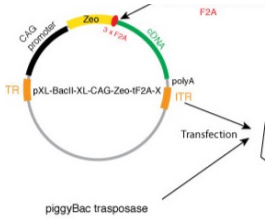
Apr 30, 2018

Cell adhesion assay for HEK-293 and Cdh2-deficient HEK-293 cells



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Goodman, KM, Yamagata, M, Jin, X, Mannepalli, S, Katsamba, PS, Ahlsén, G, Sergeeva AP, Honig, B, Sanes, JR, Shapiro, L. (2016) Molecular basis of sidekick-mediated cell-cell adhesion and specificity. *eLife*DOI: http://dx.doi.org/10.7554/eLife.19058

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We use this protocol and it's working

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Abstract

This is a protocol for cell adhesion assays using 293T cells or N-cadherin-deficient 293NC cells (Yamagata et al., 2018). HEK293T(ATCC CRL-3216) cells are easily transfectable with plasmids. The 293NC cells lack N-cadherin (Cadherin-2), but maintain the property of its parental 293T cells. Thus, this 293T variant is useful to test cell adhesion (Goodman et al., 2016; Yamagata et al., 2018). Here, I describe a protocol and some technical tips.

Guidelines

Technical inquries related to this protocol should be directed to Masahito Yamagata, PhD (e-mail: yamagatm@mcb.harvard.edu or yamagatm2@gmail.com).

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Safety warnings

Human 293 cells are not hazardous. However, it is important to use them as human cells.

Before start

293NC cells are available upon request to Joshua Sanes (sanesj@mcb.harvard.edu).

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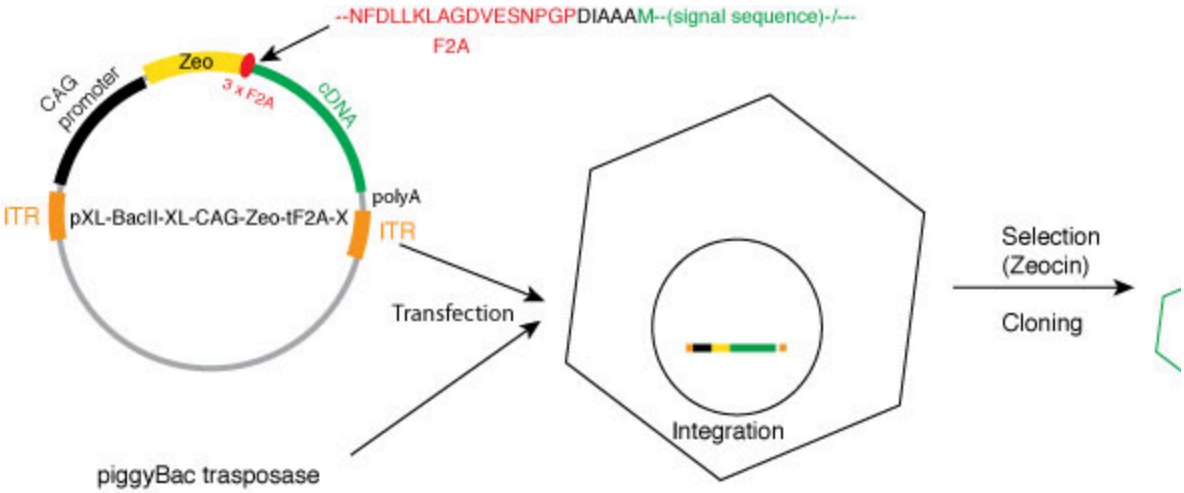
1 Generate stably transfected cells which express the adhesive protein at the homogenous level. To generate stable cell lines, it is efficient to use **pXL-BacII-XL-CAG-Zeo-tF2A-X** system (Figure 1) as described in Yamagata et al. 2018 and Goodman et al., 2016. Culture them to almost confluency in 6 well plates or 60mm dish. The cells have to be healthy right before the experiment. If you need to label cells, use CellTracker(Invitrogen). CellTrackers are available in various colors. Add the stock solution to the culture medium, and incubate for 5-15 mins at 37C.

Figure 1



2 Precoat 24 well plates with 1% BSA in PBS for at least 2-3 hours (typically, overnight) . Using non-tissue culture 24 wells is better. However, tissue-culture-treated 24 well plates also work by precoating them. (→ Step 9)

3 Warm up 'Trypsin in PBS' at 37C. Adjust concentration of trypsin, and add EDTA or CaCl2 as needed. Typically,

TE: 0.05-0.25%(w/v)Trypsin + 1mM EDTA in PBS (to remove Ca2+-dependent adhesion = cadherins)

TC: 0.05-0.25%(w/v) Trypsin + 1mM CaCl2 in PBS (to protect Ca2+-dependent adhesion)

For sensitive assay, use TPCK-treated trypsin (TPCK treatment removes chymotrypsin).

But, tissue-culture grade trypsins also work for most cases (eg, Trypsin (0.25%) Thermo-Fisher 15050065). It is necessary to optimize the concentration of trypsin.

4 Remove the medium from the cultured cells, briefly rinse with PBS(-), and add 2 ml TE or TC (each well). Incubate at 37C for 15-30 min. This dissociation depends on the condition of cells and strength of adhesion. For HEK-293T cells, extensive dissociation works better. For HEK-293NC cells, 37C incubation for 15 min works well.

5 Stop the dissociation by adding 0.2 ml 1 mg/ml trypsin inhibitor (Sigma T6522) in PBS(-), 2 ml culture medium (without serum), and 10 microlitter of 1 mg/ml DNase (Sigma DN25). Mix with 1ml pipettman, and pipet in and out 20 times. No bubbles.

6 Transfer to 15 ml tube, and spin down (→ Step 7).

Alternatively, it is possible to add this suspension to the plate (Step 10) if adhesion is fast and strong.

7 Remove the supernatant, and suspend with 0.5% BSA in PBS+20mM HEPES, 1 microgram/ml DNase ("adhesion assay solution") which had been chilled on ice. Triturate 10 times with 1ml pipetman.

8 Count cells. The cell suspensions should be kept on ice during this step.

9 Remove BSA solution from 24 well plates (from Step 2). Add 0.8ml adhesion assay solution to each well.

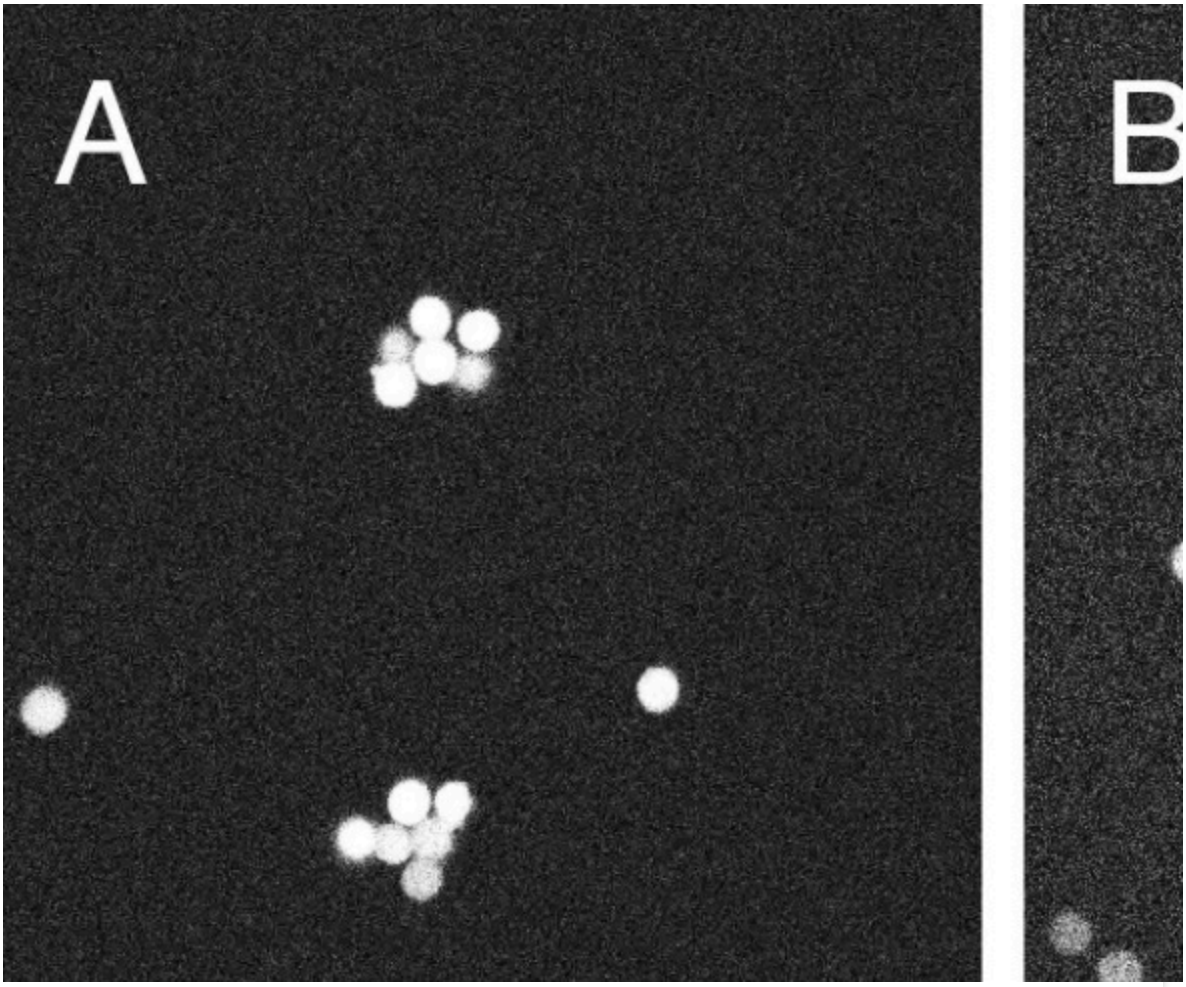
10 Add cells. 1×10(6) to each well is typical. However, different dilultion such as 1×10(5)/well may work. It depends on strength of adhesion. If the adhesion is weak, use higher concentration of cells. Mix the cells with pipetting.

11 Place the plate on a orbital shaker at room temperature or 37C. At 37C, you will observe more cadherin activity although it causes quicker recovery of cells and is sometimes sensitive to weak adhesion. This is because cadherin-dependent adhesion is enhanced at 37C. At room temperature, you can suppress cadherin-dependent background if the adhesion can be observed at this temperature.

12 Look at aggregation occasionally. For strong adhesion, you need to stop after 5-10 min of incubation. But, in most cases, incubate for 30 min to 1 hour. To stop assay, add the same volume (1ml) of 4%(w/v) paraformaldehyde in PBS to the well, and mix. You can keep this plate for a couple of days at 4C.

13 Count cells (Nt, total cell number) and aggregates (N, total particle number), and calculate Nt/N. N/Nt=1 means no aggregation. Typically, it is hard to analyze too large cell aggregates (eg, >100 cells). Thus, it is important to adjust reaction so that the cell mixtures do not contain too large aggregates.

Example



A: 293TA (parental) after 1 hour
B: 293NC (N-cadherin-deficient) after 1 hour.
Cells were labeled with Cell Tracker Green CMFDA Dye (ThermoFisher).

A: Nt=12, N=4 (Nt/N = 3) Aggregated
B: Nt=12, N-12 (Nt/N = 1) No aggregation

14 **Tips**

1) HEK293T(parental) cells have intrinsic Ca-dependent adhesion activity (cadherin-dependent adhesion). To reduce the background, the cells have to be trypsinized in the presence of EDTA. If the adhesive proteins is susceptible to Trypsin-EDTA, it is not good to use HEK-293 cells. New 293NC cells (N-cadherin is removed by CRISPR/Cas9) do not have Ca2+-dependent adhesion (Yamagata et al., 2018).

2) Adhesion depends on pH of the medium. To obtain consistent results, adding 20mM HEPES pH7.4 is important. DNA molecules from dead cells cause high background (DNA

is the most annoying "sticky molecules"). The culture has to be healthy before dissociation. The dissociated cells should be always maintained in the presence of DNase.

3) Ca2+-dependent adhesion (cadherins) is temperature-dependent whereas Ca-independent adhesion (eg, IgSF) is usually not so temperature-depedent.

References

Yamagata, M, Duan, X and Sanes, JR. (2018). Cadherins interact with synaptic organizers to promote synaptic differentiation. *Frontiers in Molecular Neuroscience*. https://doi.org/10.3389/fnmol.2018.00142

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Several tips on cell adhesion assays were based on a protocol originally from the laboratory of Dr. Masatoshi Takeichi (RIKEN).