



# PCR protocol

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RBRC 04699

Strain name A/J-7D<SM>

| Primer | primer_name | primer_seq                | length |  | Reference          |
|--------|-------------|---------------------------|--------|--|--------------------|
| 1      | D7Mit185-1  | CATTTGTTGGGAGAGAGGGA      | 20     |  | Depositor's primer |
| 2      | D7Mit185-2  | GGACAGCCAGCCATAAAAAA      | 20     |  | Depositor's primer |
| 3      | D7Mit125-1  | TATACCCATGGAAACCAAGACC    | 22     |  | Depositor's primer |
| 4      | D7Mit125-2  | GGACTCACACATTACTTCATCTGG  | 24     |  | Depositor's primer |
| 5      | D7Mit355-1  | AGTAAGGAAAATGGAAATACCAACA | 25     |  | Depositor's primer |
| 6      | D7Mit355-2  | GATACCACAGAATTTTTGGATGC   | 23     |  | Depositor's primer |
|        |             |                           |        |  |                    |
|        |             |                           |        |  |                    |
|        |             |                           |        |  |                    |

## Reaction Components

Enzyme QIAGEN Multiplex PCR Kit (Cat. No. 206143)

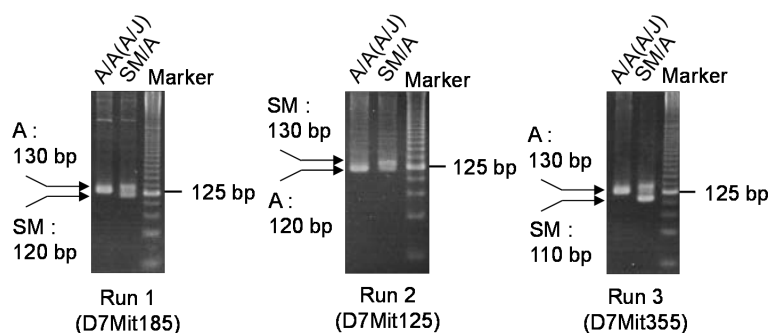
## Reaction Conditions

| step | temp (°C) | time (sec) | note                  |
|------|-----------|------------|-----------------------|
| 1    | 95        | 900        |                       |
| 2    | 94        | 30*        |                       |
| 3    | 60        | 90*        |                       |
| 4    | 72        | 60*        | *Repeat for 30 cycles |
| 5    | 60        | 1800       |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |

| Reagent      | Conc       | Reaction 1 | 2   | 3   | 4 | (μL) |
|--------------|------------|------------|-----|-----|---|------|
| H2O          |            | 2.0        | 2.0 | 2.0 |   |      |
| Master Mix   | 2 X        | 5.0        | 5.0 | 5.0 |   |      |
| primer 1     | 10 pmol/μl | 1.0        | 0   | 0   |   |      |
| primer 2     | 10 pmol/μl | 1.0        | 0   | 0   |   |      |
| primer 3     | 10 pmol/μl | 0          | 1.0 | 0   |   |      |
| primer 4     | 10 pmol/μl | 0          | 1.0 | 0   |   |      |
| primer 5     | 10 pmol/μl | 0          | 0   | 1.0 |   |      |
| primer 6     | 10 pmol/μl | 0          | 0   | 1.0 |   |      |
| DNA          |            | 1.0        | 1.0 | 1.0 |   |      |
|              |            |            |     |     |   |      |
|              |            |            |     |     |   |      |
|              |            |            |     |     |   |      |
|              |            |            |     |     |   |      |
| <b>total</b> |            | 10         | 10  | 10  |   |      |

**Marker** 25 bp DNA ladder (Invitrogen, Cat. 10597-011)

**Gel** 4% Nusieve in 0.5 X TBE (100 V, 60 min)



| primer_set | product_size | product (allele type) |            |
|------------|--------------|-----------------------|------------|
| primer 1   | primer 2     | 130, 120 bp           | A/A, SM/SM |
| primer 3   | primer 4     | 120, 130 bp           | A/A, SM/SM |
| primer 5   | primer 6     | 130, 110 bp           | A/A, SM/SM |
|            |              |                       |            |
|            |              |                       |            |

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