Transefection

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手法については以下に5通りの方法を示した。これらの試薬は、すべて現在ラボにあるが、実際、よく使用しているものは、(1) Lipofectamine PLUS (Invitrogen)と(3) Effectene (Gibco)である。また、ペプチドなど大きな分子をtransfectionするときなどは (2) DOTAP (Roche)を使用すると良い。ちなみにビーズを細胞内に導入する時も(2) DOTAP (Roche)を用いて行うことができる。現段階では、はじめて遺伝子をtransfectionする時は、まず、(1) Lipofectamine PLUS (Invitrogen) を試してみることを奨める。

(1) Lipofectamine PLUS (Invitrogen)
・Dilute 0.2~0.5 μg DNA (treated with phenol / chloroform) in 50 μl serum-free medium and mix gently.
・Add 3 μl Lipofectoamine PLUS to the tube (tube 1).
・Incubate for 15 min at R.T.
・Add 4ul Lipofectoamine to another 50 μl serum-free medium and mix gently (tube 2).
・Combine tube 1 and tube 2, and mix by vortexing.
・Incubate for 15 min at R.T.
・Add the mixture to cells with 1 ml serum-free medium (37℃) in a 35 mm glass-bottom dish .
・incubate for 1~1.5 hr at37℃ in a CO2 incubator.
・Exchange the medium for 2 ml growth medium.
・Incubate for 2 days.

(2) DOTAP (Roche)
・Dilute ~2.5 μg DNA (treated with phenol / chloroform) into 25 μl HBS buffer (tube 1).
・Dilute 15 μl DOTAP into 50 μl HBS buffer (tube 2).
・Combine tube 1 and tube 2.
・Incubate for 10~15 min.
・Add the mixture to cells with 2 ml growth medium (37℃) in a 35 mm glass-bottom dish.
・Incubate for 3~10 hr.
・Exchange medium for new 2 ml growth medium (37℃).
・Incubate for 2 days.

(3) Effectene (Gibco)
・Dilute 0.1~0.5 μg DNA (treated with phenol / chloroform) into 100 μl Buffer EC.
・Add 3.2 μl Enhancer and mix by vortexing for 1 sec.
・Incubate at R.T. for 2~5 min (tube 1).
・Add 10 μl Effectene Reagent to the tube 1.
・Mix by vortexing for 10 sec.
・Incubate for 5~10 min at R.T.
・Add the mixture to cells with 2 ml growth medium (37℃) in a 35 mm glass-bottom dish.
・Incubate for 2 days.

(4) Lipofectamine 2000 reagent (Gibco)
・Add 3~5 μg DNA into 240 μl serum-free medium and mix gently (tube 1).
・Add 20 μl (LF 2000 Reagent) into 240 μl serum-free medium and mix gently(tube 2).
・Combine tube1 and tube 2.
・Incubate at R.T. for 20 min.
・Add the mixture to cells with 2 ml growth medium (37℃) in a 35 mm glass-bottom dish.
・Incubate for 2 days.

(5) Cell Phect (Pharmacia)
・Dilute 1~10 μg DNA (treated with phenol / chloroform) into 30 μl D.W.
・Add 30 μl solution A and mix.
・Incubate for 10 min. at R.T.

- Add 60 μl solution B.
- Mix by vortexing for 5 sec.
- Incubate for 15 min at R.T.
- Add mixture to cells with 2 ml growth medium (37℃) in a 35 mm glass-bottom dish.
- Incubate for 2 days.