# PCR protocol

Sheet ID PS_06158
Release date : 10/25/2016
Print date : 10/25/2016

RBRC 01071 Strain name Floxed RBP-J

| Primer | primer_name | primer_seq | length | | |
|---|---|---|---|---|---|
| 1 | 01071 | CTGAGTAAGATGAGATGCTGACATCTGA | 28 | | RIKEN BRC |
| 2 | DY225 | ATGTACATTTTGTACTCACAGAGATGGATG | 30 | | Depositor's primer |
| 3 | WT2 | GCTTGAGGCTTGATGTTCTGTATTGC | 26 | | Int. Immunol., 14, 637-645 (2002). |

## Reaction Components

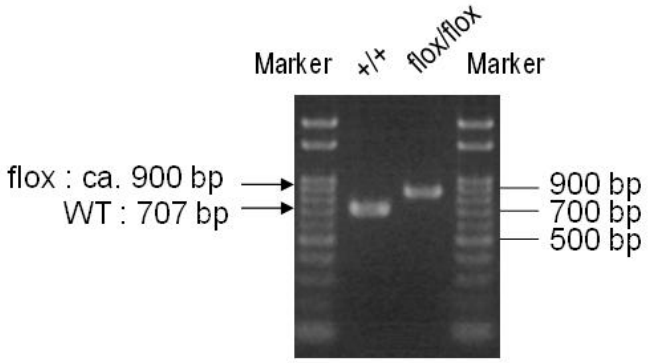
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

| | Conc | Run 1 | Run 2 | Run 3 | Run 4 |
|---|---|---|---|---|---|
| H2O | | 1.8 | | | |
| PCR buffer | 2 X | 10.0 | | | |
| dNTP | 2.0 mM | 4.0 | | | |
| primer 1 | 10 pmol/μl | 1.0 | | | |
| primer 2 | 10 pmol/μl | 1.0 | | | |
| primer 3 | 10 pmol/μl | 1.0 | | | |
| DNA | | 1.0 | | | |
| Taq | 1 U/μl | 0.2 | | | |
| | total | 20 | | | |

## Reaction Conditions

| step | temp (℃) | time (sec) | note |
|---|---|---|---|
| 1 | 94 | 120 | |
| 2 | 98 | 10 | * |
| 3 | 60 | 30 | * |
| 4 | 68 | 90 | * *Repeat for 30 cycles |

Standard PCR




**Marker**

Gene Ladder 100 (Wako, Cat. 316-06951)

**Gel**

2% agarose in 1 X TAE (100 V, 30 min)

**PCR products**

| primer_set | | product_size | product |
|---|---|---|---|
| primer 1 | primer 2 | 707 bp | WT |
| primer 1 | primer 2 | ca 900 bp | flox |
| primer 1 | primer 3 | | null (After cre mediated excision) |

**Comment** Novagen KOD Xtreme™ Hot Start DNA Polymerase can be purchased from EMD.

# PCR protocol

Sheet ID PS_05433
Release date : 3/3/2014
Print date : 10/25/2016

RBRC 01071 Strain name Floxed RBP-J

| Primer | primer_name | primer_seq | length | | |
|---|---|---|---|---|---|
| 1 | WT1 | GTTCTTAACCTGTTGGTCGGAACC | 24 | | Int. Immunol., 14, 637-645 (2002). |
| 2 | WT2 | GCTTGAGGCTTGATGTTCTGTATTGC | 26 | | Int. Immunol., 14, 637-645 (2002). |
| 3 | Neo | GCAATCCATCTTGTTCAATGGCC | 23 | | Int. Immunol., 14, 637-645 (2002). |

## Reaction Components

Enzyme: TOYOBO KOD FX (Code.No : KFX-101)

| | Conc | Run 1 | Run 2 | Run 3 | Run 4 |
|---|---|---|---|---|---|
| H2O | | 1.8 | | | |
| PCR buffer | 2 X | 10.0 | | | |
| dNTP | 2.0 mM | 4.0 | | | |
| primer 1 | 10 pmol/μl | 1.0 | | | |
| primer 2 | 10 pmol/μl | 1.0 | | | |
| primer 3 | 10 pmol/μl | 1.0 | | | |
| DNA | | 1.0 | | | |
| Taq | 1 U/μl | 0.2 | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | total | 20 | | | |

## Reaction Conditions

| step | temp (℃) | time (sec) | note |
|---|---|---|---|
| 1 | 94 | 120 | |
| 2 | 98 | 10 | * |
| 3 | 60 | 30 | * |
| 4 | 68 | 60 | * *Repeat for 30 cycles |
| | | | |
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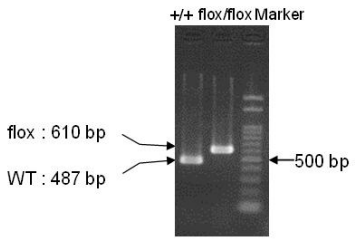
| | | | |

Standard PCR




**Marker**

Gene Ladder 100 (Wako, Cat. 316-06951)

**Gel**

2% agarose in 1 X TAE (100 V, 30 min)

**PCR products**

| primer_set | | product_size | product |
|---|---|---|---|
| primer 1 | primer 2 | 487 bp | WT |
| primer 1 | primer 3 | 610 bp | flox |
| | | | |
| | | | |
| | | | |

**Comment** Novagen KOD Xtreme™ Hot Start DNA Polymerase can be purchased from EMD.

# PCR protocol

Sheet ID PS_02494
Release date : 06/03/2009
Print date : 10/25/2016

RBRC 01071 Strain name Floxed RBP-J

| Primer | primer_name | primer_seq | length | | |
|---|---|---|---|---|---|
| 1 | WT1 | GTTCTTAACCTGTTGGTCGGAACC | 24 | | Int. Immunol., 14, 637-645 (2002). |
| 2 | WT2 | GCTTGAGGCTTGATGTTCTGTATTGC | 26 | | Int. Immunol., 14, 637-645 (2002). |
| 3 | Neo | GCAATCCATCTTGTTCAATGGCC | 23 | | Int. Immunol., 14, 637-645 (2002). |

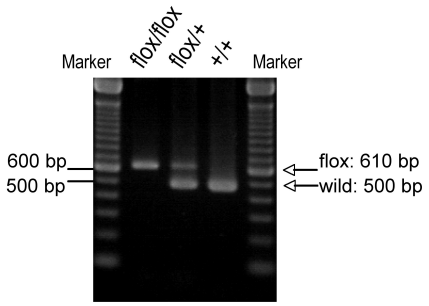
## Reaction Components

Enzyme: TaKaRa LA Taq with GC buffer (Cat. No. RR02AG)

| | Conc | Run 1 | Run 2 | Run 3 | Run 4 |
|---|---|---|---|---|---|
| H2O | | 3.8 | | | |
| PCR buffer | 2 X GC I | 10.0 | | | |
| dNTP | 2.5 mM | 2.0 | | | |
| primer 1 | 10 pmol/μl | 1.0 | | | |
| primer 2 | 10 pmol/μl | 1.0 | | | |
| primer 3 | 10 pmol/μl | 1.0 | | | |
| DNA | | 1.0 | | | |
| Taq | | 0.2 | | | |
| | total | 20 | | | |

## Reaction Conditions

| step | temp (℃) | time (sec) | note |
|---|---|---|---|
| 1 | 94 | 180 | |
| 2 | 94 | 30 | * |
| 3 | 60 | 30 | * |
| 4 | 72 | 60 | * *Repeat for 30 cycles |
| 5 | 72 | 300 | |




## Marker

100 bp ladder (Invitrogen, Cat. 15628-050)

## Gel

2% agarose in 1 X TAE (100 V, 30 min)

## PCR products

| primer_set | | product_size | product |
|---|---|---|---|
| primer 1 | primer 2 | 500 bp | WT |
| primer 1 | primer 3 | 610 bp | flox |

## Comment

# PCR protocol

Sheet ID PS_00340
Release date : 9/7/2005
Print date : 10/25/2016

RBRC 01071 Strain name Floxed RBP-J

| Primer | primer_name | primer_seq | length | | |
|---|---|---|---|---|---|
| 1 | Floxed1 | GAAGGTCGGTTGACACCAGATAGC | 24 | | Int. Immunol., 14, 637-645 (2002). |
| 2 | Neo | GCAATCCATCTTGTTCAATGGCC | 23 | | Int. Immunol., 14, 637-645 (2002). |
| 3 | WT1 | GTTCTTAACCTGTTGGTCGGAACC | 24 | | Int. Immunol., 14, 637-645 (2002). |
| 4 | WT2 | GCTTGAGGCTTGATGTTCTGTATTGC | 26 | | Int. Immunol., 14, 637-645 (2002). |

## Reaction Components

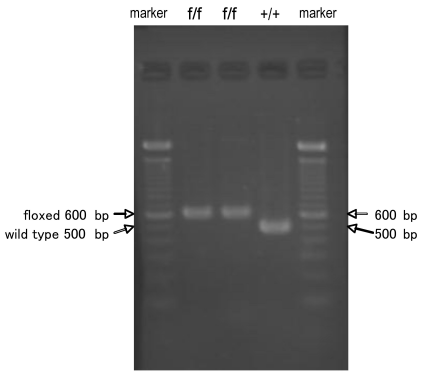
Enzyme: TaKaRa LA Taq with GC buffer (Cat. No. RR02AG)

| | Conc | Run 1 | Run 2 | Run 3 | Run 4 |
|---|---|---|---|---|---|
| H2O | | 2.8 | | | |
| PCR buffer | 2 X GC I | 10.0 | | | |
| dNTP | 2.5 mM | 2.0 | | | |
| primer 1 | 10 pmol/μl | 1.0 | | | |
| primer 2 | 10 pmol/μl | 1.0 | | | |
| primer 3 | 10 pmol/μl | 1.0 | | | |
| primer 4 | 10 pmol/μl | 1.0 | | | |
| DNA | | 1.0 | | | |
| Taq | | 0.2 | | | |
| | total | 20 | | | |

## Reaction Conditions

| step | temp (℃) | time (sec) | note |
|---|---|---|---|
| 1 | 94 | 180 | |
| 2 | 94 | 30 | * |
| 3 | 60 | 30 | * |
| 4 | 72 | 60 | * *Repeat for 30 cycles |
| 5 | 72 | 300 | |

Standard PCR




**Marker**

100 bp ladder (Invitrogen, Cat. 15628-050)

**Gel**

2% agarose in 1 X TAE (100 V, 30 min)

**PCR products**

| primer_set | | product_size | product |
|---|---|---|---|
| primer 1 | primer 2 | 600 bp | floxed |
| primer 3 | primer 4 | 500 bp | WT |

**Comment**