

美吉生物PCR扩增报告

1. 项目基本信息

| | | | |
|--------|---|---------|---------------|
| 项目名称 | MJ20171016007-MJ-M-20171022014-蔚薇-20171103PCR正式实验报告 | 项目跟进销售 | 曹维 |
| 项目联系人 | 蔚薇 | 合同编号 | MJ20171016007 |
| 收样人 | 程红 | 收样日期 | 2017-10-17 |
| PCR操作员 | 宋佳宇 | PCR报告日期 | 2017-11-03 |
| 报告撰写人 | 宋佳宇 | 报告审核人 | 张璐璐 |

2. PCR预实验

2.1 引物设计

| 测序区域 | 引物名称 | 引物序列 | 备注 |
|----------------|----------|------------------------|----|
| 338F_806R | 338F | ACTCCTACGGGAGGCAGCAG | |
| | 806R | GGACTACHVGGGTWTCTAAT | |
| AMV4-5NF_AMDGR | AMV4-5NF | AAGCTCGTAGTTGAATTCG | |
| | AMDGR | CCCAACTATCCCTATTAATCAT | |
| ITS1F_ITS2R | ITS1F | CTTGGTCATTTAGAGGAAGTAA | |
| | ITS2R | GCTGCGTCTTCATCGATGC | |

2.2 PCR扩增条件

为了保证后续数据分析的准确性及可靠性，需要两个条件，首先是尽可能使用低循环数扩增；其次保证每个样品扩增的循环数统一。为了达到以上目的，我方首先随机选取样品进行预实验，以确保在最低的循环数中绝大多数的样品能够扩增出浓度合适的产物，为所有样品的正式试验做充分准备。

在预实验完成后，PCR 正式试验采用TransGen AP221-02: TransStart Fastpfu DNA Polymerase，20μl反应体系：

5×FastPfu Buffer 4 μl
 2.5 mM dNTPs 2 μl
 Forward Primer(5 μM) 0.8 μl
 Reverse Primer(5 μM) 0.8 μl
 FastPfu Polymerase 0.4 μl
 BSA 0.2 μl
 Template DNA 10 ng

补ddH₂O至 20 μl

PCR仪：ABI GeneAmp® 9700型

PCR反应参数：

- 1 × (3 minutes at 95 °C)
- 循环数 × (30 seconds at 95 °C; 30 seconds at 退火温度 °C; 45 seconds at 72 °C)
- 10 minutes at 72 °C, 10 °C until halted by user

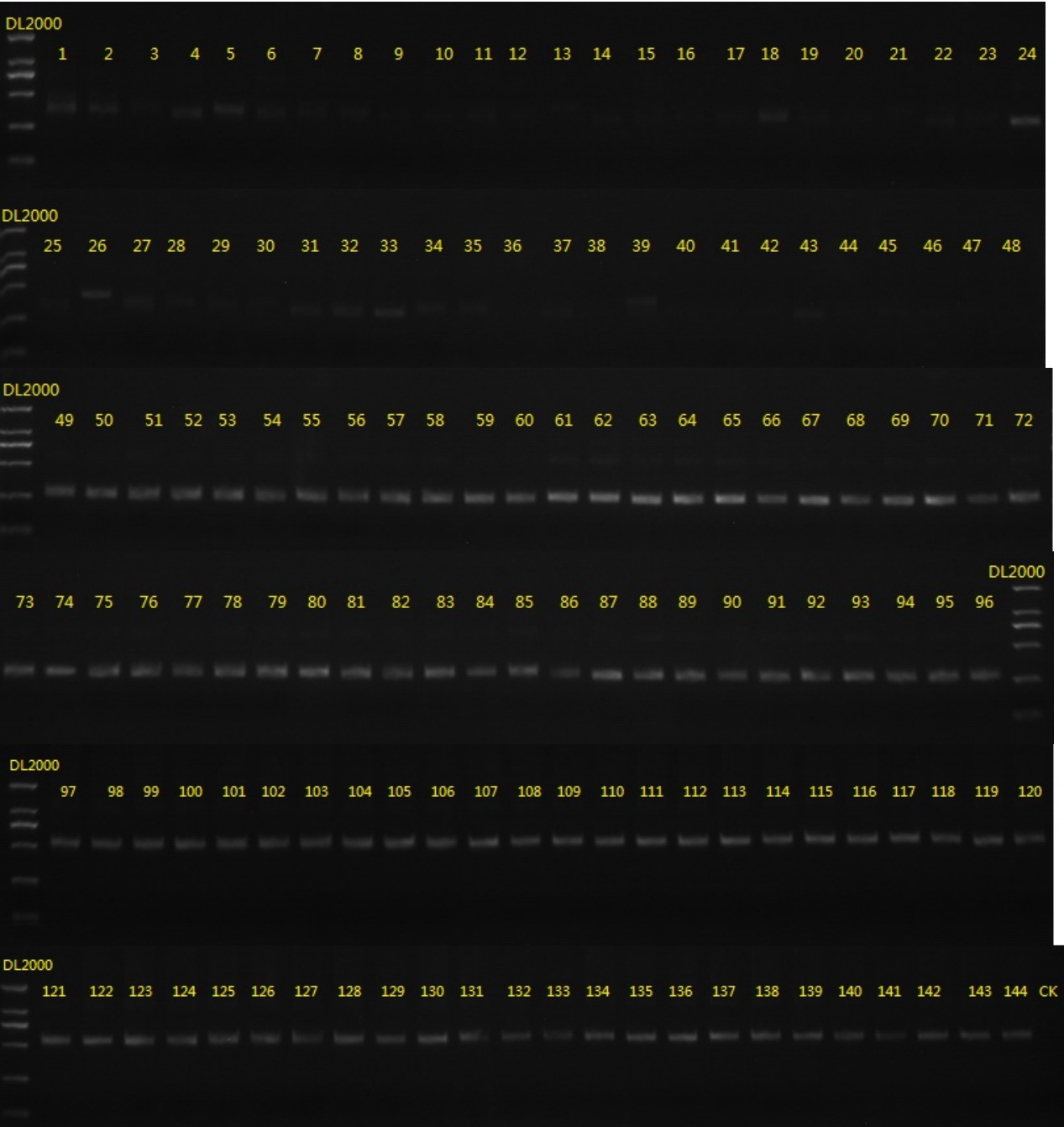
二轮扩增：

一轮引物：AML1F-AML2R 退火温度55℃，循环数32cycles

二轮引物：AMV4-5NF_AMDGR 退火温度55℃，循环数30cycles

2.3 PCR扩增结果鉴定胶图

2%琼脂糖凝胶电泳检测PCR产物，3μl上样检测电泳图：



DL2000条带分布如下：



2.4 PCR扩增结果

我方已将贵方样品进行PCR扩增条件摸索，截止目前的PCR扩增结果统计如下：

| 胶图编号 | 样品名称 | 美吉编号 | 实验名称 | 测序引物 | 退火温度(℃) | 循环数 | 结果 |
|------|-------------|-----------|------|-------------|---------|-----|----|
| 1 | J_T_YS_1_w | EBJ230037 | 蔚薇A1 | ITS1F_ITS2R | 55 | 36 | A |
| 2 | J_T_YS_2_w | EBJ230038 | 蔚薇A2 | ITS1F_ITS2R | 55 | 36 | A |
| 3 | J_T_YS_3_w | EBJ230039 | 蔚薇A3 | ITS1F_ITS2R | 55 | 36 | A |
| 4 | J_T_JJE_1_w | EBJ230040 | 蔚薇A4 | ITS1F_ITS2R | 55 | 36 | A |
| 5 | J_T_JJE_2_w | EBJ230041 | 蔚薇A5 | ITS1F_ITS2R | 55 | 36 | A |
| 6 | J_T_JJE_3_w | EBJ230042 | 蔚薇A6 | ITS1F_ITS2R | 55 | 36 | A |

| | | | | | | | |
|----|---------------|-----------|-------|----------------|----|----|---|
| 7 | J_T_SJ_1_w | EBJ230043 | 蔚薇A7 | ITS1F_ITS2R | 55 | 36 | A |
| 8 | J_T_SJ_2_w | EBJ230044 | 蔚薇A8 | ITS1F_ITS2R | 55 | 36 | A |
| 9 | J_T_SJ_3_w | EBJ230045 | 蔚薇A9 | ITS1F_ITS2R | 55 | 36 | A |
| 10 | J_T_YOUS_1_w | EBJ230046 | 蔚薇A10 | ITS1F_ITS2R | 55 | 36 | A |
| 11 | J_T_YOUS_2_w | EBJ230047 | 蔚薇A11 | ITS1F_ITS2R | 55 | 36 | A |
| 12 | J_T_YOUS_3_w | EBJ230048 | 蔚薇A12 | ITS1F_ITS2R | 55 | 36 | B |
| 13 | J_T_YWOZ_1_w | EBJ230049 | 蔚薇A13 | ITS1F_ITS2R | 55 | 36 | A |
| 14 | J_T_YWOZ_2_w | EBJ230050 | 蔚薇A14 | ITS1F_ITS2R | 55 | 36 | A |
| 15 | J_T_YWOZ_3_w | EBJ230051 | 蔚薇A15 | ITS1F_ITS2R | 55 | 36 | A |
| 16 | J_T_SX_1_w | EBJ230052 | 蔚薇A16 | ITS1F_ITS2R | 55 | 36 | B |
| 17 | J_T_SX_2_w | EBJ230053 | 蔚薇A17 | ITS1F_ITS2R | 55 | 36 | A |
| 18 | J_T_SX_3_w | EBJ230054 | 蔚薇A18 | ITS1F_ITS2R | 55 | 36 | A |
| 19 | J_T_CB_1_w | EBJ230055 | 蔚薇A19 | ITS1F_ITS2R | 55 | 36 | A |
| 20 | J_T_CB_2_w | EBJ230056 | 蔚薇A20 | ITS1F_ITS2R | 55 | 36 | B |
| 21 | J_T_CB_3_w | EBJ230057 | 蔚薇A21 | ITS1F_ITS2R | 55 | 36 | A |
| 22 | J_T_WHLS_1_w | EBJ230058 | 蔚薇A22 | ITS1F_ITS2R | 55 | 36 | A |
| 23 | J_T_WHLS_2_w | EBJ230059 | 蔚薇A23 | ITS1F_ITS2R | 55 | 36 | A |
| 24 | J_T_WHLS_3_w | EBJ230060 | 蔚薇A24 | ITS1F_ITS2R | 55 | 36 | A |
| 25 | J_T_WHJJE_1_w | EBJ230061 | 蔚薇A25 | ITS1F_ITS2R | 55 | 36 | A |
| 26 | J_T_WHJJE_2_w | EBJ230062 | 蔚薇A26 | ITS1F_ITS2R | 55 | 36 | A |
| 27 | J_T_WHJJE_3_w | EBJ230063 | 蔚薇A27 | ITS1F_ITS2R | 55 | 36 | A |
| 28 | T_ZR_1_w | EBJ230064 | 蔚薇A28 | ITS1F_ITS2R | 55 | 36 | A |
| 29 | T_ZR_2_w | EBJ230065 | 蔚薇A29 | ITS1F_ITS2R | 55 | 36 | A |
| 30 | T_ZR_3_w | EBJ230066 | 蔚薇A30 | ITS1F_ITS2R | 55 | 36 | B |
| 31 | T_YS_1_w | EBJ230067 | 蔚薇A31 | ITS1F_ITS2R | 55 | 36 | A |
| 32 | T_YS_2_w | EBJ230068 | 蔚薇A32 | ITS1F_ITS2R | 55 | 36 | A |
| 33 | T_YS_3_w | EBJ230069 | 蔚薇A33 | ITS1F_ITS2R | 55 | 36 | A |
| 34 | T_JJESJ_1_w | EBJ230070 | 蔚薇A34 | ITS1F_ITS2R | 55 | 36 | A |
| 35 | T_JJESJ_2_w | EBJ230071 | 蔚薇A35 | ITS1F_ITS2R | 55 | 36 | A |
| 36 | T_JJESJ_3_w | EBJ230072 | 蔚薇A36 | ITS1F_ITS2R | 55 | 36 | C |
| 37 | T_YOUS_1_w | EBJ230073 | 蔚薇A37 | ITS1F_ITS2R | 55 | 36 | A |
| 38 | T_YOUS_2_w | EBJ230074 | 蔚薇A38 | ITS1F_ITS2R | 55 | 36 | C |
| 39 | T_YOUS_3_w | EBJ230075 | 蔚薇A39 | ITS1F_ITS2R | 55 | 36 | A |
| 40 | T_SX_1_w | EBJ230076 | 蔚薇A40 | ITS1F_ITS2R | 55 | 36 | C |
| 41 | T_SX_2_w | EBJ230077 | 蔚薇A41 | ITS1F_ITS2R | 55 | 36 | C |
| 42 | T_SX_3_w | EBJ230078 | 蔚薇A42 | ITS1F_ITS2R | 55 | 36 | B |
| 43 | T_CBWH_1_w | EBJ230079 | 蔚薇A43 | ITS1F_ITS2R | 55 | 36 | A |
| 44 | T_CBWH_2_w | EBJ230080 | 蔚薇A44 | ITS1F_ITS2R | 55 | 36 | B |
| 45 | T_CBWH_3_w | EBJ230081 | 蔚薇A45 | ITS1F_ITS2R | 55 | 36 | B |
| 46 | T_WW_1_w | EBJ230082 | 蔚薇A46 | ITS1F_ITS2R | 55 | 36 | B |
| 47 | T_WW_2_w | EBJ230083 | 蔚薇A47 | ITS1F_ITS2R | 55 | 36 | C |
| 48 | T_WW_3_w | EBJ230084 | 蔚薇A48 | ITS1F_ITS2R | 55 | 36 | C |
| 49 | J_T_YS_1_w | EBJ230037 | 蔚薇A49 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 50 | J_T_YS_2_w | EBJ230038 | 蔚薇A50 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 51 | J_T_YS_3_w | EBJ230039 | 蔚薇A51 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 52 | J_T_JJE_1_w | EBJ230040 | 蔚薇A52 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 53 | J_T_JJE_2_w | EBJ230041 | 蔚薇A53 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 54 | J_T_JJE_3_w | EBJ230042 | 蔚薇A54 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 55 | J_T_SJ_1_w | EBJ230043 | 蔚薇A55 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 56 | J_T_SJ_2_w | EBJ230044 | 蔚薇A56 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 57 | J_T_SJ_3_w | EBJ230045 | 蔚薇A57 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 58 | J_T_YOUS_1_w | EBJ230046 | 蔚薇A58 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 59 | J_T_YOUS_2_w | EBJ230047 | 蔚薇A59 | AMV4-5NF_AMDGR | 55 | 30 | A |

| | | | | | | | |
|-----|---------------|--------------|--------|----------------|----|----|---|
| 60 | J_T_YOUS_3_w | EBJ230048 | 蔚薇A60 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 61 | J_T_YWOZ_1_w | EBJ230049 | 蔚薇A61 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 62 | J_T_YWOZ_2_w | EBJ230050 | 蔚薇A62 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 63 | J_T_YWOZ_3_w | EBJ230051 | 蔚薇A63 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 64 | J_T_SX_1_w | EBJ230052 | 蔚薇A64 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 65 | J_T_SX_2_w | EBJ230053 | 蔚薇A65 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 66 | J_T_SX_3_w | EBJ230054 | 蔚薇A66 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 67 | J_T_CB_1_w | EBJ230055 | 蔚薇A67 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 68 | J_T_CB_2_w | EBJ230056 | 蔚薇A68 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 69 | J_T_CB_3_w | EBJ230057 | 蔚薇A69 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 70 | J_T_WHLS_1_w | EBJ230058 | 蔚薇A70 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 71 | J_T_WHLS_2_w | EBJ230059 | 蔚薇A71 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 72 | J_T_WHLS_3_w | EBJ230060 | 蔚薇A72 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 73 | J_T_WHJJE_1_w | EBJ230061 | 蔚薇A73 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 74 | J_T_WHJJE_2_w | EBJ230062 | 蔚薇A74 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 75 | J_T_WHJJE_3_w | EBJ230063 | 蔚薇A75 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 76 | T_ZR_1_w | EBJ230064 | 蔚薇A76 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 77 | T_ZR_2_w | EBJ230065 | 蔚薇A77 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 78 | T_ZR_3_w | EBJ230066 | 蔚薇A78 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 79 | T_YS_1_w | EBJ230067 | 蔚薇A79 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 80 | T_YS_2_w | EBJ230068 | 蔚薇A80 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 81 | T_YS_3_w | EBJ230069 | 蔚薇A81 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 82 | T_JJESJ_1_w | EBJ230070 | 蔚薇A82 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 83 | T_JJESJ_2_w | EBJ230071 | 蔚薇A83 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 84 | T_JJESJ_3_w | EBJ230072 | 蔚薇A84 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 85 | T_YOUS_1_w | EBJ230073 | 蔚薇A85 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 86 | T_YOUS_2_w | EBJ230074 | 蔚薇A86 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 87 | T_YOUS_3_w | EBJ230075 | 蔚薇A87 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 88 | T_SX_1_w | EBJ230076 | 蔚薇A88 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 89 | T_SX_2_w | EBJ230077 | 蔚薇A89 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 90 | T_SX_3_w | EBJ230078 | 蔚薇A90 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 91 | T_CBWH_1_w | EBJ230079 | 蔚薇A91 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 92 | T_CBWH_2_w | EBJ230080 | 蔚薇A92 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 93 | T_CBWH_3_w | EBJ230081 | 蔚薇A93 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 94 | T_WW_1_w | EBJ230082 | 蔚薇A94 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 95 | T_WW_2_w | EBJ230083 | 蔚薇A95 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 96 | T_WW_3_w | EBJ230084 | 蔚薇A96 | AMV4-5NF_AMDGR | 55 | 30 | A |
| 97 | J_T_YS_1_w | EBJ230037_B1 | 蔚薇A97 | 338F_806R | 55 | 27 | A |
| 98 | J_T_YS_2_w | EBJ230038_B1 | 蔚薇A98 | 338F_806R | 55 | 27 | A |
| 99 | J_T_YS_3_w | EBJ230039_B1 | 蔚薇A99 | 338F_806R | 55 | 27 | A |
| 100 | J_T_JJE_1_w | EBJ230040_B1 | 蔚薇A100 | 338F_806R | 55 | 27 | A |
| 101 | J_T_JJE_2_w | EBJ230041_B1 | 蔚薇A101 | 338F_806R | 55 | 27 | A |
| 102 | J_T_JJE_3_w | EBJ230042_B1 | 蔚薇A102 | 338F_806R | 55 | 27 | A |
| 103 | J_T_SJ_1_w | EBJ230043_B1 | 蔚薇A103 | 338F_806R | 55 | 27 | A |
| 104 | J_T_SJ_2_w | EBJ230044_B1 | 蔚薇A104 | 338F_806R | 55 | 27 | A |
| 105 | J_T_SJ_3_w | EBJ230045_B1 | 蔚薇A105 | 338F_806R | 55 | 27 | A |
| 106 | J_T_YOUS_1_w | EBJ230046_B1 | 蔚薇A106 | 338F_806R | 55 | 27 | A |
| 107 | J_T_YOUS_2_w | EBJ230047_B1 | 蔚薇A107 | 338F_806R | 55 | 27 | A |
| 108 | J_T_YOUS_3_w | EBJ230048_B1 | 蔚薇A108 | 338F_806R | 55 | 27 | A |
| 109 | J_T_YWOZ_1_w | EBJ230049_B1 | 蔚薇A109 | 338F_806R | 55 | 27 | A |
| 110 | J_T_YWOZ_2_w | EBJ230050_B1 | 蔚薇A110 | 338F_806R | 55 | 27 | A |
| 111 | J_T_YWOZ_3_w | EBJ230051_B1 | 蔚薇A111 | 338F_806R | 55 | 27 | A |
| 112 | J_T_SX_1_w | EBJ230052_B1 | 蔚薇A112 | 338F_806R | 55 | 27 | A |

| | | | | | | | |
|-----|---------------|--------------|--------|-----------|----|----|---|
| 113 | J_T_SX_2_w | EBJ230053_B1 | 蔚薇A113 | 338F_806R | 55 | 27 | A |
| 114 | J_T_SX_3_w | EBJ230054_B1 | 蔚薇A114 | 338F_806R | 55 | 27 | A |
| 115 | J_T_CB_1_w | EBJ230055_B1 | 蔚薇A115 | 338F_806R | 55 | 27 | A |
| 116 | J_T_CB_2_w | EBJ230056_B1 | 蔚薇A116 | 338F_806R | 55 | 27 | A |
| 117 | J_T_CB_3_w | EBJ230057_B1 | 蔚薇A117 | 338F_806R | 55 | 27 | A |
| 118 | J_T_WHLS_1_w | EBJ230058_B1 | 蔚薇A118 | 338F_806R | 55 | 27 | A |
| 119 | J_T_WHLS_2_w | EBJ230059_B1 | 蔚薇A119 | 338F_806R | 55 | 27 | A |
| 120 | J_T_WHLS_3_w | EBJ230060_B1 | 蔚薇A120 | 338F_806R | 55 | 27 | A |
| 121 | J_T_WHJJE_1_w | EBJ230061_B1 | 蔚薇A121 | 338F_806R | 55 | 27 | A |
| 122 | J_T_WHJJE_2_w | EBJ230062_B1 | 蔚薇A122 | 338F_806R | 55 | 27 | A |
| 123 | J_T_WHJJE_3_w | EBJ230063_B1 | 蔚薇A123 | 338F_806R | 55 | 27 | A |
| 124 | T_ZR_1_w | EBJ230064_B1 | 蔚薇A124 | 338F_806R | 55 | 27 | A |
| 125 | T_ZR_2_w | EBJ230065_B1 | 蔚薇A125 | 338F_806R | 55 | 27 | A |
| 126 | T_ZR_3_w | EBJ230066_B1 | 蔚薇A126 | 338F_806R | 55 | 27 | A |
| 127 | T_YS_1_w | EBJ230067_B1 | 蔚薇A127 | 338F_806R | 55 | 27 | A |
| 128 | T_YS_2_w | EBJ230068_B1 | 蔚薇A128 | 338F_806R | 55 | 27 | A |
| 129 | T_YS_3_w | EBJ230069_B1 | 蔚薇A129 | 338F_806R | 55 | 27 | A |
| 130 | T_JJESJ_1_w | EBJ230070_B1 | 蔚薇A130 | 338F_806R | 55 | 27 | A |
| 131 | T_JJESJ_2_w | EBJ230071_B1 | 蔚薇A131 | 338F_806R | 55 | 27 | A |
| 132 | T_JJESJ_3_w | EBJ230072_B1 | 蔚薇A132 | 338F_806R | 55 | 27 | A |
| 133 | T_YOUS_1_w | EBJ230073_B1 | 蔚薇A133 | 338F_806R | 55 | 27 | A |
| 134 | T_YOUS_2_w | EBJ230074_B1 | 蔚薇A134 | 338F_806R | 55 | 27 | A |
| 135 | T_YOUS_3_w | EBJ230075_B1 | 蔚薇A135 | 338F_806R | 55 | 27 | A |
| 136 | T_SX_1_w | EBJ230076_B1 | 蔚薇A136 | 338F_806R | 55 | 27 | A |
| 137 | T_SX_2_w | EBJ230077_B1 | 蔚薇A137 | 338F_806R | 55 | 27 | A |
| 138 | T_SX_3_w | EBJ230078_B1 | 蔚薇A138 | 338F_806R | 55 | 27 | A |
| 139 | T_CBWH_1_w | EBJ230079_B1 | 蔚薇A139 | 338F_806R | 55 | 27 | A |
| 140 | T_CBWH_2_w | EBJ230080_B1 | 蔚薇A140 | 338F_806R | 55 | 27 | A |
| 141 | T_CBWH_3_w | EBJ230081_B1 | 蔚薇A141 | 338F_806R | 55 | 27 | A |
| 142 | T_WW_1_w | EBJ230082_B1 | 蔚薇A142 | 338F_806R | 55 | 27 | A |
| 143 | T_WW_2_w | EBJ230083_B1 | 蔚薇A143 | 338F_806R | 55 | 27 | A |
| 144 | T_WW_3_w | EBJ230084_B1 | 蔚薇A144 | 338F_806R | 55 | 27 | A |

2.5 结果说明

| 结果代码 | 预实验结果说明 |
|------|-------------------------------------|
| A | PCR产物目的条带大小正确，浓度合适，可进行后续实验； |
| B | PCR产物目的条带大小正确，浓度偏低，可尝试进行后续实验； |
| C | PCR产物目的条带太弱或未检测到，无法进行后续实验，需要重新提供样品； |