BacterioMatch® II Electrocompetent Reporter Cells

Catalog #200195

Storage Store the cells immediately at the bottom of a –80°C freezer. Do not store the cells in liquid nitrogen. Store the control plasmid DNA at –80°C.

Introduction

The BacterioMatch® II two-hybrid system reporter strain is a histidine auxotrophic mutant derived from XL1-Blue MRF'. This strain harbors the genetic elements necessary to report the interaction between recombinant bait and target proteins expressed from BacterioMatch II two-hybrid system plasmids. Episomally expressed $LaqI^q$ represses synthesis of the bait and target until induction. Positive protein-protein interactions are indicated by the expression of a reporter gene cassette that includes the *HIS3* gene (conferring the ability to grow in the presence of the compound 3-AT) and *aadA* gene (conferring streptomycin resistance).

BacterioMatch II electrocompetent reporter cells (catalog #200195) are optimized for high-efficiency, large-scale cotransformation reactions, performed during BacterioMatch II two-hybrid library screening. Performing library cotransformation by electroporation greatly increases the transformation efficiency, decreasing the number of cotransformation reactions required for library coverage. For the small-scale cotransformation of purified plasmids performed during BacterioMatch II two-hybrid system validation steps, Stratagene offers the chemically-competent BacterioMatch II validation reporter competent cells (catalog #200192). Stratagene also offers chemically-competent cells for library screening (BacterioMatch II screening reporter competent cells, catalog #200190).

Materials Provided

Materials Provided	Quantity	Transformation Efficiency (cfu/μg of pUC18 DNA)[a]
BacterioMatch® II electrocompetent reporter cells	5 × 100 μl	1×10^9
pUC18 plasmid DNA (0.1 ng/μl in TE buffer)	10 μl	—

[a] Stratagene guarantees this efficiency when the cells are used according to the protocol in this instruction manual.

Additional Materials Required

Sterile electroporation cuvettes, 0.1-cm gap width

Sterile microcentrifuge tubes

14-ml BD Falcon polypropylene round-bottom tubes (BD Biosciences, Catalog # 352059)

Genotype

Δ(*mcrA*)*183* Δ(*mcrCB-hsdSMR-mrr*)*173 endA1 hisB supE44 thi-1 recA1 gyrA96 relA1 lac* [F´ *laqI*^{q} *HIS3 aadA* Kan^r].
BacterioMatch II electrocompetent cells are kanamycin resistant/

Transformation Guidelines for BacterioMatch® II Two-Hybrid System Reporter Strain Competent Cells

Storage Conditions

Electrocompetent cells are sensitive to even small variations in temperature and must be stored at the bottom of a –80°C freezer. Transferring tubes from one freezer to another may result in a loss of efficiency. Competent cells should be placed at –80°C directly from the dry ice shipping container.

Aliquoting Cells

Keep the cells on ice at all times during aliquoting. It is essential that the microcentrifuge tubes that the cells will be aliquoted into are placed on ice before the cells are thawed and that the cells are aliquoted directly into the pre-chilled tubes.

Cuvette Gap Width

Use a cuvette with a 0.1-cm gap to maximize the transformation efficiency and to minimize the possibility of arcing. A cuvette with a 0.2-cm gap is not recommended because the transformation efficiency is lower and the possibility of arcing is higher.

Ionic Strength of DNA Solution

The sample DNA to be transformed by electroporation must be in a low-ionic-strength buffer, such as TE buffer or water. DNA samples containing too much salt will cause arcing at high voltage, possibly damaging both the sample and the electroporator.

Plating Media

For the efficiency determination assay described in the protocol on the reverse page, it is important to plate the cells transformed with the pUC18 plasmid on LB–ampicillin medium.

For plating after cotransformation of the bait plasmid (carrying Cam^r) and a target plasmid library (carrying Tet^r) during two-hybrid interaction screens, plating should be performed according to the instruction manual provided with the BacterioMatch II Two-Hybrid System Library Construction Kit with Electrocompetent Cells (Catalog #200414; available online at http://www.stratagene.com/manuals/index.asp).

Electroporation Protocol for Determination of Transformation Efficiency

Note *For the efficiency determination, it is important to use pUC18 plasmid DNA. Do not substitute with pBT, pTRG or other plasmids.*

For a protocol for cotransformation of reporter strain electrocompetent cells with Bait and Target plasmids, see the BacterioMatch II Two-Hybrid System Library Construction Kit with Electrocompetent Cells Instruction Manual, *available online at http://www.stratagene.com/manuals/index.asp*. (Search for catalog #200414.)

1. Pre-chill a sterile electroporation cuvette (0.1-cm gap) and a sterile 1.5-ml microcentrifuge tube thoroughly on ice. Preheat sterile SOC medium to 37°C.
2. Set the electroporator to a voltage setting of 1500 V (15 kV/cm field strength). Set the resistance to 400 Ω and the capacitance to 25 µF.
3. Thaw the BacterioMatch II electrocompetent reporter cells on ice. After mixing the cells gently, aliquot 40 µl of cells into the pre-chilled microcentrifuge tube. Keep the tube on ice throughout the procedure.
4. Dilute the pUC18 control DNA 1:10 with sterile dH_2O. Add 1 µl of the diluted pUC18 DNA to the cells with gentle mixing.
5. Transfer the cell-DNA mixture to a chilled electroporation cuvette, tapping the cuvette until the mixture settles evenly to the bottom.
6. Slide the cuvette into the electroporation chamber until the cuvette sits flush against the electrical contacts.
7. Pulse the sample once, then quickly remove the cuvette. Immediately add 960 µl of SOC medium (held at 37°C) to resuspend the cells.
8. Transfer the cells to a sterile 14-ml BD Falcon polypropylene round-bottom tube (BD Biosciences Catalog #352059). Incubate the tube at 37°C for 60 minutes with shaking at 225 rpm.
9. Following the incubation period, plate 5 µl of the transformation mixture onto each of two LB-ampicillin agar plates. To accommodate this small plating volume, first place a 200-µl pool of SOC medium on the plate. Pipet 5 µl of the cells into the pool and then spread the mixture with a sterile spreader.
10. Incubate the plates overnight at 37°C, inverted.
11. Count the number of colonies on each plate. Expect an average of ≥50 cfu, indicating an efficiency of $\geq 1 \times 10^9$ cfu/µg pUC18 DNA.

Preparation of Media and Reagents

SOB Medium (per Liter)	**SOC Medium (per 100 ml)**
20.0 g of tryptone 5.0 g of yeast extract 0.5 g of NaCl Add deionized H_2O to a final volume of 1 liter Autoclave Add 10 ml of filter-sterilized 1 M $MgCl_2$ and 10 ml of filter-sterilized 1 M $MgSO_4$ prior to use	**Note** *This medium should be prepared immediately before use.* 2 ml of filter-sterilized 20% (w/v) glucose or 1 ml of filter-sterilized 2 M glucose SOB medium (autoclaved) to a final volume of 100 ml
LB Agar (per Liter)	**LB–Ampicillin Agar (per Liter)**
10 g of NaCl 10 g of tryptone 5 g of yeast extract 20 g of agar Adjust pH to 7.0 with 5 N NaOH Add deionized H_2O to a final volume of 1 liter Autoclave	Prepare 1 liter of LB agar Autoclave Cool to 55°C Add 10 ml of 10-mg/ml-filter-sterilized ampicillin Pour into petri dishes (~25 ml/100-mm plate)

Quality Control Testing

Transformations are performed both with and without pUC18 DNA using 40-µl aliquots of cells and 1 µl of pUC18 plasmid (10 pg/µl) following the protocol outlined above. Following transformation, 5-µl samples of the culture are plated in duplicate on LB agar plates with 100 µg/ml of ampicillin. The plates are incubated at 37°C overnight and the efficiency is calculated based on the average number of colonies per plate.

Full activity is guaranteed through two years.

Lot #

Efficiency $\geq 1 \times 10^9$ cfu/µg pUC18 DNA

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Quality Controlled By

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Endnotes

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