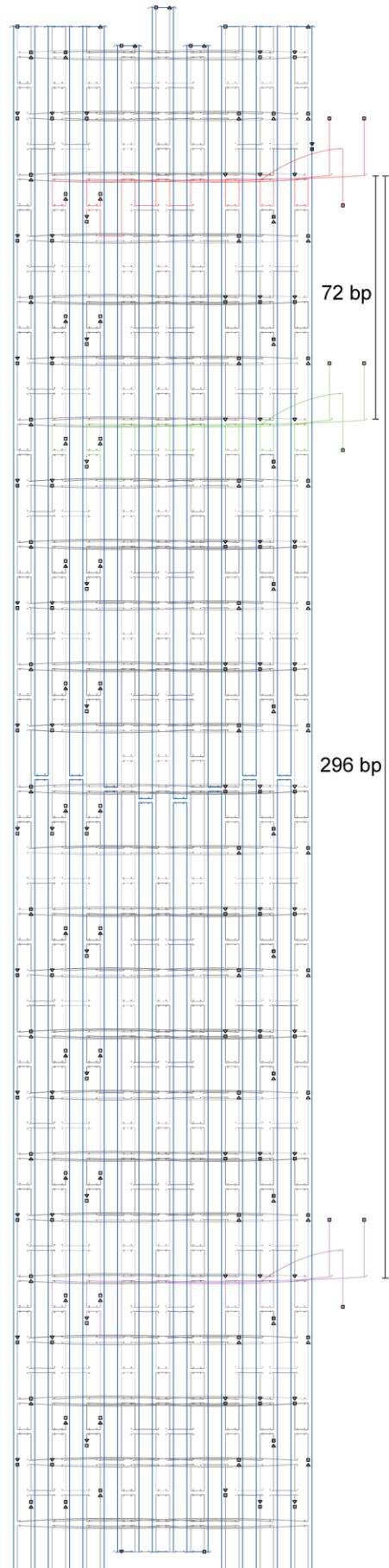
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Supporting Data S1.

A plane diagram of the three-layered rectangular structure (plotted by the software caDNAno). The essential elements for assembling DNA nanocalipers include M13mp18 scaffold ssDNA (blue) and massive staple strands, in which strands colored gray (named as structural strands) are designed to form the stable structure and 9 strands (colored red, green, and purple; named as positioning strands) are designed to create different given distances of EGF molecules. After being decorated by external nucleotide residues (5'-AAAAAAAAAAAAAAA-3'), all positioning strands are bound to polyT-linked EGF molecules. For NC-25, the distance between red and green positioning strands is 72 bp \approx 25 nm; For NC-100, the distance between red and purple positioning strands is 296 bp \approx 100 nm.



Supporting Data S2. Detailed sequences of structural strands and positioning strands.

Staple strands	Sequence (5'-3')
structural strand 1	AACGGGTATTAACCAAGTACAAATAACGTTAATTGTAGCGGGAGCACGTATAAC
structural strand 2	TTGTAGCGTAAATGCTTAAAATATTCCAGGAAGAAAT
structural strand 3	CGGAACGAGGGTACAACCTCTGTTAGAAAAAATCAGGTACGTATCGATGA
structural strand 4	CGGAGTGAGAATACAGCAGCGCTCATTAATTCTAGCGGGAGAGGCAGGTT
structural strand 5	GATGATACAGGAGTGTCAAATGGTAGTTGACCTATCAGGTGC
structural strand 6	TGCGTATTCCTGTGTAGGACGTTTGCAACAACTAAAAACCCATG
structural strand 7	TAATTGCGTTGCGAACGAGTA
structural strand 8	CCGATAGTTGCGCATCAGCTGCCAAAAGAACGAAACGGAACGCCATTGCA
structural strand 9	CCACCCTCAGAGCCACATAGATAAAATACCAATCACACACGACCAGTA
structural strand 10	CAGTATTAACACCGCCACAGAGGTTCTGATTATTAATTACAGAAAGTAAGCA
structural strand 11	CGGTCCACAAAACGACACATTCAACTAATCTAACAGCTTGGAG
structural strand 12	CAAAGGCCCTAAAGGTAGGGCTTTTCAAAAATAGCACCCAAAAG
structural strand 13	AGCAATACTCCATCACGATAGCTTATGTAATTATCAAGATTGCGGGAGGTTGA
structural strand 14	TGAATCTTACCAAAAACAATGAATCATAGCAATATAACTCAAACAAAATC
structural strand 15	GAATTCTTAACATAAAACAGTTAATTTCAGAGGCGCAGATTCACTGCT
structural strand 16	TAGGGTTGGAGAAAAAACGCTGCTATATGTCTAATATCATACTAA
structural strand 17	AGAGCTTAAATTGAGAATTGATCCGGCTTAATTACCTGAACAATATATTAGTC
structural strand 18	CACCAAGCTGAGCTGAAACGAACTAGACGACGACGTTAACCTCATT
structural strand 19	AGAACAGCAAGCCCTTTTATTCAATACTAGAACGAAAGGCAGAGCGGGAGCT
structural strand 20	ATAAAAGGACAACAAAGGATTAGAAGTATTGCCACCTAAAGGGCG
structural strand 21	AAACAGGATGCAATGCGGCAAAGAATTAGAACAGGTCAAGCGGGGTT
structural strand 22	ACCCTCTGAAGGTTATCGACAACCTCGTATTAGAGCCACCAAGGTAAAT
structural strand 23	AGGAAACGAGGCCGGATATACTTCTTATCAGATGCCACGCTGAGAGCC
structural strand 24	AAAACACTCATTTGACCCCAACCGGATTACCAAGTCATTAGGTGCG
structural strand 25	AAGTCAGAGGGTACCCAATCCATCATAATCAATAGATAAGAACGCCGAGA
structural strand 26	TGACAGGAGGTTGAGGAATTAGCGGCTTAGATAAGCAAAGCCAGCTT
structural strand 27	TGTACCCCCAAAGGGTAGCATTAAACATCCAACAAATAAGCTGAGAC
structural strand 28	GCCATTAAATGAAAAAGCGGAATTATCATCATTAGCGTCACCATTAC
structural strand 29	GGATTGCTAAAGCAACGGCTGTGAATTCACTCAGCGTGCAGCTGCATTA
structural strand 30	GCGCATCGGCTGTCTTCCTTAGAAACCGAGTTGAACCGCCCGCTAACAC
structural strand 31	GGAATCATTACCGCGCCAATACAACATGTTACAAACGGCGAACGGATTAGACA
structural strand 32	AGTCCACTGGAAAGCATTCTACTTTAACCGTTAACGCTTACGCAG

structural strand 33	AGGTGGCAAATTATCGTCAGATGCCGAACAATCAACAGTTGAAAG
structural strand 34	TACCGTTCCAGTAAGCCATTGGGTTGATTACAAGAGATGGTAG
structural strand 35	AGGCTTGCAGGAATTTCATAAAAACAGAGGAAGTGAGCGAGAGCCCCAA
structural strand 36	TCACCGCCGCAGGTCGGAAAGATTAAAGAGCAAGGAGCCTCACCCCTCA
structural strand 37	TAATAAGAGCAAGCGCTAACGAGCCAACGATTGTCACGTGGACTCCAACGT
structural strand 38	TCTGAAACCAGTATTAGTTGCCAGACCATTGCTATCCGCT
structural strand 39	CGCCTCCCTCAGAGCCAGACTTAAGAAACAAGGCCAACATCTGAAAT
structural strand 40	AGAATCAAGTTGCCTATTCTGATGAATAAACGAACCAACATCAC
structural strand 41	TATGTTAGACCGACTTAATAAGAAACATTATCAAACCTCAATCAAT
structural strand 42	TTGTAAACTAAAATTCTCAGAGCATAAGCTGAGCGCCGCCGTCGA
structural strand 43	GAGGGTTGCCACCCCTCCAGACCGGGATTACGGCGATTAACCAAGCTT
structural strand 44	ACGGTAATTACCATCTGAAAAGGTGGCATCGCAGTCCCTATTAT
structural strand 45	AGAGTCTGTTCTTGACGCTATTAGTTGGATAACGTCACGTAGCGAC
structural strand 46	CCATCGATAACAAAGTTCCCTAGAACATCC
structural strand 47	AACCTAAAACGAAAGAGGCCAAAAGCTGCTACCTTATACGAGCTGGGCCGAAAC
structural strand 48	ACTACAACCCCTGCCTAATATAACAGCGCAGCAATATGATATTCAACC
structural strand 49	TGCGTCTTCCAGACGT
structural strand 50	TAGACGGGAGAACGTCAAAAAATAAGTCCCATTGCTCCGAAATCGGCAA
structural strand 51	TTCAGGAAAGAGGATTAATGCTGACAGGCAACTGAGTAATGTGAGG
structural strand 52	CAGCCTTACAGAGAGATAAAATTAAATCAATAAGCCCCAGCAGGCGAAA
structural strand 53	ACAGTATCGGCCTATTGAATCGTAAATTGTGAGGAAGTTCC
structural strand 54	CAGTCGGAAAGCCTGATTCAACACTGCGGATTTCTGTAGACAGGCC
structural strand 55	CGCCCACGCATAAGCTCCAAACACTATCTTAATTCTCCTGTATATTAAA
structural strand 56	ATTAACGGTAAAATACGTA
structural strand 57	TAGCTTTTCAGGCTTGAGAACACCAGCTCACTGCCGCTTTC
structural strand 58	ACATTTCGACTGGTAATAAGT
structural strand 59	TTAAATTTCAGGAAACATTATGACCTGCACCCCTCAGAATAGGT
structural strand 60	TGAGGACTAAAGATAATGAAATCGTCATAACAGTTCCGTGATCTCATTGCC
structural strand 61	ACATTCAATAAGTTACCTGAGGAAACTTGCTTACTCACGCTG
structural strand 62	AGAAAATTCAATTCAAAATCGGCATCTCTAGTTGCCGCTACAGGGCGC
structural strand 63	TCACCGTCCAAACGTATTGAGGTTGGCGATTAAGTGGCGAG
structural strand 64	GAGTGAGCTAACTCACAT
structural strand 65	CCATATTATTATATTGAGCGAAATGCTGACAAATTAAACGCTAACGTG
structural strand 66	AGATCTACAAAGGCATTAGATCCCCTCAACGATCTAAAGTT
structural strand 67	ATTTGCAAGCCCTTACGCTGAGTAATCGTTAGTAATACCAGCAG

structural strand 68	AGAGCCAGTGATTAAGTACATAAAGTCTGAGACCAGAACATCGATTAGA
structural strand 69	TCCTCAAGTAGCAAGCAAAGATTACAAAATAGAAGGGCGACATGGTCA
structural strand 70	TACCGTAAATGAAAGTAACAAAGAATAGTAGTGAGAAAGGCCGGAGA
structural strand 71	TTAACCGGGTCAGTGCC
structural strand 72	CAGATATAGAAGGCTTATCCGTAAAGTACTCAACAGGAGCCCCCTGAGAAGTGT
structural strand 73	AAAATCACCGAACCAAATCCTTGAATATACAGCGTAAGCATGGAAA
structural strand 74	ATTGACGGACATATAATCAAGAAAATGCAAATGTTAGAATAGCGGGCG
structural strand 75	GAGATAACCCACACCAGTTACCATATGCAGTCAGCTATCCAGTTGAAACAAG
structural strand 76	AACTGGCACAAAATCAACCATATCCAGAAGGATCTAAAGCATCACCTT
structural strand 77	GGATCGTCACCCTGAAAGGAAAAGAAGTTAGAAGCAGGAACAAACAATCATA
structural strand 78	ATGAATCGCACACAACCGCATTTAGTAAAAAACAGTTAGTACAA
structural strand 79	CAGTTGAGGGACGACG
structural strand 80	CATTAGCACAATAATATGCTCTGAAGAGTCAGAGGCCACAGCACTAA
structural strand 81	AGGAACCAATATCCAACAGGTAGACACCGTACTCGGT
structural strand 82	TGAGAGTCTGATAAATTGTTAGCTATTTGTACATACATAAACAGT
structural strand 83	TATCATCGCCTGATAAATTGTGATGAACGTACAGGTAACCTAGAGGCAGGGAAAGGGGA
structural strand 84	ATTGTATCGGTTTCGACAATGGAGATTAACTGACCAAGAGAGTTGCAGCAAG
structural strand 85	CCAAGCGCAGAACAAAGTACAGGCTGACCGTTATAATTGTAATTGGTGCAGGCCT
structural strand 86	AATCCCCTCTGGCAAGAAGAATAAAAGACAAACACCCTGACAAAGACA
structural strand 87	AAAGCCAGAATGGAAAATTCTACTTACGGTGTAGCATGTCGGCGGAT
structural strand 88	ACCGAGGCAGTTAGCGAACAGTAATAAATTAAACATGCCGTACAGAGTAA
structural strand 89	TTGAAAACATAGCGCAAATTAAATCAAG
structural strand 90	GTAGGTTTACCGCGCTTGTACATTGTAGATTAGAGCCGTCA
structural strand 91	CCACGGAACCGATTGAACATCGGCAAACAATTCTAAAATATCTTAG
structural strand 92	GCTATCTTACCGACCCAGCTAACGCCATGAGAATATGTCTATGGCGAT
structural strand 93	TAATGCCCGCCTGTAGCATAAATCCTGGATAGACCGCTCCGGAAAGCA
structural strand 94	CTCCAAAAAAAGCCGATATAAACATTATGTGTACAGAACAGCTGATTGCCCT
structural strand 95	ATCCTGTTACCCACACATACCGACATATTAGGGAAGCGCAATCAAT
structural strand 96	TTGCTCAGACCCCTCAGTATCGGTATAACCCTGCCAGCTGGATCCCC
structural strand 97	GGCCCACGGTCGAGGACGCCAACAGATTAAGTTAGAAAGGAAACCG
structural strand 98	CCCTTATTAGCGTTGGTTGAGTAATTGCGTTGAATGGCTTACCGCC
structural strand 99	ATTGCGAATAATAGTAAAGGAAATCTACTTCATCAAGGGTGGTTTCTTT
structural strand 100	GAACCGCCTACCAGGCTGCGGATAATAAGCTTAGAACCCCTCATAT
structural strand 101	TAAGAGGCAGGTACCGTTGT
structural strand 102	GATTTTTGTTATAACTGAAGAACCGAACAAAGAACTCAATCGGAGATAGA

structural strand 103	CCGCCACCAGAACCAACCAACCAAAATCGGTGCTCCTTTTGTAAATCAAAAA
structural strand 104	ATGCCACTTGACGAGAATGGTTAGGGTGCCTCGCACTCCAGCC
structural strand 105	GAGAGGGTAGCTATTTTGAG
structural strand 106	CTCATAGTAGTAACAGGTAGATTCAATAACCTAATGCCG
structural strand 107	CCTTGATATTCAAATAATCATTAGCTCAAATCAGAAATAACAACC
structural strand 108	AAGATAAAATGCAACAGTGATGGCAATTCATCACGTAATCACAATGAAA
structural strand 109	TTTTTGGACGTGAACGAGGCATTTGAGGCCCTCCGACTAGTTGCT
structural strand 110	TTTAATGCTCAAATATCATTTCGGAACAAAATTCGGTGGATT
structural strand 111	AGGTTTAGTACCGATATAAGCTTTAATTGTACCAAGAACGCTTATTCAA
structural strand 112	GCGCGTTTCATCGCGAAACCACAAAATTATAGCCCTATCGGCC
structural strand 113	GATAGCCGAGCCTTAATAG
structural strand 114	CGACCTGCTCCATGTTACTTAGGAACCGAGGAATACCGGCCAGTGGTGGTAACGCC
structural strand 115	GCACAGACAGTGGCAGTTATTAAATTAAAACCATCTTGTGAATT
structural strand 116	GCTTGACGATTAAAGACAGACGACGACAATAAGCAAGCATCC
structural strand 117	GAGCACTAGACATTCTAACGGATATTCAATTTCGTCACAT
structural strand 118	CGCAAGGAGTTAATATTGATAAGAAAGCGAACAGAACCGCTTA
structural strand 119	CGTCGGATCTGTTGGCGAGAGGGCGGAAGAACCGCTTTATA
structural strand 120	GGATTATTCTATGGTTTTCAAATCGTGTGATAATGAAAAAAG
structural strand 121	TAATTCGCGCTGCAAGAGGCATAGCATCAGTTACAACAACCCG
structural strand 122	GCATGCCTGGCCCTGACTTGAAGAGGACAGTCGAAATCAT
structural strand 123	ATGGCGCTTCCGGCGTCCAATTAAATCATACAGAGGACC
structural strand 124	TAAAGATTGGTTGATACATGTTTGATCAACCAATAGGGGA
structural strand 125	TACCTACACTTCCCTCCAATCGCACACCGGAAATAAGATCG
structural strand 126	TTGCTGGTACGGTACGGACTACCTCAGTATAAGCGTCTTAAT
structural strand 127	GCTGAACCGCGAACTGTTGCACGTGGAAACAGACTCCTACAA
structural strand 128	CTAGGGCGATAAATCAAAGTCCTGAACAAGAACGCACCAAAC
structural strand 129	TAAAGTGTAAACCTGTTGAATAAGGCTTCCCACGAAGGCCTT
structural strand 130	CGCGTAACTGATGGTGAATTACGAGCATGTATCATTCTAG
structural strand 131	TAGCTGTTGGCGCCAGAGTAATCTGACAAGCAGCGATTGCG
structural strand 132	GTTCTAGCTGGAGCAACCAATTCTAGAATGACCATTCCACATG
structural strand 133	GAATTGAGGACCTGAAAGTAACAGAACAAACAAAGAACGACA
structural strand 134	AAACAAACAGGAAATTACATAGGTTAACATTAAAGTCATAATC
structural strand 135	AGCATCGTAACAGAAAACGGCGAACGATGCCGTATGGCTTT
structural strand 136	CTTATTAAATGCCGAAAGGCTGAATAGGATTAACGATTGG
structural strand 137	TTAGTAGAAGATGTGAGTGTAGAACCTCCAGTAGCAGACTGTA

structural strand 138	CAATCTCCGTGAAGCGGATAAAATATGCATTAAGAGTCCTCATT
structural strand 139	GGAAATATCCATTAAATAAAAACAGAGAGCCATTCATAGCC
structural strand 140	GTGTTTGACGGATGATGATACCTTGGGAGGGAACCGGAAC
structural strand 141	CAGATGGATAGGTCTTATTCAITCCTTCGGAATCTGAATT
structural strand 142	GTATACATTGAAATTATTCTGCCCTGACAAAGACACAGAACCG
structural strand 143	TGTGTCTGCCGAGCTCAGGTCAITGGATAAGTGCCAGCAT
structural strand 144	TAATACTTTGTTAAAGTTGATTAGAGAGTACATAGCCCCGAG
structural strand 145	CATTTTGTTTCCGACGCAGATAACATAACGCTTCGAAGG
structural strand 146	GACGTTGTGCTGGTTGACGGTCAATCATAAGGCCGGAACATA
structural strand 147	ATTTAAAAGATTGTAGCTAATTACTCAAAAGCCACCAAAT
structural strand 148	TCATCAACCGCTATTACGTTACCAACGGAACCTCGGTCTTG
structural strand 149	GGGTACCGAGACGGGACCAGCGCATAGGCTACGGAGATCTG
0 nm positioning strand 1	AGCAGCAAAATACCGGGAAAGGTAATAACCTACGGAATAATA
0 nm positioning strand 2	TTGCCTGATAATCAGTATAGTAAAATTGAGACAATTAAAGA
0 nm positioning strand 3	ATCGGAACGAAAAACCAAAAGTACCGACAAAGGTATTCTATCC
0 nm-A positioning strand 1	AAAAAAAAAAAAAAAGCAGCAAAATACCGGGAAAGGTAATAACCTACGGAATAATA
0 nm-A positioning strand 2	AAAAAAAAAAAAAAATTGCCTGATAATCAGTATAGTAAAATTGAGACAATTAAAGA
0 nm-A positioning strand 3	AAAAAAAAAAAAAAATCGGAACGAAAAACCAAAAGTACCGACAAAGGTATTCTATCC
25 nm positioning strand 1	ATCTGGTCAATATTTAGATTTCTAACAAATGAAAATACAGA
25 nm positioning strand 2	AGCCATTGCAGGAGGCTTATATAATTAGTATAAAAATAAGTA
25 nm positioning strand 3	AAAGGAAGAGTGTGTATGCAGAACCGCGCTGTTTCATCCAG
25 nm-A positioning strand 1	AAAAAAAAAAAAAAATCTGGTCAATATTTAGATTTCTAACAAATGAAAATACAGA
25 nm-A positioning strand 2	AAAAAAAAAAAAAAAGCCATTGCAGGAGGCTTATATAATTAGTATAAAAATAAGTA
25 nm-A positioning strand 3	AAAAAAAAAAAAAAAGGAAGAGTGTGTATGCAGAACCGCGCTGTTTCATCCAG
100 nm positioning strand 1	CAGTCAAACGTAAAACCTGGAAGTCCTGACTTCGTCACCAG
100 nm positioning strand 2	TGACCGTAGCAAAGCGGGGGTAAAGAACTGAAAGACAGACT
100 nm positioning strand 3	CACAATTGCCAACGCCAAATCACGTAACAAGAACCAT
100 nm-A positioning strand 1	AAAAAAAAAAAAACAGTCACCGTAAACCTGGAAGTCCTGACTTCGTCACCAG
100 nm-A positioning strand 2	AAAAAAAAAAAAATGACCGTAGCAAAGCGGGGGTAAAGAACTGAAAGACAGACT
100 nm-A positioning strand 3	AAAAAAAAAAAAACACAATTGCCAACGCCAAATCACGTAACAAGAACCAT
PolyT	TTTTTTTTTTTTT-SH-C6

A

Genetic Site	Cell Bank information		Customer sample	
(Locus)	A-549		A549	
Amelogenin	X	Y	X	X
CSF1PO	10	12	10	12
D13S317	11	11	11	11
D16S539	11	12	11	12
D5S818	11	11	11	11
D7S820	8	11	8	11
TH01	8	9,3	8	9,3
TPOX	8	11	8	11
vWA	14	14	14	14
D21S11			29	29
Percent match between the sample and the database profile: 94.44%				

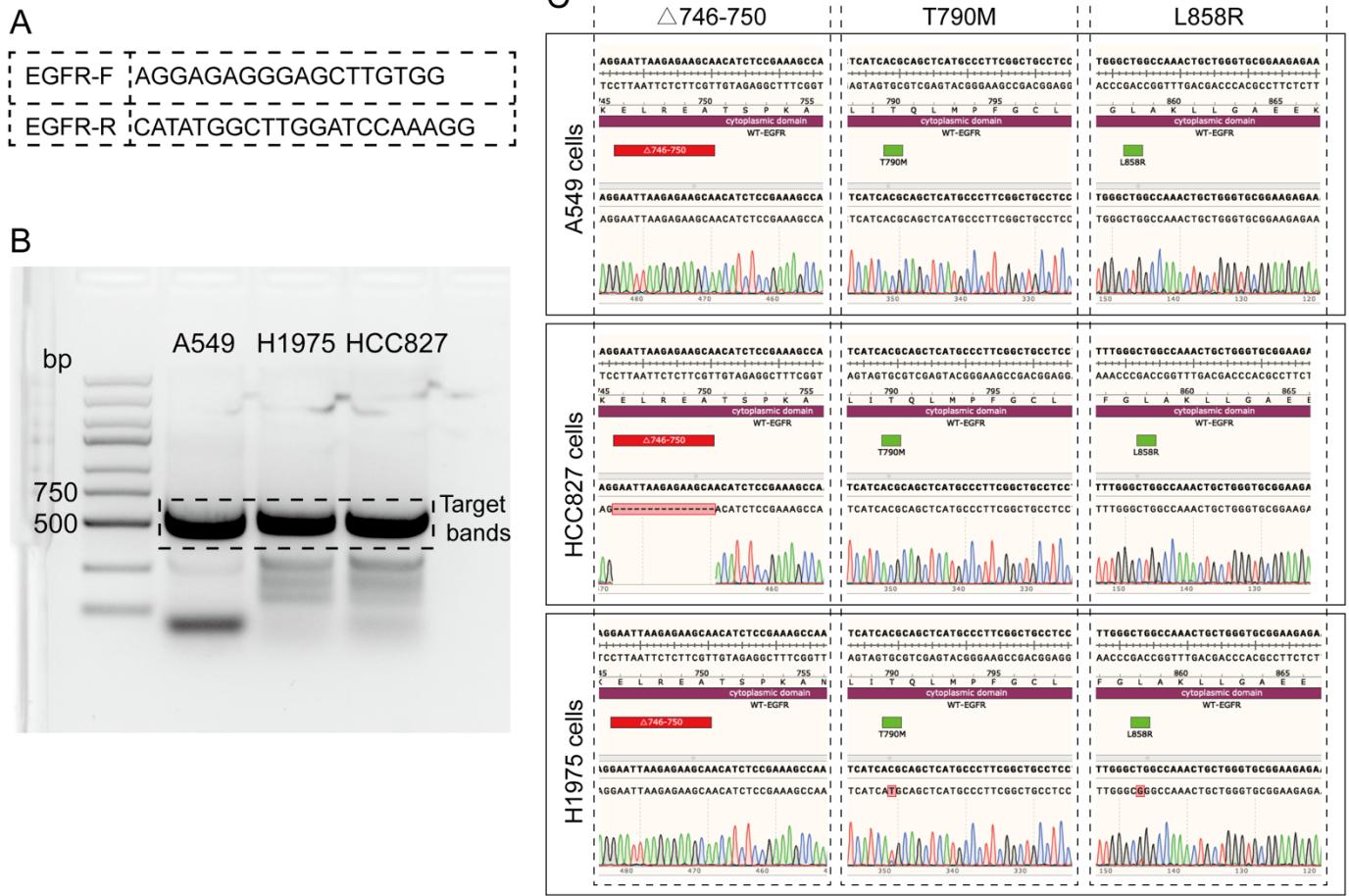
B

Genetic Site	Cell Bank information		Customer sample	
(Locus)	HCC827		HCC827	
Amelogenin	X	X	X	X
CSF1PO	11	11	11	11
D13S317	9	9	9	9
D16S539	12	12	12	12
D5S818	12	12	12	12
D7S820	11	12	11	12
TH01	6	6	6	6
TPOX	8	8	8	8
vWA	18	18	18	18
D21S11			31	31
Percent match between the sample and the database profile: 100%				

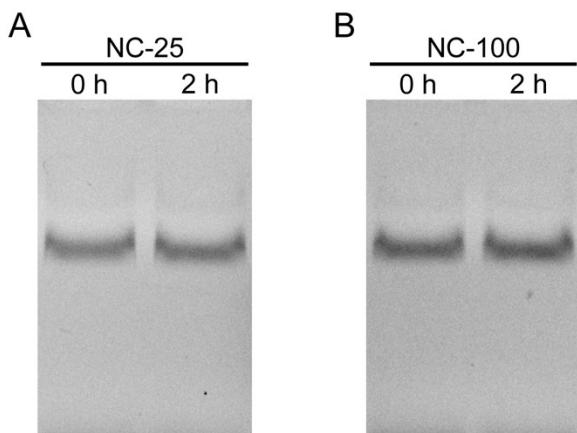
C

Genetic Site	Cell Bank information		Customer sample	
(Locus)	H1975		H1975	
Amelogenin	X	X	X	X
CSF1PO	12	12	12	12
D13S317	10	13	10	13
D16S539	9	12	9	12
D5S818	11	12	11	12
D7S820	8	11	8	8
TH01	7	7	7	7
TPOX	8	11	8	11
vWA	18	18	18	18
D21S11			28	28
Percent match between the sample and the database profile: 94%				

Supporting Data S3. STR identification results of various lung cancer cell lines. The purities of A549 (A), HCC827 (B) and H1975 (C) were verified by STR identification.



Supporting Data S4. Identification of A549, H1975, and HCC827 cell lines. (A) Nucleotide sequences of EGFR-F and EGFR-R primers. (B) The separation of PCR products (in 1% agarose gel) from EGFR-F and EGFR-R primers extensions. Marker: D2000 plus DNA Ladder. (C) Sequencing results of A549, HCC827, and H1975 cells exhibit their EGFR mutation status. The nucleotide sequence of EGFR (Human Gene, NM_005228.5) was downloaded from the *Ensembl website* and observed by the SnapGene software. Then, EGFR-F primers and EGFR-R primers at the corresponding positions of the shortest sequence fragments (approximately 500 bp) containing the E746_A750 deletion, L858R, and T790M were designed to compare the differences in the nucleotide sequence of EGFR in the three cell lines. These results demonstrate that no unwanted mutations or cell blending occurred during the culture period of three cell lines.



Supporting Data S5. Gel electrophoresis (1% agarose) results show the stability of DNA nanocalipers. (A) NC-25 was incubated in the detection environment for 0 h and 2 h. (B) NC-100 was incubated in the detection environment for 0 h and 2 h. The representative gel electrophoresis images show the respective signal expressions among various samples. The signal expression of DNA nanocalipers incubated in the detection environment for 0 h was consistent with that of the 2 h incubation, demonstrating that DNA nanocalipers remained structurally stable within the detection period.

Supporting Data S6. Identification of EGFR mutations by NGS and DNA nanocalipers.

Sample number	NGS		DNA nanocalipers
	Mutation type	Amino acid change	
1	Mutated EGFR	p. Leu858Arg	Mutated EGFR
	Mutated PTEN	p. Glu314fs	
2	Mutated EGFR	p. Glu746_Ser752delinsVal	Mutated EGFR
	Mutated BAP1	p. Ile47fs	
3	Mutated EGFR	E21	Mutated EGFR
	Mutated MET	E2	
4	Mutated EGFR	p. Glu746_AlA750del	Mutated EGFR
	Mutated TP53	p. Arg175His	
5	Mutated EGFR	p. Gly719Ser p. Glu709Ala	Mutated EGFR
	Mutated PMS1	p. Gln226*	
	Mutated RB1	p. Ser565*	
	Mutated WRN	p. Ser1141*	
6	Mutated STK11	deletion	Mutated EGFR
	Mutated EGFR	p. Leu861Gln	
	Mutated TP53	p. Trp53*	
7	Mutated ERBB2	Amplification	Wild-type EGFR
	Mutated TP53	p. Ser166*	
	Wild-type EGFR	No mutation	
8			Wild-type EGFR
9	Mutated KIF5B-RET	Fusion	Wild-type EGFR

Note: The standard of the quality examination offered by the service provider: The minimum DNA content requirement is 10 ng for NGS. The typical cancer/normal cell proportion should reach 25% for NGS.