Mycoplasma Test Report

Information introduction

| Company Code: | 20211029_1 |
|---------------|-------------|
| Sample Code:  | AC16        |
| Sample To:    | Shanghai University |
| Testing Type: | Detection of Mycoplasma |
| Testing Method: | TaqMan-qPCR |

Testing Method

1. Based on the fluorescent quantitative PCR platform, the mycoplasma DNA can be quantitatively detected by the FAM probe in a single reaction.
2. The internal standard is introduced in the multiplex PCR process, the VIC probe is used to detect the internal standard gene to monitor the experimental process to avoid false negatives.
3. The QPCR amplification reaction is in a closed environment throughout the entire process, which effectively avoids false positive results caused by PCR product contamination, and at the same time uses negative reference products to control false positive.
4. Quantify the mycoplasma DNA in the sample by the positive reference concentration standard curve to obtain the concentration of mycoplasma DNA in the sample and give the reference degree of mycoplasma contamination.
5. This method is highly sensitive and can detect as few as 10 copies of mycoplasma DNA.

Quality Control

1. The positive detection channel of the standard product shows an "S" type amplification curve, and the Ct value of each reference product has a good linearity, $R^2=0.9998$. 
2. The negative reference detection channel (blue) has no amplification performance, the internal standard channel (green) shows a standard "S" type amplification curve, and the negative control is established.

Test Results

1. Sample detection channel amplification curve (left), internal standard channel amplification curve (right). The internal standard channel shows an "S" type amplification curve, indicating that the PCR process of the sample is normal.

2. Qualitative standards
### Conclusion

1. The amplification result of the positive reference product was normal, and the Ct value between each reference product was linear. No amplification in the detection channel of the negative reference product, and the internal standard gene amplification was normal. No abnormality in the experimental process.

2. The internal reference gene amplification of the tested sample is normal, the Ct value is 25.08, and the PCR process of the tested product is normal.

3. The Ct value of the sample was NoCt, so the mycoplasma test result of the sample was **negative**.
**Mycoplasma Test Report**

**Information introduction**

| Company Code:          | 20211109_1 |
|------------------------|------------|
| Sample Code:           | H9         |
| Sample To:             | Shanghai University |
| Testing Type:          | Detection of Mycoplasma |
| Testing Method:        | TaqMan-qPCR |

**Testing Method**

1. Based on the fluorescent quantitative PCR platform, the mycoplasma DNA can be quantitatively detected by the FAM probe in a single reaction.
2. The internal standard is introduced in the multiplex PCR process, the VIC probe is used to detect the internal standard gene to monitor the experimental process to avoid false negatives.
3. The QPCR amplification reaction is in a closed environment throughout the entire process, which effectively avoids false positive results caused by PCR product contamination, and at the same time uses negative reference products to control false positive.
4. Quantify the mycoplasma DNA in the sample by the positive reference concentration standard curve to obtain the concentration of mycoplasma DNA in the sample and give the reference degree of mycoplasma contamination.
5. This method is highly sensitive and can detect as few as 10 copies of mycoplasma DNA.

**Quality Control**

1. The positive detection channel of the standard product shows an "S" type amplification curve, and the Ct value of each reference product has a good linearity, $R^2=0.9998$. 
2. The negative reference detection channel (blue) has no amplification performance, the internal standard channel (green) shows a standard "S" type amplification curve, and the negative control is established.

Test Results

1. Sample detection channel amplification curve (left), internal standard channel amplification curve (right). The internal standard channel shows an "S" type amplification curve, indicating that the PCR process of the sample is normal.

2. Qualitative standards
Detection channel  | Internal reference channel  | Qualitative results
--- | --- | ---
Ct<19  |  | Strong positive (+++)
19≤Ct<25  |  | Positive (++)
25≤Ct<30  |  | Weak positive (+)
Ct≥30  | Ct<30  | Negative (-)

3. Summary of test results

| Inspection Channel | Detection channel Ct range | Internal reference channel Ct range | Qualitative results | Reference concentration |
|---|---|---|---|---|
| Positive | Ct ∈ (18-30) | 25.1 | - | - |
| Negative | undet | 24.7 | - | - |
| Sample | NoCt | 25.86 | Negative | - |

**Conclusion**

1. The amplification result of the positive reference product was normal, and the Ct value between each reference product was linear. No amplification in the detection channel of the negative reference product, and the internal standard gene amplification was normal. No abnormality in the experimental process.

2. The internal reference gene amplification of the tested sample is normal, the Ct value is 25.08, and the PCR process of the tested product is normal.

3. The Ct value of the sample was NoCt, so the mycoplasma test result of the sample was Negative.
Cell Line Authentication Service

STR Profiling Report

Sample Code: AC16
Sample Type: Cell Line
Testing Method: STR Genotyping
Report Time: June 10, 2021
COMPANY STATEMENT

1. THIS REPORT IS ONLY RESPONSIBLE FOR THE SAMPLES ANALYZED.
2. THE TESTING RESULTS AND THE ORGANIZATION NAME WILL NOT BE USED FOR ADVERTISEMENT, COMMERCIAL EXHIBITIONS, COMMERCIAL PERFORMANCE AND OTHER COMMERCIAL ACTIVITIES.
3. OBJECTIONS SHOULD BE RAISED WITHIN FIFTEEN DAYS AFTER THE RECEIPT OF THIS REPORT.
4. THE PAPER REPORT WITH CONTENT ALTERING, ADDING OR WITHOUT THE STAMPED SEAL OF THE COMPANY ARE INVALID.

Testing Company: Shanghai Biowing Applied Biotechnology Co. Ltd
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Contact: Wenyao Zhang
E-mail: market@biowing.com.cn
Cell Line Authentication – STR Profiling Report

Sample code

| Table 1. Sample Code |
|----------------------|
| Customer’s code | Company Code |
| AC16 | 20210607-05 |

Sample Number: 1

Sample Type: Cell line

Testing Type: STR

Testing Method:

DNA was extracted by a commercial kit from CORNING (AP-EMN-BL-GDNA-250G). The twenty STRs including Amelogenin locus were amplified by six multiplex PCR and separated on ABI 3730XL Genetic Analyzer. The signals were then analyzed by the software GeneMapper.

Data Interpretation:

Cell lines were authenticated using Short Tandem Repeat (STR) analysis as described in 2012 in ANSI Standard (ASN-0002) by the ATCC Standards Development Organization (SDO) and in Capes-Davis et al., Match criteria for human cell line authentication: Where do we draw the line? Int J Cancer. 2013;132(11):2510-9.
## Test Results

### 1. STR profile

Table 2. STR and Amelogenin Genotyping Results of Cell line.

| Loci     | Sample information | Cell Bank information |
|----------|--------------------|-----------------------|
|          | Sample name: AC16  | Cell line name: AC16 [Human hybrid] |
|          | Allele1 | Allele2 | Allele3 | Allele1 | Allele2 | Allele3 |
| D5S818   | 9       | 11      |         | 9       | 11      |         |
| D13S317  | 12      | 13      |         | 12      | 13      |         |
| D7S820   | 10      | 11      | 12      | 10      | 11      | 12      |
| D16S539  | 11      | 13      |         | 11      | 13      |         |
| VWA      | 16      | 18      |         | 16      | 18      |         |
| TH01     | 7       | 8       | 9.3     | 7       | 8       | 9.3     |
| AMEL     | X       | X       |         | X       | X       |         |
| TPOX     | 11      | 11      |         | 11      | 11      |         |
| CSF1PO   | 9       | 11      | 12      | 9       | 11      | 12      |
| D12S391  | 18      | 21      |         |         |         |         |
| FGA      | 21      | 25      |         |         |         |         |
| D2S1338  | 20      | 23      | 26      |         |         |         |
| D21S11   | 32.2    | 32.2    |         |         |         |         |
| D18S51   | 12      | 17      |         |         |         |         |
| D8S1179  | 14      | 15      |         |         |         |         |
| D3S1358  | 17      | 18      |         |         |         |         |
| D6S1043  | 14      | 17      |         |         |         |         |
| PENTAE   | 7       | 12      | 16      |         |         |         |
| D19S433  | 15.2    | 15.2    |         |         |         |         |
| PENTAD   | 3.2     | 9       |         |         |         |         |
| D1S1656  | 12      | 12      |         |         |         |         |
2. database annotation

Figure 1. STR matching analysis

| EV | Cell No. | Cell name       | Locus names     |
|----|---------|-----------------|----------------|
|    |         |                 | D5S818 | D13S317 | D7S820 | D16S539 | VWA | THO1 | AM | TPOX | CSF1PO |
| L0(36/36) CVCL.4U18 | AC16 [Human hybrid] | Query (Your Cell) | 9,11. | 12,13. | 10,11,12 | 11,13. | 16,18. | 7,8,0,3 | X.X. | 11,11. | 9,11,12 |

Note: The STR online match analysis of the test cell against EXPASY database, showing cell number (Cell No.) and cell name.

3. Authentication

☐ The submitted sample profile is human, but not a match for any profile in the DSMZ STR database.

☑ The submitted profile is exact match for the following human cell line(s) in the EXPASY STR database (8 core loci plus Amelogenin): AC16 [Human hybrid].

☐ The submitted profile is similar to the following DSMZ human cell line: /

● Note: A cell line can be considered to be authenticated when 80% (exact match) of the alleles in its STR profile match profiles from tissue or other cell line samples from that donor or from database. Cell lines with between a 55% to 80% (similar) match require further profiling for investigation of relatedness.

Figure 2. STR profiles of sample cell line
Appendix

1. Genotyping Strategy and Site Distribution

Table S1. Experimental Strategy and Sites

|     | Strategy 1   | Strategy 2   | Strategy 3   | Strategy 4   |
|-----|--------------|--------------|--------------|--------------|
| 1   | D3S1358      | D8S1179      | D19S433      | AMEL         |
| 2   | VWA          | D21S11       | TH01         | D1S1656      |
| 3   | D7S820       | D16S539      | D13S317      | D5S818       |
| 4   | CSF1PO       | D2S1338      | TPOX         | D12S391      |
| 5   | PENTAE       | PENTAD       | D18S51       | FGA          |
| 6   |              |              |              | D6S1043      |

The allele match algorithm compares the 8 core loci plus amelogenin only, even though alleles from all loci will be reported when available.

2. DSMZ tools was used to carry on the cell line comparison, which contains 2455 cell lines STR data from ATCC, DSMZ, JCRB, ECACC, GNE and RIKEN databases. If the cell is not included in the above cell library, it needs to be compared with other databases.

Technician: Jianan Zhang
Checked by: Chen Qian
Issued by: Yang Bai
Issue date: June 10, 2021
Cell Line Authentication Service

STR Profiling Report

Sample Code: H9
Sample Type: Cell Line
Testing Method: STR Genotyping
Report Time: October 26, 2021
COMPANY STATEMENT

1. THIS REPORT IS ONLY RESPONSIBLE FOR THE SAMPLES ANALYZED.
2. THE TESTING RESULTS AND THE ORGANIZATION NAME WILL NOT BE USED FOR ADVERTISEMENT, COMMERCIAL EXHIBITIONS, COMMERCIAL PERFORMANCE AND OTHER COMMERCIAL ACTIVITIES.
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Contact: Wenyao Zhang
E-mail: market@biowing.com.cn
Cell Line Authentication – STR Profiling Report

Sample code

| Customer’s code | Company Code   |
|-----------------|---------------|
| H9              | 20211021-01   |

Sample Number :1
Sample Type: Cell line
Testing Type: STR

Testing Method:

DNA was extracted by a commercial kit from CORNING (AP-EMN-BL-GDNA-250G). The twenty STRs including Amelogenin locus were amplified by six multiplex PCR and separated on ABI 3730XL Genetic Analyzer. The signals were then analyzed by the software GeneMapper.

Data Interpretation:

Cell lines were authenticated using Short Tandem Repeat (STR) analysis as described in 2012 in ANSI Standard (ASN-0002) by the ATCC Standards Development Organization (SDO) and in Capes-Davis et al., Match criteria for human cell line authentication: Where do we draw the line? Int J Cancer.2013;132(11):2510-9.
## Test Results

### 1. STR profile

Table 2. STR and Amelogenin Genotyping Results of Cell line.

| Loci     | Sample information | Cell Bank information |
|----------|--------------------|-----------------------|
|          | Sample name: H9    | Cell line name: WA09,H9|
|          | Allele1 | Allele2 | Allele3 | Allele1 | Allele2 | Allele3 |
| D5S818   | 11      | 12      |         | 11      | 12      |         |
| D13S317  | 9       | 9       |         | 9       | 9       |         |
| D7S820   | 9       | 11      |         | 9       | 11      |         |
| D16S539  | 12      | 13      |         | 12      | 13      |         |
| VWA      | 17      | 17      |         | 17      | 17      |         |
| TH01     | 9.3     | 9.3     |         | 9.3     | 9.3     |         |
| AMEL     | X       | X       |         | X       | X       |         |
| TPOX     | 10      | 11      |         | 10      | 11      |         |
| CSF1PO   | 11      | 11      |         | 11      | 11      |         |
| D12S391  | 15      | 19      |         |         |         |         |
| FGA      | 26      | 28      |         |         |         |         |
| D2S1338  | 18      | 24      |         |         |         |         |
| D21S11   | 30      | 30      |         |         |         |         |
| D18S51   | 13      | 13      |         |         |         |         |
| D8S1179  | 8       | 14      |         |         |         |         |
| D3S1358  | 13      | 16      |         |         |         |         |
| D6S1043  | 12      | 19      |         |         |         |         |
| PENTAE   | 11      | 14      |         |         |         |         |
| D19S433  | 12      | 15      |         |         |         |         |
| PENTAD   | 9       | 13      |         |         |         |         |
| D1S1656  | 12      | 13      |         |         |         |         |
2. database annotation

Figure 1. STR matching analysis

| EV | Cell No. | Cell name | Locus names |
|----|----------|-----------|-------------|
| Query (Your Cell) |          |           | D5S818 | D13S317 | D7S820 | D16S539 | VWA | TH01 | AM | TPOX | CSF1PO |
| L053636 | CYCL 9773 | WA09 | 11 12 | 9 10 | 9 11 | 12 13 | 17 17 | 9 10 | 11 | 10 | 11 11 |

Note: The STR online match analysis of the test cell against EXPASY database, showing cell number (Cell No.) and cell name.

3. Authentication

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☐ The submitted profile is similar to the following DSMZ human cell line: /

★ Note: A cell line can be considered to be authenticated when 80% (exact match) of the alleles in its STR profile match profiles from tissue or other cell line samples from that donor or from database. Cell lines with between a 55% to 80% (similar) match require further profiling for investigation of relatedness.

Figure 2. STR profiles of sample cell line
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|   | Strategy 1 | Strategy 2 | Strategy 3 | Strategy 4 |
|---|------------|------------|------------|------------|
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| 2 | VWA        | D21S11     | TH01       | D1S1656    |
| 3 | D7S820     | D16S539    | D13S317    | D5S818     |
| 4 | CSF1PO     | D2S1338    | TPOX       | D12S391    |
| 5 | PENTAE     | PENTAD     | D18S51     | FGA        |
| 6 |            |            |            | D6S1043    |

The allele match algorithm compares the 8 core loci plus amelogenin only, even though alleles from all loci will be reported when available.

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