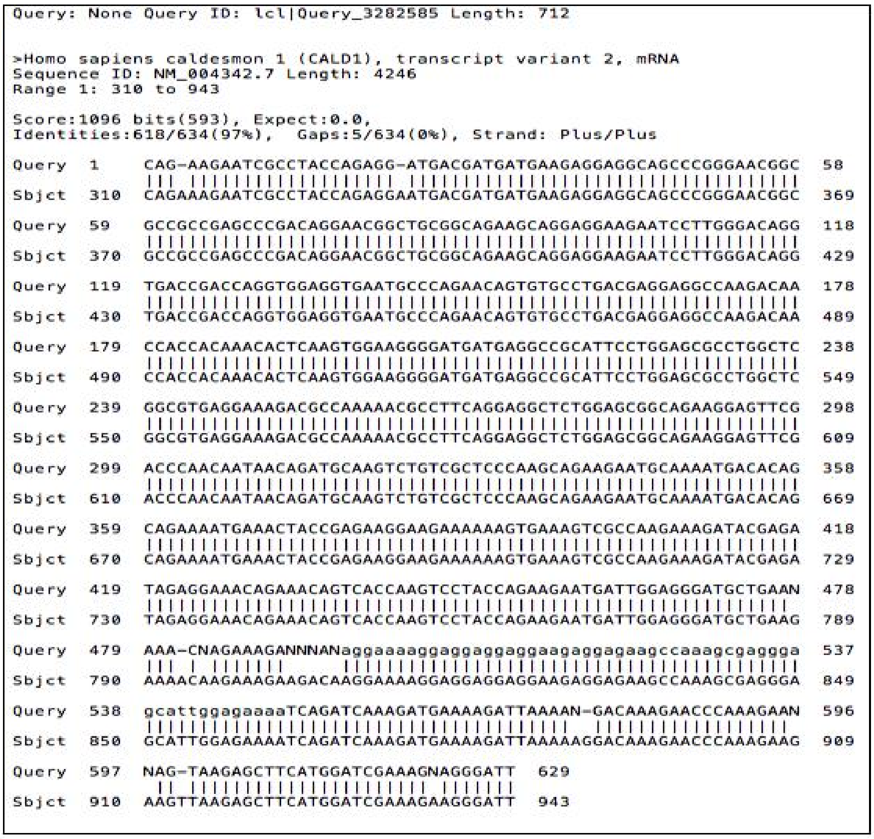
CAL-51



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Query: None Query ID: lcl|Query_3282585 Length: 712

>Homo sapiens caldesmon 1 (CALD1), transcript variant 2, mRNA
Sequence ID: NM_004342.7 Length: 4246
Range 1: 310 to 943

Score:1096 bits(593), Expect:0.0,
Identities:618/634(97%),  Gaps:5/634(0%), Strand: Plus/Plus

Query  1    CAG-AAGAATCGCCTACCAGAGG-ATGACGATGATGAAGAGGAGGCAGCCCGGGAACGGC  58
            ||| ||||||||||||||||||| ||||||||||||||||||||||||||||||||||||
Sbjct  310  CAGAAAGAATCGCCTACCAGAGGAATGACGATGATGAAGAGGAGGCAGCCCGGGAACGGC  369

Query  59   GCCGCCGAGCCCGACAGGAACGGCTGCGGCAGAAGCAGGAGGAAGAATCCTTGGGACAGG  118
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  370  GCCGCCGAGCCCGACAGGAACGGCTGCGGCAGAAGCAGGAGGAAGAATCCTTGGGACAGG  429

Query  119  TGACCGACCAGGTGGAGGTGAATGCCCAGAACAGTGTGCCTGACGAGGAGGCCAAGACAA  178
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  430  TGACCGACCAGGTGGAGGTGAATGCCCAGAACAGTGTGCCTGACGAGGAGGCCAAGACAA  489

Query  179  CCACCACAAACACTCAAGTGGAAGGGGATGATGAGGCCGCATTCCTGGAGCGCCTGGCTC  238
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  490  CCACCACAAACACTCAAGTGGAAGGGGATGATGAGGCCGCATTCCTGGAGCGCCTGGCTC  549

Query  239  GGCGTGAGGAAAGACGCCAAAAACGCCTTCAGGAGGCTCTGGAGCGGCAGAAGGAGTTCG  298
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  550  GGCGTGAGGAAAGACGCCAAAAACGCCTTCAGGAGGCTCTGGAGCGGCAGAAGGAGTTCG  609

Query  299  ACCCAACAATAACAGATGCAAGTCTGTCGCTCCCAAGCAGAAGAATGCAAAATGACACAG  358
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  610  ACCCAACAATAACAGATGCAAGTCTGTCGCTCCCAAGCAGAAGAATGCAAAATGACACAG  669

Query  359  CAGAAAATGAAACTACCGAGAAGGAAGAAAAAAGTGAAAGTCGCCAAGAAAGATACGAGA  418
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  670  CAGAAAATGAAACTACCGAGAAGGAAGAAAAAAGTGAAAGTCGCCAAGAAAGATACGAGA  729

Query  419  TAGAGGAAACAGAAACAGTCACCAAGTCCTACCAGAAGAATGATTGGAGGGATGCTGAAN  478
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| 
Sbjct  730  TAGAGGAAACAGAAACAGTCACCAAGTCCTACCAGAAGAATGATTGGAGGGATGCTGAAG  789

Query  479  AAA-CNAGAAAGANNNANaggaaaaggaggaggaggaagaggagaagccaaagcgaggga  537
            ||| | |||||||     ||||||||||||||||||||||||||||||||||||||||||
Sbjct  790  AAAACAAGAAAGAAGACAAGGAAAAGGAGGAGGAGGAAGAGGAGAAGCCAAAGCGAGGGA  849

Query  538  gcattggagaaaaTCAGATCAAAGATGAAAAGATTAAAAN-GACAAAGAACCCAAAGAAN  596
            |||||||||||||||||||||||||||||||||||||||  |||||||||||||||||| 
Sbjct  850  GCATTGGAGAAAATCAGATCAAAGATGAAAAGATTAAAAAGGACAAAGAACCCAAAGAAG  909

Query  597  NAG-TAAGAGCTTCATGGATCGAAAGNAGGGATT  629
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Sbjct  910  AAGTTAAGAGCTTCATGGATCGAAAGAAGGGATT  943
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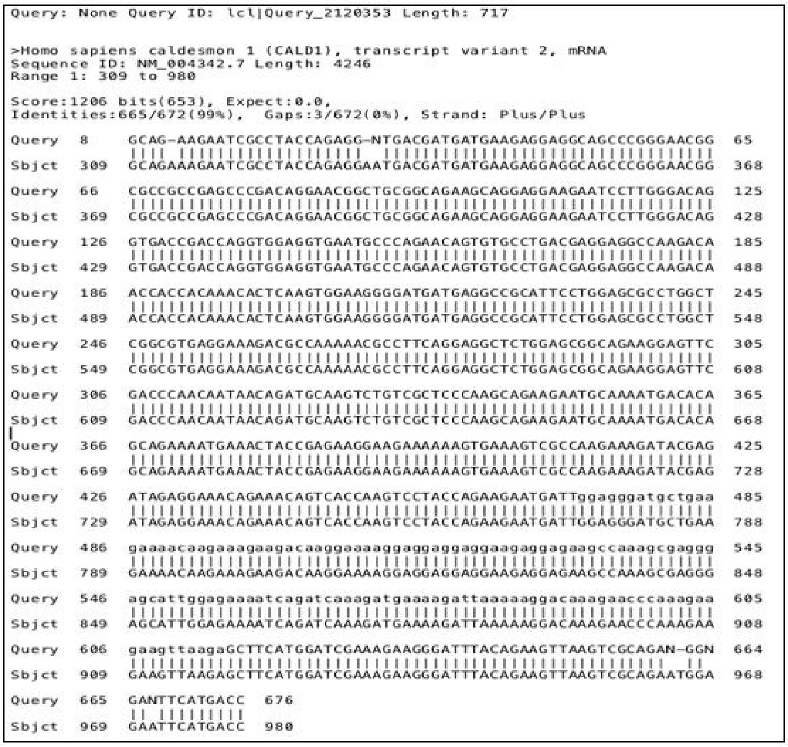
BT549



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Query: None Query ID: lcl|Query_2120353 Length: 717

>Homo sapiens caldesmon 1 (CALD1), transcript variant 2, mRNA
Sequence ID: NM_004342.7 Length: 4246
Range 1: 309 to 980

Score:1206 bits(653), Expect:0.0,
Identities:665/672(99%),  Gaps:3/672(0%), Strand: Plus/Plus

Query  8    GCAG-AAGAATCGCCTACCAGAGG-NTGACGATGATGAAGAGGAGGCAGCCCGGGAACGG  65
            |||| |||||||||||||||||||  ||||||||||||||||||||||||||||||||||
Sbjct  309  GCAGAAAGAATCGCCTACCAGAGGAATGACGATGATGAAGAGGAGGCAGCCCGGGAACGG  368

Query  66   CGCCGCCGAGCCCGACAGGAACGGCTGCGGCAGAAGCAGGAGGAAGAATCCTTGGGACAG  125
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  369  CGCCGCCGAGCCCGACAGGAACGGCTGCGGCAGAAGCAGGAGGAAGAATCCTTGGGACAG  428

Query  126  GTGACCGACCAGGTGGAGGTGAATGCCCAGAACAGTGTGCCTGACGAGGAGGCCAAGACA  185
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  429  GTGACCGACCAGGTGGAGGTGAATGCCCAGAACAGTGTGCCTGACGAGGAGGCCAAGACA  488

Query  186  ACCACCACAAACACTCAAGTGGAAGGGGATGATGAGGCCGCATTCCTGGAGCGCCTGGCT  245
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  489  ACCACCACAAACACTCAAGTGGAAGGGGATGATGAGGCCGCATTCCTGGAGCGCCTGGCT  548

Query  246  CGGCGTGAGGAAAGACGCCAAAAACGCCTTCAGGAGGCTCTGGAGCGGCAGAAGGAGTTC  305
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  549  CGGCGTGAGGAAAGACGCCAAAAACGCCTTCAGGAGGCTCTGGAGCGGCAGAAGGAGTTC  608

Query  306  GACCCAACAATAACAGATGCAAGTCTGTCGCTCCCAAGCAGAAGAATGCAAAATGACACA  365
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  609  GACCCAACAATAACAGATGCAAGTCTGTCGCTCCCAAGCAGAAGAATGCAAAATGACACA  668

Query  366  GCAGAAAATGAAACTACCGAGAAGGAAGAAAAAAGTGAAAGTCGCCAAGAAAGATACGAG  425
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  669  GCAGAAAATGAAACTACCGAGAAGGAAGAAAAAAGTGAAAGTCGCCAAGAAAGATACGAG  728

Query  426  ATAGAGGAAACAGAAACAGTCACCAAGTCCTACCAGAAGAATGATTggagggatgctgaa  485
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  729  ATAGAGGAAACAGAAACAGTCACCAAGTCCTACCAGAAGAATGATTGGAGGGATGCTGAA  788

Query  486  gaaaacaagaaagaagacaaggaaaaggaggaggaggaagaggagaagccaaagcgaggg  545
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  789  GAAAACAAGAAAGAAGACAAGGAAAAGGAGGAGGAGGAAGAGGAGAAGCCAAAGCGAGGG  848

Query  546  agcattggagaaaatcagatcaaagatgaaaagattaaaaaggacaaagaacccaaagaa  605
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  849  AGCATTGGAGAAAATCAGATCAAAGATGAAAAGATTAAAAAGGACAAAGAACCCAAAGAA  908

Query  606  gaagttaagaGCTTCATGGATCGAAAGAAGGGATTTACAGAAGTTAAGTCGCAGAN-GGN  664
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||  || 
Sbjct  909  GAAGTTAAGAGCTTCATGGATCGAAAGAAGGGATTTACAGAAGTTAAGTCGCAGAATGGA  968

Query  665  GANTTCATGACC  676
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Sbjct  969  GAATTCATGACC  980
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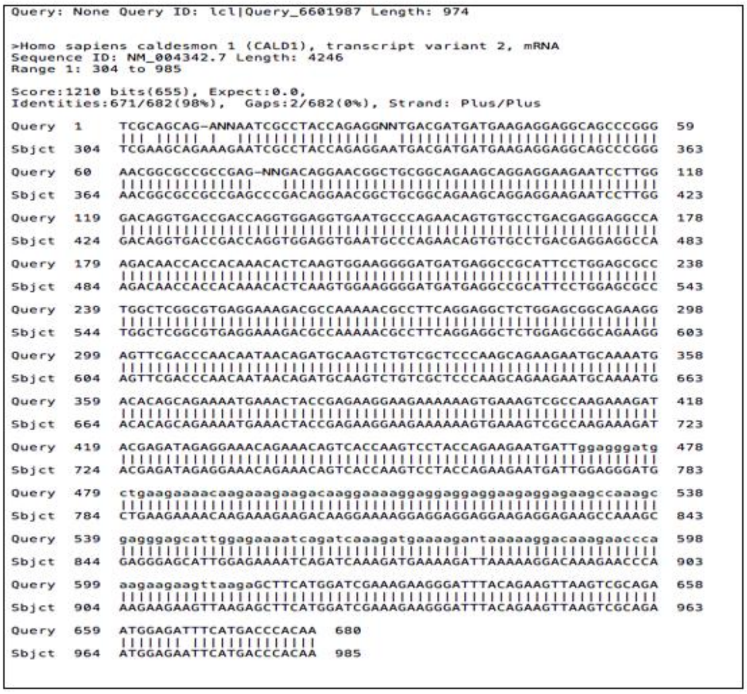
MDA-MB-231



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Query: None Query ID: lcl|Query_6601987 Length: 974

>Homo sapiens caldesmon 1 (CALD1), transcript variant 2, mRNA
Sequence ID: NM_004342.7 Length: 4246
Range 1: 304 to 985

Score:1210 bits(655), Expect:0.0,
Identities:671/682(98%),  Gaps:2/682(0%), Strand: Plus/Plus

Query  1    TCGCAGCAG-ANNAATCGCCTACCAGAGGNNTGACGATGATGAAGAGGAGGCAGCCCGGG  59
            ||| ||||| |  ||||||||||||||||  |||||||||||||||||||||||||||||
Sbjct  304  TCGAAGCAGAAAGAATCGCCTACCAGAGGAATGACGATGATGAAGAGGAGGCAGCCCGGG  363

Query  60   AACGGCGCCGCCGAG-NNGACAGGAACGGCTGCGGCAGAAGCAGGAGGAAGAATCCTTGG  118
            |||||||||||||||   ||||||||||||||||||||||||||||||||||||||||||
Sbjct  364  AACGGCGCCGCCGAGCCCGACAGGAACGGCTGCGGCAGAAGCAGGAGGAAGAATCCTTGG  423

Query  119  GACAGGTGACCGACCAGGTGGAGGTGAATGCCCAGAACAGTGTGCCTGACGAGGAGGCCA  178
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  424  GACAGGTGACCGACCAGGTGGAGGTGAATGCCCAGAACAGTGTGCCTGACGAGGAGGCCA  483

Query  179  AGACAACCACCACAAACACTCAAGTGGAAGGGGATGATGAGGCCGCATTCCTGGAGCGCC  238
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  484  AGACAACCACCACAAACACTCAAGTGGAAGGGGATGATGAGGCCGCATTCCTGGAGCGCC  543

Query  239  TGGCTCGGCGTGAGGAAAGACGCCAAAAACGCCTTCAGGAGGCTCTGGAGCGGCAGAAGG  298
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  544  TGGCTCGGCGTGAGGAAAGACGCCAAAAACGCCTTCAGGAGGCTCTGGAGCGGCAGAAGG  603

Query  299  AGTTCGACCCAACAATAACAGATGCAAGTCTGTCGCTCCCAAGCAGAAGAATGCAAAATG  358
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  604  AGTTCGACCCAACAATAACAGATGCAAGTCTGTCGCTCCCAAGCAGAAGAATGCAAAATG  663

Query  359  ACACAGCAGAAAATGAAACTACCGAGAAGGAAGAAAAAAGTGAAAGTCGCCAAGAAAGAT  418
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  664  ACACAGCAGAAAATGAAACTACCGAGAAGGAAGAAAAAAGTGAAAGTCGCCAAGAAAGAT  723

Query  419  ACGAGATAGAGGAAACAGAAACAGTCACCAAGTCCTACCAGAAGAATGATTggagggatg  478
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  724  ACGAGATAGAGGAAACAGAAACAGTCACCAAGTCCTACCAGAAGAATGATTGGAGGGATG  783

Query  479  ctgaagaaaacaagaaagaagacaaggaaaaggaggaggaggaagaggagaagccaaagc  538
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  784  CTGAAGAAAACAAGAAAGAAGACAAGGAAAAGGAGGAGGAGGAAGAGGAGAAGCCAAAGC  843

Query  539  gagggagcattggagaaaatcagatcaaagatgaaaagantaaaaaggacaaagaaccca  598
            ||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||||
Sbjct  844  GAGGGAGCATTGGAGAAAATCAGATCAAAGATGAAAAGATTAAAAAGGACAAAGAACCCA  903

Query  599  aagaagaagttaagaGCTTCATGGATCGAAAGAAGGGATTTACAGAAGTTAAGTCGCAGA  658
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  904  AAGAAGAAGTTAAGAGCTTCATGGATCGAAAGAAGGGATTTACAGAAGTTAAGTCGCAGA  963

Query  659  ATGGAGATTTCATGACCCACAA  680
            ||||||| ||||||||||||||
Sbjct  964  ATGGAGAATTCATGACCCACAA  985
```

Supplementary Figure 1 Sequencing analysis of low-molecular-weight isoform of caldesmon transcript variants in triple-negative breast cancer cell lines. Sanger sequencing was performed to identify the low-molecular-weight isoform of caldesmon (l-CAD) transcript variant expressed in triple-negative breast cancer (TNBC) cell lines. Sequencing and alignment analyses were conducted by Molecular Cloning Laboratories (San Francisco, CA, United States). BLAST sequence alignment of RT-PCR amplicons demonstrated that all analyzed TNBC cell lines matched to Homo sapiens caldesmon 1 (CALD1) transcript variant 2 (mRNA Sequence ID: NM_004342.7). For CAL-51 cells, sequence alignment showed 97% identity (618/634) with a score of 1096 bits and no significant gaps. BT-549 cells exhibited 99% identity (665/672) with a score of 1206 bits, while MDA-MB-231 cells demonstrated 98% identity (671/682) with a score of 1210 bits. All alignments had an expectation value of 0.0, confirming high-confidence identification of CALD1 transcript variant 2 as the predominant l-CAD isoform expressed in these TNBC cell lines.