Supplementary Materials for

Kinectin 1 promotes the growth of triple-negative breast cancer via directly co-activating NF-kappaB/p65 and enhancing its transcriptional activity

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Report of Human Cell Line Authentication
Supplementary Methods

Ethics statement

The obtained BC tissue arrays was supported by the National Human Genetic Resources Sharing Service Platform (2005DKA21300). This study was approved by the Institutional Review Board of the Second Clinical Medicine College of Jinan University, and all patients provided written informed consent for the use of surgical samples (LL-KY-2019435). Mouse transportation, housing, and breeding were conducted according to recommendations of “The use of non-human animals in research”. Mice were killed by cervical dislocation to prevent suffering. All mouse experiments were conducted in accordance with the ethical standards and guidelines of the Asian Federation of Laboratory Animal Science Associations and were approved by the Second Clinical Medicine College of Jinan University (20200316-23).

Cell Culture

All cell lines were purchased from CellCook Biotech CO., LTD. MCF10A cells were grown in DMEM/F12 medium (Gibco) supplemented with 5% horse serum (Gibco), 10 µg/ml insulin (Gibco), 20 ng/ml epidermal growth factor (EGF), 0.5 µg/ml hydrocortisone. MCF7 cells were grown in MEM medium (Gibco) supplemented with 10% fetal bovine serum (FBS) and 10 µg/ml insulin, 1× nonessential amino acid (CellCook). ZR75-1 and HCC38 cells in RPMI1640 medium supplemented with 10% FBS. T47D and BT459 cells were maintained in RPMI1640 medium supplemented with 10% FBS and 10 µg/ml insulin. MDA-MB-231 cells were maintained in DMEM medium supplemented with 10% FBS. All media were supplemented with 5,000U/ml penicillin/streptomycin (Invitrogen). All cells were maintained at 37°C with 5% CO₂. All cell lines used in this study were authenticated using short tandem repeat typing (results are presented in the Supplementary data 2) and tested to exclude mycoplasma contamination.

Cell Proliferation and Colony Formation Assay
For cell proliferation assay, MDA-MB-231 (8000 per well) or BT549 cells (6000 per well) cultivated on 96-well plates (Corning) were transfected with siRNA oligos, and cell proliferation were determined after 0h, 24h, 48h, and 72h by CCK-8 kit (MCE) at 450nm according to the manufacturers’ instructions. For cell colony formation assay, MDA-MB-231 (1000 per well) or BT549 cells (1000 per well) were cultivated on 6-well plates. Attached cells were transfected with siRNA oligos, and cells were collected at 7 day. Cells were washed twice with PBS and fixed in 4% paraformaldehyde for 1h. Cell colonies were photographed and counted after staining with 0.1% crystal violet.

**Cell Migration and Invasiveness**

For cell migration assay treated with KTN1 shRNA vectors, KTN1 overexpression with minicircle KTN1 vectors, and CXCL8 siRNA oligos, 1×10^5 cells were seeded in Transwell chambers (Merck Millipore, 8µm) of 24-well plates (Corning). The complete medium (10% FBS) was added to the bottom of Transwell chambers, while the serum-free medium was added to the top of Transwell chambers. After being cultured between 16h and 20h, the cells were fixed with 4% paraformaldehyde, photographed, and calculated for statistical analysis. Each experiment was repeated at least three times.

For cell invasion assay treated with KTN1 shRNA vectors, KTN1 overexpression with MC KTN1 vectors, and CXCL8 siRNA oligos, 1×10^5 cells were seeded in Matrigel-coated Transwell chambers (BD, 8µm) of 24-well plates. Other measures were the same as cell migration assay.

For cell migration assay and cell invasion assay treated with KTN1 siRNA oligos or CXCL8 protein, 1×10^5 cells were seeded in Transwell chambers (Merck Millipore, 8µm) of 24-well plates (Corning), respectively. The complete medium (5% FBS) containing 800 ng/ml CXCL8 protein was added to the bottom of Transwell chambers, while the serum-free medium containing cells was added to the top of Transwell chambers. Other measures were the same as the above contents.
RNA extraction, Quantitative Reverse Transcription-PCR (qRT-PCR), and RNA sequencing

Total RNA was extracted from cells using TRIzol (Life Technologies) according to the manufacturer’s instructions. Reverse transcription was conducted using TransScript One-Step RT-PCR SuperMix (Transgen Biotech), and qPCRs were run using SYBR Green PCR Master Mix (Thermo Fisher Scientific) in a 96-well plate (Bio-Rad). The mRNA relative expression was calculated by the comparative Ct values. Each experiment was repeated at least three times. The primer sequences for qRT-PCR were listed in Table S1. For RNA sequencing, NEB library were prepared to obtain mRNA, subjected to quality control using an Agilent 2100 bioanalyzer. RNA sequencing was performed at Beijing Novogene, using the Illumina Novaseq 6000 platform.

Table S1. Sequences of qRT-PCR primers used in this study.

| Primers   | Forward              | Reverse             |
|-----------|----------------------|---------------------|
| GAPDH     | 5’- ACATCGCTCAGACACCAGATG-3’ | 5’- TGTAGTTGAGGTCAATGAAGG-3’ |
| β-Actin   | 5’- ACCTTCTACAATGAGCTGCG-3’   | 5’- CCTGGATAGCAACCGTACATGG-3’   |
| KTN1      | 5’- TGGAAATCAGAGCAGAAAAGGGG-3’ | 5’- GAACTGAAGCGGAGGTCTG-3’ |
| NF-κB/p65 | 5’- AATCCAGTGTGTGAAAGAC-3’   | 5’- GCTGCTCTTCTATAGGAACT-3’   |
| CXCL8     | 5’- ATACTCCAACCTTTCCACCC-3’  | 5’- TCTGCCACCAGTTTCTTTTG-3’  |
| IL-24     | 5’- GAGGAACACGAGACTGAGAG-3’  | 5’- TCCAGAGAAGGATGTAAAAC-3’  |
| CCL5      | 5’- TGCCCACTCAAAGGAGTATTC-3’   | 5’- CCATCCTAGCTCATCTCACAAG-3’   |
| CXCL16    | 5’- CATCTTCATCCTCAACCGCAG-3’   | 5’- CATCTTCATCCTCAACCGCAG-3’   |
**Western blot, Enzyme-linked immunosorbent assay (ELISA), and antibodies**

The following primary antibodies were used: anti-KTN1 [Cell Signaling Technology (CST), #13243, 1:3000], anti-NF-κB/p65 (CST, #8242, 1:3000), anti-phospho-NF-κB/p65 (CST, #3033, 1:1000), anti-β-actin (CST, #3700, 1:3000), anti-GAPDH (CST, #5174, 1:3000), anti-β-Tubulin (CST, #2128S, 1:3000), anti-β-Tubulin (CST, #2128, 1:3000), anti-Lamin B1 (CST, #13435, 1:1000), anti-Vimentin (CST, #5741, 1:3000), anti-E-cadherin (CST, #14472, 1:3000), anti-N-cadherin (CST, #13116, 1:3000). The following secondary antibodies were also used: anti-rabbit IgG-HRP (CST, #7074, 1:2000) and anti-mouse IgG-HRP (CST, #7076, 1:2000); For ChIP and Co-IP assay antibodies: normal mouse IgG (Merck, #12-371). Recombinant Human IL-8 (CXCL8) (PeproTech, 200-08).

**Table S2.** Sequences of ChIP-qPCR primers used in this study.

| Primers | Forward | Reverse |
|---------|---------|---------|
| CXCL8-NF-κB/p65_1 | 5’- CTCAAAGATCATGTGTTTGTGTA-3’ | 5’- GTAGGAGATGTAATGCTATG-3’ |
| CXCL8-NF-κB/p65_2 | 5’- GTCTTTGGATAAAGAGCATG-3’ | 5’- AGGTATGTTATGCTCCAGAA-3’ |
| CXCL8-NF-κB/p65_3 | 5’- CTTTCGTCATACCTCCGTATTTG-3’ | 5’- TCATCACCCTACTAGAGGAACCT-3’ |

**Table S3.** Sequences of siRNA or shRNA used in this study.

| Sequences | 5’- GAGTGATCTTTCTAGCAAA-3’ |
|-----------|----------------------------|
| siKTN1_1  | 5’- GAATCTGGGTGTAATACAA-3’ |
| Name            | Sequence                                                                 |
|-----------------|--------------------------------------------------------------------------|
| shKTN1_1        | 5’-CCGGCCTCTCTAGTTGAAGAATTACTCGAGTAAGTTCTTCAACTAGAGGTGGTTTTTG-3’         |
| shKTN1_2        | 5’-CCGGCGGTAAACCAACAGCTCACAACTCGAGTTGTGAGCTGTTGGTTTACCGTTTTTG-3’         |
| siCXCL8_1       | 5’- GTCAGTGCATAAAGACATA-3’                                              |
| siCXCL8_2       | 5’- GCCAAGGAGTGCTAAAGAA-3’                                              |
| siNF-κB/p65_1   | 5’- CTCAAGATCTGCCGAGTG-3’                                               |
| siNF-κB/p65_2   | 5’- AGACGATCGTCACC GGATT-3’                                             |
Figure S1. The top 10 Gene Ontology (GO) analysis with the molecular function of basal BC compared with other subtypes of BC (including Lunimal A, Lunimal B and Her2).
Figure. S2. Association of KTN1 IHC-staining scores with BCs tumor grades (I-II, II, II-III, III). The positive staining was indicated by ‘red’ arrows. The numbers of cases were showed below. Data were plotted as the means of 95% confidence interval ± s.d. One-Way ANOVA and Dunnett’s multiple comparison test were used to analyze the data. *P < 0.05, **P < 0.01, ***P < 0.001. Scale bars, 100 µm.
Figure S3. KTN1 knockdown inhibited the tumorigenesis and invasion of BT549 cells. Knockdown of KTN1 in BT549 cell by KTN1_1 and KTN1_2 shRNA vectors was determined by qRT-PCR. (a) and Western blot assay (b) compared with negative control vector (NC). c Measurement of cell proliferation by CCK-8 assay cells with NC, shKTN1_1 and shKTN1_2 plasmids treatment. d Measurement of cell colony forming efficiency by colony formation assay cells with NC, shKTN1_1 and shKTN1_2 plasmids treatment. e Transwell assay analysis of migration and invasiveness cells with NC, shKTN1_1 and shKTN1_2 plasmids treatment. f Western blot analysis results of EMT markers in KTN1 knockdown cells. All statistical data represent the average of three independent experiments ±s.d. * P < 0.05, ** P < 0.01, *** P < 0.001. Scale bars, 100 µm.
Figure. S4. RNA-seq analysis of KTN1-regulated downstream gene profile changes in TNBC cells. a Represent differential signaling pathways were highlighted by KEGG pathway analysis in MDA-MB-231 cells. b A heat map analysis of represent differential cytokine genes in BT549 cells treated by KTN1 siRNA oligos compared with control oligos. c Analysis of the 209 basal breast cancer subtypes of patients from TCGA dataset, based on PAM50 breast cancer gene signature classifier. All groups were stratified into ‘High’ and ‘Low’ KTN1 expression based on autoselect best cutoff. $P<0.001$. 
Figure. S5. Knockdown of CXCL8 inhibited BT549 tumor growth and invasion. a QRT-PCR analysis of RNA expression of CXCL8 in BT549 cells by CXCL8 siRNA oligos compared with control oligos. b CXCL8 knockdown inhibited MDA-MB-231 cell proliferation by CCK-8 assay at 0h, 24h, 48h and 72h. c CXCL8 knockdown inhibited cell migration and invasion by Transwell assay. Each experiment was performed in triplicate and data a represented as mean ± s.d. One-Way ANOVA and Dunnett’s multiple comparison test were used to analyze the data (*P < 0.05, **P < 0.01, ***P < 0.001). Scale bars, 100 µm.
Figure. S6. High CXCL8 mRNA expression revealed a shorter overall survival, relapse-free survival, and distant metastasis-free survival in BC patients from Kaplan-Meier plotter dataset.
Figure. S7. KTN1 regulated CXCL8 mRNA via phosphorylating NF-κB/p65 pathway in TNBC cells. 
a Western blot analysis of protein expression of KTN1, phosphorylated NF-κB/p65 (Ser536), NF-κB/p65 in MDA-MB-231 and BT549 cells transfected with NC siRNA oligo, KTN1_1 and KTN1_2 siRNA oligos, respectively. 
b ELISA assay showing the secretion of CXCL8 in MDA-MB-231 cells and BT549 cells transfected with negative control oligos, KTN1 siRNA oligos, NF-κB/p65 siRNA oligos or minicircle KTN1. Each experiment was performed in triplicate and data a represented as mean ± s.d. One-Way ANOVA and Dunnett’s multiple comparison test were used to analyze the data (*P < 0.05, **P < 0.01, ***P < 0.001).
Figure. S8. Rescue experiments by restoring CXCL8 expression after KTN1 depletion re-increased the TNBC cell growth and invasion. **a** Proliferation analysis of KTN1 depleted MDA-MB-231 and BT549 cells added with CXCL8 protein as determined by a CCK-8 assay. **b** Migration and invasiveness of KTN1 depleted MDA-MB-231 and BT549 cells added with CXCL8 protein as determined by Transwell assays. Each experiment was performed in triplicate and data represented as mean ± s.d. One-Way ANOVA and Dunnett’s multiple comparison test were used to analyze the data (*P < 0.05, **P < 0.01, ***P < 0.001). Scale bars, 100 µm.
Figure S9. Co-IP assay and western blot analysis of both KTN1, NF-κB/p65 proteins using specific antibodies was performed to confirm their direct interactions.
Report of Human Cell Line Authentication

(Notice: This authentication report is restricted to the cell sold from Guangzhou Cellcook Biotech Co., Ltd, and the date with seal is the date of delivery.)

I. Sample

Sample Name: labeled as ‘MCF-10A’

II. Method and Procedure

1. PCR is amplified with STR Multi-amplification Kit (PowerPlex™16HS System);
2. PCR products are assayed with 3100 DNA Analyzer (Applied Biosystems®).
3. Amplification of gene COX1 and electrophoresis are employed to survey the species of the sample.

III. Results

1. The STR profiles of the cell line sample are in the attached table and figure.
2. The search result in ATCC and DSMZ databases.
3. The electrophoresis figure of gene COX1.

MCF-10A: ① No loci has tri-alleles or tetra-alleles. Contamination of other human cell line is not found (Figure 1 & Table 1). ② 100% matched cell lines are found in ATCC and DSMZ data banks. And the cell line named as “MCF-10A” et al. (Figure 2 & Figure 3). ③ The sample is a human cell line. Contamination of other species cells are not found in the sample (Figure 4).

Operator: Xiaohua Mo  
Auditor: Xuanyi Liang

Guangzhou Cellcook Biotech Co., Ltd
Figure 1. STR profiles of MCF-10A cell line

[Image of STR profiles]
Table 1. STR profiles of MCF-10A cell line

| Locus     | Allele 1 | Allele 2 |
|-----------|----------|----------|
| D3S1358   | 14       | 18       |
| TH01      | 8        | 9.3      |
| D21S11    | 28       | 30       |
| D18S51    | 19       |          |
| Penta E   | 13       | 14       |
| D5S818    | 10       | 13       |
| D13S317   | 8        | 9        |
| D7S820    | 10       | 11       |
| D16S539   | 11       | 12       |
| CSF1PO    | 10       | 12       |
| Penta D   | 10       | 12       |
| AMEL      | x        |          |
| vWA       | 15       | 17       |
| D8S1179   | 14       | 16       |
| TPOX      | 9        | 11       |
| FGA       | 22       | 24       |

Figure 2. Search result in ATCC database

SEARCH THE STR DATABASE

As part of our continuing efforts to characterize and authenticate the cell lines in the Cell Biology collection, ATCC has developed a comprehensive database of short tandem repeat (STR) DNA profiles for all of our human cell lines. View our brief tutorial before starting.

1. STR Profiling Analysis
2. Matching Algorithm
3. Interrogating the Database
Figure 3. Search result in DSMZ database

| EV      | Cell No. | Cell name | Locus names | Figures |
|---------|----------|-----------|-------------|---------|
|         |          |           | D5S818     |         |
| 1:01(36:35) | CRL-10317 | MCF 10A | 10.13  8.9  10.18  11.12  15.17 | XX  9.11  10.12 |
| 1:02(36:35) | CRL-10318 | MCF 10F | 10.13  8.9  10.18  11.12  15.17 | XX  9.11  10.12 |
| 1:03(36:35) | CRL-10081 | MCF 10-2A | 10.13  9.9  10.18  11.12  15.17 | XX  9.11  10.12 |
| 1:03(36:35) | CRL-10080 | MCF 10-2F | 10.13  9.9  10.18  11.12  15.17 | XX  9.11  10.12 |
| 0:06(36:35) | CRL-9445 | NCI-H2464 [H2444] | 10.10  11.12  8.10  12.12  17.17 | 9.3  9.4  9.5 |
| 0:06(36:35) | 439 | CAL-12 | 13.13  8.8  11.12  10.10  15.17 | XX  9.11  11.12 |
| 0:06(36:35) | 658 | UPCL-SCC-024 | 13.13  8.11  10.10  11.12  15.17 | 9.3  9.4  9.5 |
| 0:06(36:35) | CCL-312 | MRC-9 | 12.13  8.11  10.13  11.12  15.17 | 7.8  9.11  10.12 |
| 0:08(36:35) | CRL-2943 | UACC-3199 | 13.13  11.11  10.10  11.12  15.17 | 9.3  9.4  9.5  9.6  10.13 |
| 0:08(36:35) | HTB-127 | MDA-MB-330 | 13.13  9.9  10.13  9.12  14.16 | 9.3  9.4  9.5  9.6  9.7  9.8  9.9  9.10  9.11  9.12 |
| 0:08(36:35) | IFO50072 | MRC-9 | 12.13  8.11  10.13  11.12  15.17 | 7.8  9.11  10.12 |

Figure 4. Authentication of the species of the sample

M: Marker. As the size of 700, 600, 500, 400, 300, 200 and 100bp from up to down.

Nine species are checked, as follow: Homo sapiens 391bp, Cricetulus griseus 315bp, Macaca mulatta 287bp, Cercopithecus aethiops 222bp, Rattus norvegicus 196bp, Canis familiaris 172bp, Mus musculus 150bp, Bos Taurus 102bp, IC 70bp

The sample: The band size is 391bp which matches the size of human.
Report of Human Cell Line Authentication

(Notice: This authentication report is restricted to the cell sold from Guangzhou Cellcook Biotech Co., Ltd, and the date with seal is the date of delivery.)

I. Sample
   Sample Name: labeled as ‘MCF7’

II. Method and Procedure
   1. PCR is amplified with STR Multi-amplification Kit (PowerPlex™16HS System);
   2. PCR products are assayed with 3100 DNA Analyzer (Applied Biosystems®).
   3. Amplification of gene COX1 and electrophoresis are employed to survey the species of the sample.

III. Results
   1. The STR profiles of the cell line sample are in the attached table and figure.
   2. The search result in ATCC and DSMZ databases.
   3. The electrophoresis figure of gene COX1.

MCF7: ①No loci has tri-alleles or tetra-alleles. Contamination of other human cell line is not found (Figure 1 & Table 1). ②100% matched cell lines are found in ATCC and DSMZ data banks. And the cell line named as “MCF7” et al. (Figure 2 & Figure 3). ③The sample is a human cell line. Contamination of other species cells are not found in the sample (Figure 4).

Operator: Xiaohua Mo

Auditor: Xuan yi Liang

Guangzhou Cellcook Biotech Co., Ltd.
Table 1. STR profiles of MCF7 cell line

|         | Allele1 | Allele2 |
|---------|---------|---------|
| D3S1358 | 16      |         |
| TH01    | 6       |         |
| D21S11  | 30      |         |
| D18S51  | 14      |         |
| Penta_E | 7       | 12      |
| D5S818  | 11      | 12      |
| D13S317 | 11      |         |
| D7S820  | 8       | 9       |
| D16S539 | 11      | 12      |
| CSF1PO  | 10      |         |
| Penta_D | 12      |         |
| AMEL    | x       |         |
| vWA     | 14      | 15      |
| D8S1179 | 10      | 14      |
| TPOX    | 9       | 12      |
| FGA     | 23      | 25      |

Figure 2. Search result in ATCC database
Figure 3. Search result in DSMZ database

Result of STR matching analysis by your data.

| EV       | Cell No. | Cell name | Locus names | Figures |
|----------|----------|-----------|-------------|---------|
| 1.12/36/32 | 115      | MCF-7     | D5S818 11,11, 8,9, 11,12, 14,15 | 6, X, 9,12, 10,10 |
| 1.12/36/32 | HTB-22   | MCF-7     | D3S1358 11,11, 8,9, 11,12, 14,15 | 6, X, 9,12, 10,10 |
| 1.12/36/32 | JCRB0134 | MCF-7     | D7S820 11,11, 8,9, 11,12, 14,15 | 6, X, 9,12, 10,10 |
| 1.12/36/32 | RCEB1904 | MCF-7     | D16S539 11,11, 8,9, 11,12, 14,15 | 6, X, 9,12, 10,10 |
| 1.12/36/32 | KPL-1    | 317       | YW A TH01 AM TPOX CSF1PO | Figures |
| 0.94/36/32 | CRL-9000 | NCI-H1869 | D5S818 11,11, 8,9, 12,12, 16,16 | 6, X, 9,12, 10,10 |
| 0.81/26/32 | 203      | SK-N-MC   | D3S1358 11,11, 8,9, 12,12, 17,17 | 9,9, 9, X, 9,11, 10,10 |
| 0.81/26/32 | 254      | BEN       | D7S820 11,11, 8,9, 12,12, 15,15 | 6, X, 9,12, 10,10 |
| 0.81/26/32 | 377      | YCC-SUP   | D16S539 11,11, 8,9, 9,11, 14,16 | 6,9, X, 9,12, 10,10 |
| 0.81/26/32 | CRL-2207 | RSE21-M17 | YW A TH01 AM TPOX CSF1PO | Figures |
| 0.81/26/32 | CRL-2270 | MC-1XIC   | D5S818 11,11, 8,9, 12,12, 17,17 | 9,9, 9, X, 9,11, 10,10 |
| 0.81/26/32 | CRL-7721 | MB-157    | D3S1358 11,11, 8,9, 12,12, 15,15 | 7,8, X, 9,12, 10,10 |

Figure 4. Authentication of the species of the sample

M: Marker. As the size of 700, 600, 500, 400, 300, 200 and 100bp from up to down.

Nine species are checked, as follow: *Homo sapiens* 391bp, *Crassipinnis griseus* 315bp, *Macaca mulatta* 287bp, *Cercopithecus aethiops* 222bp, *Rattus norvegicus* 196bp, *Canis familiaris* 172bp, *Mus musculus* 150bp, *Bos Taurus* 102bp, 1C 70bp

The sample: The band size is 391bp which matches the size of human.
Report of Human Cell Line Authentication

(Notice: This authentication report is restricted to the cell sold from Guangzhou Cellcook Biotech Co., Ltd, and the date with seal is the date of delivery.)

I. Sample
   Sample Name: labeled as ‘T47D’

II. Method and Procedure
   1. PCR is amplified with STR Multi-amplification Kit (PowerPlex™16 HS System);
   2. PCR products are assayed with 3100 DNA Analyzer (Applied Biosystems®).
   3. Amplification of gene COX1 and electrophoresis are employed to survey the species of the sample.

III. Results
   1. The STR profiles of the cell line sample are in the attached table and figure.
   2. The search result in ATCC and DSMZ databases.
   3. The electrophoresis figure of gene COX1.

T47D: ①No loci has tri-alleles or tetra-alleles. Contamination of other human cell line is not found (Figure 1 & Table 1). ②100% matched cell lines are found in ATCC and DSMZ data banks. And the cell line named as “T47D” et al. (Figure 2 & Figure 3). ③The sample is a human cell line. Contamination of other species cells are not found in the sample (Figure 4).

Operator: Xiaohua Mo
Auditor: Xuan yi Liang
Guangzhou Cellcook Biotech Co., Ltd.
Table 1. STR profiles of T47D cell line

|          | Allele1 | Allele2 |
|----------|---------|---------|
| D3S1358  | 15      | 17      |
| TH01     | 6       |         |
| D21S11   | 28      | 31      |
| D18S51   | 17      |         |
| Penta_E  | 7       | 14      |
| D5S818   | 12      |         |
| D13S317  | 12      |         |
| D7S820   | 11      |         |
| D16S539  | 10      |         |
| CSF1PO   | 11      | 13      |
| Penta_D  | 10      | 12      |
| AMEL     | x       |         |
| vWA      | 14      |         |
| D8S1179  | 9       | 12      |
| TPOX     | 11      |         |
| FGA      | 23      |         |

Figure 2. Search result in ATCC database

SEARCH THE STR DATABASE

As part of our continuing efforts to characterize and authenticate the cell lines in the Cell Biology collection, ATCC has developed a comprehensive database of short tandem repeat (STR) DNA profiles for all of our human cell lines. View our brief tutorial before starting.

1. STR Profiling Analysis
2. Matching Algorithm
3. Interrogating the Database

Showing 1 - 2 Of 2

| Add to Cart | %Match | ATCC® Number | Designation          | D5S818 | D13S317 | D7S820 | D16S539 | vWA | TH01 | AMEL | TPOX | CSF1PO |
|-------------|--------|---------------|-----------------------|--------|---------|--------|---------|-----|------|------|------|--------|
|             | 100.0  | HTB-133       | T-47D Breast CancerHuman | 12     | 12      | 11     | 10      | 14  | 6    | X     | 11   | 11.13  |
|             | 100.0  | CRL-2865      | T47D-KBluc Breast Ductal CarcinomaHuman | 12     | 12      | 11     | 10      | 14  | 6    | X     | 11   | 11.13  |

Disclaimer: Reference to this database and the data contained therein may be cited in publications, and ATCC encourages such citation or reference. While every reasonable effort has been made to assure the accuracy of these data, no warranty, express or implied, is made by ATCC as to their accuracy.
**Figure 3. Search result in DSMZ database**

| EV  | Cell No. | Cell name | D5S818 | D13S317 | D16S539 | D7S820 | TH01 | Amel | TPOX | CSF1PO | Figures |
|-----|----------|-----------|--------|---------|--------|--------|------|------|------|--------|---------|
| 1.20(36-28) | 717 | T47D | 7.10 | 7.17 | 7.17 | 7.17 | 7.10 | 7.14 | 7.14 | 7.15 | 7.15 | - |
| 1.29(36-28) | CRL-2865 | T47D-KBhce | 12.12 | 12.12 | 12.12 | 12.12 | 12.12 | 12.12 | 12.12 | 12.12 | 12.12 | - |
| 1.29(36-28) | HTB-133 | T-47D | 12.12 | 12.12 | 12.12 | 12.12 | 12.12 | 12.12 | 12.12 | 12.12 | 12.12 | - |
| 0.95(26-28) | 601 | KYO-1 | 10.10 | 10.10 | 10.10 | 10.10 | 10.10 | 10.10 | 10.10 | 10.10 | 10.10 | - |
| 0.85(24-28) | 266 | S-117 | 11.11 | 11.11 | 11.11 | 11.11 | 11.11 | 11.11 | 11.11 | 11.11 | 11.11 | - |
| 0.85(24-28) | RCB2097 | TE-4 | 9.10 | 9.10 | 9.10 | 9.10 | 9.10 | 9.10 | 9.10 | 9.10 | 9.10 | - |
| 0.79(22-28) | 68 | NAMALWA-KN2 | 12.12 | 12.12 | 12.12 | 12.12 | 12.12 | 12.12 | 12.12 | 12.12 | 12.12 | - |
| 0.79(22-28) | 443 | CAL-112T | 10.10 | 10.10 | 10.10 | 10.10 | 10.10 | 10.10 | 10.10 | 10.10 | 10.10 | - |
| 0.79(22-28) | 461 | UPCI-S2026 | 9.10 | 9.10 | 9.10 | 9.10 | 9.10 | 9.10 | 9.10 | 9.10 | 9.10 | - |
| 0.79(22-28) | 674 | LA-N-8 | 12.12 | 12.12 | 12.12 | 12.12 | 12.12 | 12.12 | 12.12 | 12.12 | 12.12 | - |
| 0.79(22-28) | 721 | NCEB-1 | 9.11 | 9.11 | 9.11 | 9.11 | 9.11 | 9.11 | 9.11 | 9.11 | 9.11 | - |
| 0.79(22-28) | CRL-2283 | SQO | 10.10 | 10.10 | 10.10 | 10.10 | 10.10 | 10.10 | 10.10 | 10.10 | 10.10 | - |

A graphical presentation is shown at the bottom of this page.

**Figure 4. Authentication of the species of the sample**

M: Marker. As the size of 700, 600, 500, 400, 300, 200 and 100bp from up to down.

Nine species are checked, as follow: *Homo sapiens* 391bp, *Cricetulus griseus* 315bp, *Macaca mulatta* 287bp, *Cercopithecus aethiops* 222bp, *Rattus norvegicus* 196bp, *Canis familiaris* 172bp, *Mus musculus* 150bp, *Boa constrictor* 102bp, 1C 70bp

The sample: The band size is 391bp which matches the size of human.
Report of Human Cell Line Authentication
(Notice: This authentication report is restricted to the cell sold from Guangzhou Cellcook Biotech Co., Ltd, and the date with seal is the date of delivery.)

I. Sample
   Sample Name: labeled as ‘ZR-75-1’

II. Method and Procedure
   1. PCR is amplified with STR Multi-amplification Kit (PowerPlex™ 16HS System);
   2. PCR products are assayed with 3100 DNA Analyzer (Applied Biosystems®);
   3. Amplification of gene COX1 and electrophoresis are employed to survey the species of the sample.

III. Results
   1. The STR profiles of the cell line sample are in the attached table and figure.
   2. The search result in ATCC and DSMZ databases.
   3. The electrophoresis figure of gene COX1.

   ZR-75-1: ①No loci has tri-alleles or tetra-alleles. Contamination of other human cell line is not found (Figure 1 & Table 1). ②100% matched cell lines are found in ATCC and DSMZ data banks. And the cell line named as “ZR-75-1” et al. (Figure 2 & Figure 3). ③The sample is a human cell line. Contamination of other species cells are not found in the sample (Figure 4).
Figure 1: STR profiles of ZR-75-1 cell line
Table 1. STR profiles of ZR-75-1 cell line

| Allele1 | Allele2 |
|---------|---------|
| D3S1358 | 15      | 16    |
| TH01    | 7       | 9.3   |
| D21S11  | 31      |       |
| D18S51  | 13      | 14    |
| Penta_E |         | 14    |
| D5S818  | 13      |       |
| D13S317 | 9       |       |
| D7S820  | 10      | 11    |
| D16S539 | 11      |       |
| CSF1PO  | 10      | 11    |
| Penta_D | 14      |       |
| AMEL    | x       |       |
| vWA     | 16      | 18    |
| D8S1179 | 11      | 13    |
| TPOX    | 8       |       |
| FGA     | 20      | 22    |

Figure 2. Search result in ATCC database

SEARCH THE STR DATABASE

As part of our continuing efforts to characterize and authenticate the cell lines in the Cell Biology collection, ATCC has developed a comprehensive database of short tandem repeat (STR) DNA profiles for all of our human cell lines. View our brief tutorial before starting.

1. STR Profiling Analysis
2. Matching Algorithm
3. Interrogating the Database

Showing 1 - 1 Of 1
Figure 3. Search result in DSMZ database

![Result of STR matching analysis by your data.](image)

| EV    | Cell No. | Cell name | Locus names | Figures |
|-------|----------|-----------|-------------|---------|
|       |          |           | D5S818 | D13S317 | D7S829 | D16S539 | VWA | TH01 | AM | TPOX | CSF1PO |         |
| 1.16(3631) | CRL-1500 | ZR-75-1 | 13.13 | 9.9 | 10.11 | 11.11 | 16.18 | 7.9 | XX | XX | XX | 10.11 | - |
| 1.16(3631) | SCRM5923 | YNB-1 | 13.13 | 9.9 | 10.11 | 11.11 | 16.18 | 7.9 | XX | XX | XX | 10.11 | - |
| 1.16(3631) | SCRM5925 | YMB-1-E | 13.13 | 9.9 | 10.11 | 11.11 | 16.18 | 7.9 | XX | XX | XX | 10.11 | - |
| 1.16(3631) | SCRM596 | ZR-75-1 | 13.13 | 9.9 | 10.11 | 11.11 | 16.18 | 7.9 | XX | XX | XX | 10.11 | - |
| 0.84(3631) | 450 | BC-3C | 11.13 | 9.9 | 11.11 | 11.11 | 17.16 | 6.9 | XX | XX | XX | 11.11 | - |
| 0.84(3631) | 494 | HDQ-P1 | 11.11 | 12.12 | 11.12 | 11.11 | 16.18 | 9.3 | 9.3 | XX | XX | 11.11 | - |
| 0.84(3631) | CRL-10762 | MCF-12A | 11.13 | 9.11 | 11.11 | 9.12 | 19.10 | 7.7 | XX | XX | XX | 10.11 | - |
| 0.84(3631) | CRL-10763 | MCF-12F | 11.13 | 9.11 | 11.11 | 9.12 | 19.10 | 7.7 | XX | XX | XX | 10.11 | - |
| 0.84(3631) | CRL-2020 | DDBTRG-6550 | 12.13 | 9.9 | 11.11 | 10.12 | 15.16 | 7.8 | XX | XX | XX | 10.11 | - |
| 0.84(3631) | CRL-2090 | CCD-1074sk | 10.11 | 9.9 | 11.11 | 11.11 | 16.17 | 9.9 | XX | XX | XX | 11.12 | - |
| 0.84(3631) | CRL-5934 | NCI-H2227 | 13.13 | 8.8 | 10.12 | 9.9 | 16.18 | 9.3 | 9.3 | XX | XX | 11.11 | - |

Figure 4. Authentication of the species of the sample

![Image of gel electrophoresis](image)

M: Marker. As the size of 700, 600, 500, 400, 300, 200 and 100bp from up to down.

Nine species are checked, as follow: *Homo sapiens* 391bp, *Cricetulus griseus* 315bp, *Macaca mulatta* 287bp, *Cercopithecus aethiops* 222bp, *Rattus norvegicus* 196bp, *Canis familiaris* 172bp, *Mus musculus* 150bp, *Boo Taurus* 102bp, 1C 70bp

The sample: The band size is 391bp which matches the size of human.
Report of Human Cell Line Authentication

I. Sample

Sample Name: labeled as ‘MDA-MB-453’

II. Method and Procedure

1. PCR is amplified with STR Multi-amplification Kit (PowerPlex™16HS System);
2. PCR products are assayed with 3100 DNA Analyzer (Applied Biosystems®).
3. Amplification of gene COX1 and electrophoresis are employed to survey the species of the sample.

III. Results

1. The STR profiles of the cell line sample are in the attached table and figure.
2. The search result in ATCC and DSMZ databases.
3. The electrophoresis figure of gene COX1.

MDA-MB-453: ①No loci has tri-alleles or tetra-alleles. Contamination of other human cell lines are not found (Figure 1 & Table 1). ②Compared the STR data of MDA-MB-453 cell line in the databases of ATCC and DSMZ, all the alleles of MDA-MB-453 were exactly matched with the alleles of MDA-MB-453 cells found in both cell banks (Figure 2&3). ③The sample is a human cell line. Contamination of other species cells are not found in the sample (Figure 4).

To all above, the sample is a single cell line, and it is MDA-MB-453 cell line.

Operator: Xiaohua Mo
Auditor: Xunyi Liang
Guangzhou Cellcook Biotech Co., Ltd

(Notice: This authentication report is restricted to the cell sold from Guangzhou Cellcook Biotech Co., Ltd, and the date with seal is the date of delivery.)
Figure 1. STR profiles of MDA-MB-453 cell line
Table 1. STR profiles of MDA-MB-453 cell line

|     | Allele1 | Allele2 |
|-----|---------|---------|
| D3S1358 | 15      |         |
| TH01    | 6       |         |
| D21S11  | 29      | 31      |
| D18S51  | 15      | 20      |
| Penta_E | 11      |         |
| D6S18  | 11      |         |
| D13S317 | 12      |         |
| D7S820  | 10      |         |
| D16S539 | 9       |         |
| CSF1PO | 10      | 12      |
| Penta_D | 9       | 10      |
| AMEL    | x       |         |
| VWA     | 17      | 18      |
| D8S1179 | 10      | 12      |
| TPOX    | 10      |         |
| FGA     | 18      | 23      |

Figure 2. Search result in ATCC database

As part of our continuing efforts to characterize and authenticate the cell lines in the Cell Biology collection, ATCC has developed a comprehensive database of short tandem repeat (STR) DNA profiles for all of our human cell lines. View our brief tutorial before starting.

1. STR Profiling Analysis
2. Matching Algorithm
3. Interrogating the Database

Showing 1 - 2 Of 2

Disclaimer: Reference to this database and the data contained therein may be cited in publications, and ATCC encourages such citation or reference. While every reasonable effort has been made to assure the accuracy of these data, no warranty, express or implied, is made by ATCC as to their accuracy.
### Figure 3. Search result in DSMZ database

**Result of STR matching analysis by your data.**

A graphical presentation is shown at the bottom of this page.

| EV  | Cell No. | Cell name         | Loci names          | Figures |
|-----|----------|-------------------|---------------------|---------|
|     |          |                   | D5S818              |         |
|     |          |                   | D13S317             |         |
|     |          |                   | D7S820              |         |
|     |          |                   | D16S539             |         |
|     |          |                   | VWA                 |         |
|     |          |                   | TH01                |         |
|     |          |                   | AM                  |         |
|     |          |                   | TPOX                |         |
|     |          |                   | CSF1PO              |         |
|     |          |                   | 11,11               |         |
|     |          |                   | 12,12               |         |
| 1.00(14/30) | 65 | MDA-MB-453        | 11,11               |         |
|     |          |                   | 12,12               |         |
|     |          |                   | 10,10               |         |
|     |          |                   | 9,9                 |         |
|     |          |                   | 17,18               |         |
|     |          |                   | 6,6                 |         |
|     |          |                   | XX                  |         |
|     |          |                   | 10,10               |         |
|     |          |                   | 10,12               |         |
|     |          |                   |                     |         |
| 1.00(14/30) | CRL-2713 | MDA-MB-453       | 11,11               |         |
|     |          |                   | 12,12               |         |
|     |          |                   | 10,10               |         |
|     |          |                   | 9,9                 |         |
|     |          |                   | 17,18               |         |
|     |          |                   | 6,6                 |         |
|     |          |                   | XX                  |         |
|     |          |                   | 10,10               |         |
|     |          |                   | 10,12               |         |
|     |          |                   |                     |         |
| 1.00(14/30) | HTB-151 | MDA-MB-453       | 11,11               |         |
|     |          |                   | 12,12               |         |
|     |          |                   | 10,10               |         |
|     |          |                   | 9,9                 |         |
|     |          |                   | 17,18               |         |
|     |          |                   | 6,6                 |         |
|     |          |                   | XX                  |         |
|     |          |                   | 10,10               |         |
|     |          |                   | 10,12               |         |
|     |          |                   |                     |         |
| 1.00(14/30) | RCB1192 | MDA-MB-453       | 11,11               |         |
|     |          |                   | 12,12               |         |
|     |          |                   | 10,10               |         |
|     |          |                   | 9,9                 |         |
|     |          |                   | 17,18               |         |
|     |          |                   | 6,6                 |         |
|     |          |                   | XX                  |         |
|     |          |                   | 10,10               |         |
|     |          |                   | 10,12               |         |
|     |          |                   |                     |         |
| 0.70(20/37) | 529 | OCI-MI           | 11,11               |         |
|     |          |                   | 11,12               |         |
|     |          |                   | 10,10               |         |
|     |          |                   | 9,9                 |         |
|     |          |                   | 17,18               |         |
|     |          |                   | 9,9                 |         |
|     |          |                   | XX                  |         |
|     |          |                   | 11,11               |         |
|     |          |                   | 10,12,14            |         |
|     |          |                   |                     |         |
| 0.67(24/36) | HTB-4 | T24              | 10,12               |         |
|     |          |                   | 12,12               |         |
|     |          |                   | 10,11               |         |
|     |          |                   | 9,9                 |         |
|     |          |                   | 17,17               |         |
|     |          |                   | 6,6                 |         |
|     |          |                   | XX                  |         |
|     |          |                   | 8,11                |         |
|     |          |                   | 10,12               |         |
|     |          |                   |                     |         |
| 0.67(24/36) | JCRB0133 | KHS-1B       | 11,11               |         |
|     |          |                   | 11,11               |         |
|     |          |                   | 10,10               |         |
|     |          |                   | 9,9                 |         |
|     |          |                   | 14,17               |         |
|     |          |                   | 6,6                 |         |
|     |          |                   | XX                  |         |
|     |          |                   | 11,11               |         |
|     |          |                   | 10,10               |         |
|     |          |                   |                     |         |
| 0.67(24/36) | JCRB0710 | EZ-1           | 10,12               |         |
|     |          |                   | 12,12               |         |
|     |          |                   | 10,11               |         |
|     |          |                   | 9,9                 |         |
|     |          |                   | 17,17               |         |
|     |          |                   | 6,6                 |         |
|     |          |                   | XX                  |         |
|     |          |                   | 8,11                |         |
|     |          |                   | 10,12               |         |
|     |          |                   |                     |         |
| 0.67(24/36) | JCRB0711 | T24          | 10,12               |         |
|     |          |                   | 12,12               |         |
|     |          |                   | 10,11               |         |
|     |          |                   | 9,9                 |         |
|     |          |                   | 17,17               |         |
|     |          |                   | 6,6                 |         |
|     |          |                   | XX                  |         |
|     |          |                   | 8,11                |         |
|     |          |                   | 10,12               |         |
|     |          |                   |                     |         |

### Figure 4. Authentication of the species of the sample

![Cell authentication image](image)

M: Marker. As the size of 700, 600, 500, 400, 300, 200 and 100bp from up to down.

Nine species are checked, as follow: *Homo sapiens* 391bp, *Cricetus griseus* 315bp, *Macaca mulatta* 287bp, *Cercopithecus aethiops* 222bp, *Rattus norvegicus* 196bp, *Canis familiaris* 172bp, *Mus musculus* 150bp, *Bos Taurus* 102bp, IC 70bp

The sample: The band size is 391bp which matches the size of human.
Report of Human Cell Line Authentication

I. Sample
   Sample Name: labeled as ‘HCC38’

II. Method and Procedure
   1. PCR is amplified with STR Multi-amplification Kit (PowerPlex™16HS System);
   2. PCR products are assayed with 3100 DNA Analyzer (Applied Biosystems®).
   3. Amplification of gene COX1 and electrophoresis are employed to survey the species of the sample.

III. Results
   1. The STR profiles of the cell line sample are in the attached table and figure.
   2. The search result in ATCC and DSMZ databases.
   3. The electrophoresis figure of gene COX1.

HCC38: ①No loci has tri-alleles or tetra-alleles. Contamination of other human cell lines are not found (Figure 1 & Table 1). ②Compared the STR data of HCC38 cell line in the databases of ATCC and DSMZ, all the alleles of HCC38 were exactly matched with the alleles of HCC38 cells found in both cell banks (Figure 2&3). ③The sample is a human cell line. Contamination of other species cells are not found in the sample (Figure 4).

   To all above, the sample is a single cell line, and it is HCC38 cell line.

Operator: Xiaohua Mo  Auditor: Xuanyi Liang
Guangzhou Cellcook Biotech Co., Ltd

(Notice:This authentication report is restricted to the cell sold from Guangzhou Cellcook Biotech Co., Ltd, and the date with seal is the date of delivery)
Table 1. STR profiles of HCC38 cell line

|                | Allele1 | Allele2 |
|----------------|---------|---------|
| D3S1358        | 18      |         |
| TH01           | 9.3     |         |
| D21S11         | 27      | 28      |
| D18S51         | 15      |         |
| Penta E        | 5       | 11      |
| D5S818         | 9       |         |
| D13S317        | 12      | 14      |
| D7S820         | 10      |         |
| D16S539        | 10      | 14      |
| CSF1PO         | 12      |         |
| Penta D        | 9       |         |
| AMEL           | x       |         |
| vWA            | 16      | 17      |
| D8S1179        | 10      |         |
| TPOX           | 9       | 12      |
| FGA            | 24      |         |

Figure 2. Search result in ATCC database

SEARCH THE STR DATABASE

As part of our continuing efforts to characterize and authenticate the cell lines in the Cell Biology collection, ATCC has developed a comprehensive database of short tandem repeat (STR) DNA profiles for all of our human cell lines. View our brief tutorial before starting.

1. STR Profiling Analysis
2. Matching Algorithm
3. Interrogating the Database
Figure 3. Search result in DSMZ database

![Result of STR matching analysis by your data.](image)

Figure 4. Authentication of the species of the sample

M: Marker. As the size of 700, 600, 500, 400, 300, 200 and 100bp from up to down.

Nine species are checked, as follow: Homo sapiens 391bp, Cricetulus griseus315bp, Macaca mulatta287bp, Cercopithecus aethiops222bp, Rattus norvegicus196bp, Canis familiaris172bp, Mus musculus150bp, Bos Taurus102bp, IC 70bp

The sample: The band size is 391bp which matches the size of human.
Report of Human Cell Line Authentication

I. Sample
Sample Name: labeled as ‘BT-20’

II. Method and Procedure
1. PCR is amplified with STR Multi-amplification Kit (PowerPlex 21 System);
2. PCR products are assayed with 3100 DNA Analyzer (Applied Biosystems®).
3. Amplification of gene COX1 and electrophoresis are employed to survey the species of the sample.

III. Results
1. The STR profiles of the cell line sample are in the attached table and figure.
2. The search result in ATCC and DSMZ databases.
3. The electrophoresis figure of gene COX1.

BT-20: ① No loci has tri-alleles or tetra-alleles. Contamination of other human cell lines are not found (Figure 1 & Table 1). ② Compared the STR data of BT-20 cell line in the databases of ATCC and DSMZ, all the alleles of BT-20 were exactly matched with the alleles of BT-20 cells found in both cell banks (Figure 2&3). ③ The sample is a human cell line. Contamination of other species cells are not found in the sample (Figure 4).

To all above, the sample is a single cell line, and it is BT-20 cell line.

Operator: Xiaohua Mo
Auditor: Xuanyi Liang
Guangzhou Cellcook Biotech Co., Ltd

(Notice: This authentication report is restricted to the cell sold from Guangzhou Cellcook Biotech Co., Ltd, and the date with seal is the date of delivery.)
Table 1. STR profiles of BT-20 cell line

|            | Allele1 | Allele2 |
|------------|---------|---------|
| AMEL       | X       |         |
| D3S1358    | 17      |         |
| D1S1656    | 17, 3   |         |
| D6S1043    | 19      |         |
| D13S317    | 11      |         |
| Penta E    | 11      | 13      |
| D16S539    | 11      | 14      |
| D18S51     | 17      |         |
| D2S1338    | 19      |         |
| CSF1PO     | 12      |         |
| Penta D    | 10      | 11      |
| TH01       | 7       | 9, 3    |
| vWA        | 16      | 17      |
| D21S11     | 28      | 29      |
| D7S820     | 10      |         |
| D5S818     | 12      |         |
| TPOX       | 11      |         |
| D8S1179    | 12      |         |
| D12S391    | 18.3    | 24      |
| D19S433    | 15      |         |
| FGA        | 22      | 24      |

Figure 2. Search result in ATCC database

SEARCH THE STR DATABASE

As part of our continuing efforts to characterize and authenticate the cell lines in the Cell Biology collection, ATCC has developed a comprehensive database of short tandem repeat (STR) DNA profiles for all of our human cell lines. View our brief tutorial before starting.

1. STR Profiling Analysis
2. Matching Algorithm
3. Interrogating the Database
### Figure 3. Search result in DSMZ database

#### Result of STR matching analysis by your data.

- DSMZ Profile Database

| EV  | Cell No. | Cell name | Query/Your Cell | DSSS13 | D13S317 | D18S510 | D16S539 | VWA | TPOX | CSF1PO |
|-----|----------|-----------|-----------------|--------|---------|---------|---------|-----|------|--------|
|     |          |           |                 | 12.12  | 11.11   | 16.10   | 11.14   | 16.37| 7.5  |        |
|     | 1.00(63.36) | HTB-10  |                 | 12.12  | 11.11   | 10.10   | 11.14   | 16.37| 7.5  | XX    |
|     | 1.00(63.36) | HTB-20  |                 |        |      |        |         |      |      |        |

### Figure 4. Authentication of the species of the sample

M: Marker. As the size of 700, 600, 500, 400, 300, 200 and 100bp from up to down.

Nine species are checked, as follow: *Homo sapiens* 391bp, *Cricetulus griseus* 315bp, *Macaca mulatta* 287bp, *Cercopithecus aethiops* 222bp, *Rattus norvegicus* 196bp, *Canis familiaris* 172bp, *Mus musculus* 150bp, *Bos Taurus* 102bp, IC 70bp

The sample: The band size is 391bp which matches the size of human.
Report of Human Cell Line Authentication

(Notice: This authentication report is restricted to the cell sold from Guangzhou Cellcoo Biotech Co., Ltd, and the date with seal is the date of delivery.)

I. Sample

Sample Name: labeled as ‘MDA-MB-231’

II. Method and Procedure

1. PCR is amplified with STR Multi-amplification Kit (PowerPlex™16HS System);
2. PCR products are assayed with 3100 DNA Analyzer (Applied Biosystems®).
3. Amplification of gene COX1 and electrophoresis are employed to survey the species of the sample.

III. Results

1. The STR profiles of the cell line sample are in the attached table and figure.
2. The search result in ATCC and DSMZ databases.
3. The electrophoresis figure of gene COX1.

MDA-MB-231: ① No loci has tri-alleles or tetra-alleles. Contamination of other human cell line is not found (Figure 1 & Table 1). ② 100% matched cell lines are found in ATCC and DSMZ data banks. And the cell line named as “MDA-MB-231” et al. (Figure 2 & Figure 3). ③ The sample is a human cell line. Contamination of other species cells are not found in the sample (Figure 4).

Operator: Xiaohua Mo
Auditor: Xuanyi Liang
Guangzhou Cellcoo Biotech Co., Ltd
Table 1. STR profiles of MDA-MB-231 cell line

| Locus     | Allele1 | Allele2 |
|-----------|---------|---------|
| D3S1358   | 16      | 17      |
| TH01      | 7       | 9, 3    |
| D21S11    | 33, 2   |         |
| D18S51    | 11      |         |
| Penta_E   | 11      |         |
| D5S818    | 12      |         |
| D13S317   | 13      |         |
| D7S820    | 8       | 9       |
| D16S539   | 12      | 13      |
| CSF1P0    | 12      | 13      |
| Penta_D   | 14      |         |
| AMEL      | x       |         |
| vWA       | 15      | 18      |
| D8S1179   | 13      |         |
| TPOX      | 8       | 9       |
| FGA       | 22      | 23      |

Figure 2. Search result in ATCC database
**Figure 3.** Search result in DSMZ database

**Result of STR matching analysis by your data.**

- DSMZ Profile Database -

| RV   | Cell No.    | Cell name       | D5S818 | D13S317 | D16S539 | D14S604 | VWA  | TPOX | AM  | TPXO | CSF1PO | Figures |
|------|-------------|-----------------|--------|---------|---------|---------|------|------|-----|------|--------|---------|
| 1.12236/232 | HTB-26     | HDA-05-0251     | 12.12  | 13.13   | 8.9     | 12.12   | 15.13 | 7.9  | 3.7 | 8.9  | 12.13  | -       |
| 1.12236/232 | T22        | HDA-05-0251     | 12.12  | 13.13   | 8.9     | 12.12   | 15.13 | 7.9  | 3.7 | 8.9  | 12.13  | -       |
| 1.12236/232 | 293        | BFL-01-0701     | 13.13  | 12.12   | 8.9     | 11.12   | 19.16 | 9.9  | 9.9 | 8.9  | 13.13  | -       |
| 1.12236/232 | 293        | BFLC-0701       | 13.13  | 12.12   | 8.9     | 11.12   | 19.16 | 9.9  | 9.9 | 8.9  | 13.13  | -       |
| 1.12236/232 | U-4697     | U-4697          | 12.12  | 12.12   | 8.8     | 9.9     | 18.18 | 7.7  | 8.9 | 8.9  | 12.12  | -       |
| 1.12236/232 | HTM-132    | MDA-RD-468      | 12.12  | 12.12   | 8.8     | 9.9     | 18.18 | 7.7  | 8.9 | 8.9  | 12.12  | -       |
| 1.12236/232 | T28        | MDA-RD-468      | 12.12  | 12.12   | 8.8     | 9.9     | 18.18 | 7.7  | 8.9 | 8.9  | 12.12  | -       |
| 1.12236/232 | HCL-12757  | HCL-12757       | 12.12  | 12.12   | 8.8     | 9.9     | 18.18 | 7.7  | 8.9 | 8.9  | 12.12  | -       |
| 1.12236/232 | KIC-10256  | KIC-10256       | 12.12  | 13.13   | 9.9     | 11.12   | 17.18 | 9.9  | 9.9 | 8.9  | 10.12  | -       |
| 1.12236/232 | KCL-2322   | KCL-2322        | 12.12  | 13.13   | 9.9     | 11.12   | 17.18 | 9.9  | 9.9 | 8.9  | 10.12  | -       |
| 1.12236/232 | KCL-5000   | KCL-5000        | 11.12  | 13.13   | 8.11    | 9.12    | 18.18 | 7.7  | 8.9 | 8.9  | 12.12  | -       |
| 1.12236/232 | KCL-5915   | KCL-5915        | 12.12  | 13.13   | 12.12   | 12.12   | 17.17 | 9.9  | 9.9 | 13.13 | -       | -       |

**Figure 4.** Authentication of the species of the sample

M: Marker. As the size of 700, 600, 500, 400, 300, 200 and 100bp from up to down.

Nine species are checked, as follow: *Homo sapiens* 391bp, *Cricetus griseus* 315bp, *Macaca mulatta* 287bp, *Cercopithecus aethiops* 222bp, *Rattus norvegicus* 196bp, *Canis familiaris* 172bp, *Mus musculus* 150bp, *Bos taurus* 102bp, 1C 70bp

The sample: The band size is 391bp which matches the size of human.
Report of Human Cell Line Authentication

(Notice: This authentication report is restricted to the cell sold from Guangzhou Cellcook Biotech Co., Ltd, and the date with seal is the date of delivery.)

I. Sample

Sample Name: labeled as ‘BT-549’

II. Method and Procedure

1. PCR is amplified with STR Multi-amplification Kit (PowerPlex™16HS System);
2. PCR products are assayed with 3100 DNA Analyzer (Applied Biosystems®).
3. Amplification of gene COX1 and electrophoresis are employed to survey the species of the sample.

III. Results

1. The STR profiles of the cell line sample are in the attached table and figure.
2. The search result in ATCC and DSMZ databases.
3. The electrophoresis figure of gene COX1.

BT-549: ① No loci has tri-alleles or tetra-alleles. Contamination of other human cell line is not found (Figure 1 & Table 1). ② 100% matched cell lines are found in ATCC and DSMZ data banks. And the cell line named as “BT-579” et al. (Figure 2 & Figure 3). ③ The sample is a human cell line. Contamination of other species cells are not found in the sample (Figure 4).

Operator: Xiaohua Mo  Auditor: Xuanyi Liang

Guangzhou Cellcook Biotech Co., Ltd
Figure 1. STR profiles of BT-549 cell line
Table 1. STR profiles of BT-549 cell line

|            | Allele1 | Allele2 |
|------------|---------|---------|
| D3S1358    | 16      |         |
| TH01       | 8       | 9.3     |
| D21S11     | 29      |         |
| D18S51     | 14      | 17      |
| Penta_E    | 7       | 11      |
| D5S818     | 11      |         |
| D13S317    | 11      |         |
| D7S820     | 8       | 11      |
| D16S539    | 11      | 12      |
| CSF1PO     | 10      | 12      |
| Penta_D    | 9       |         |
| AMEL       | x       | y       |
| vWA        | 14      |         |
| D8S1179    | 13      | 14      |
| TPOX       | 8       | 11      |
| FGA        | 23      |         |

Figure 2. Search result in ATCC database

SEARCH THE STR DATABASE

As part of our continuing efforts to characterize and authenticate the cell lines in the Cell Biology collection, ATCC has developed a comprehensive database of short tandem repeat (STR) DNA profiles for all of our human cell lines. View our brief tutorial before starting.

1. STR Profiling Analysis
2. Matching Algorithm
3. Interrogating the Database

Showing 1 - 1 Of 1

| Add to Cart | %Match | ATCC® Number | Designation | D5S818 | D13S317 | D7S820 | D16S539 | vWA | TH01 | AMEL | TPOX | CSF1PO |
|------------|--------|---------------|-------------|--------|---------|--------|---------|-----|-----|------|------|--------|
|            | 100.0  | HTB-122       | BT-549Breast Ductal CarcinomaHuman | 11    | 11      | 9,10   | 8       | 15  | 9.3 | X    | 8  | 10,12  |

[Add to Cart] [Export to Excel]
M: Marker. As the size of 700, 600, 500, 400, 300, 200 and 100bp from up to down.

Nine species are checked, as follow: Homo sapiens 391bp, Cricetulus griseus 315bp, Macaca mulatta 287bp, Cercopithecus aethiops 222bp, Rattus norvegicus 196bp, Canis familiaris 172bp, Mus musculus 150bp, Bos Taurus 102bp, IC 70bp

The sample: The band size is 391bp which matches the size of human.