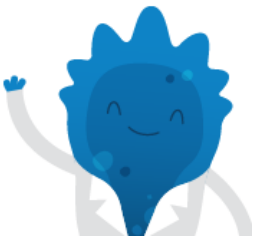
Dec 31, 2019 Version 2

Bacterial transformation V.2



In 2 collections

DOI
dx.doi.org/10.17504/protocols.io.bawgifbw

Addgene The Nonprofit Plasmid Repository[1]

[1]Addgene

Addgene The Nonprofit Plasmid Repository
Addgene

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.bawgifbw

External link: https://www.addgene.org/protocols/bacterial-transformation/

Protocol Citation: Addgene The Nonprofit Plasmid Repository 2019. Bacterial transformation. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bawgifbw

Protocol status: Working
We use this protocol and it's working

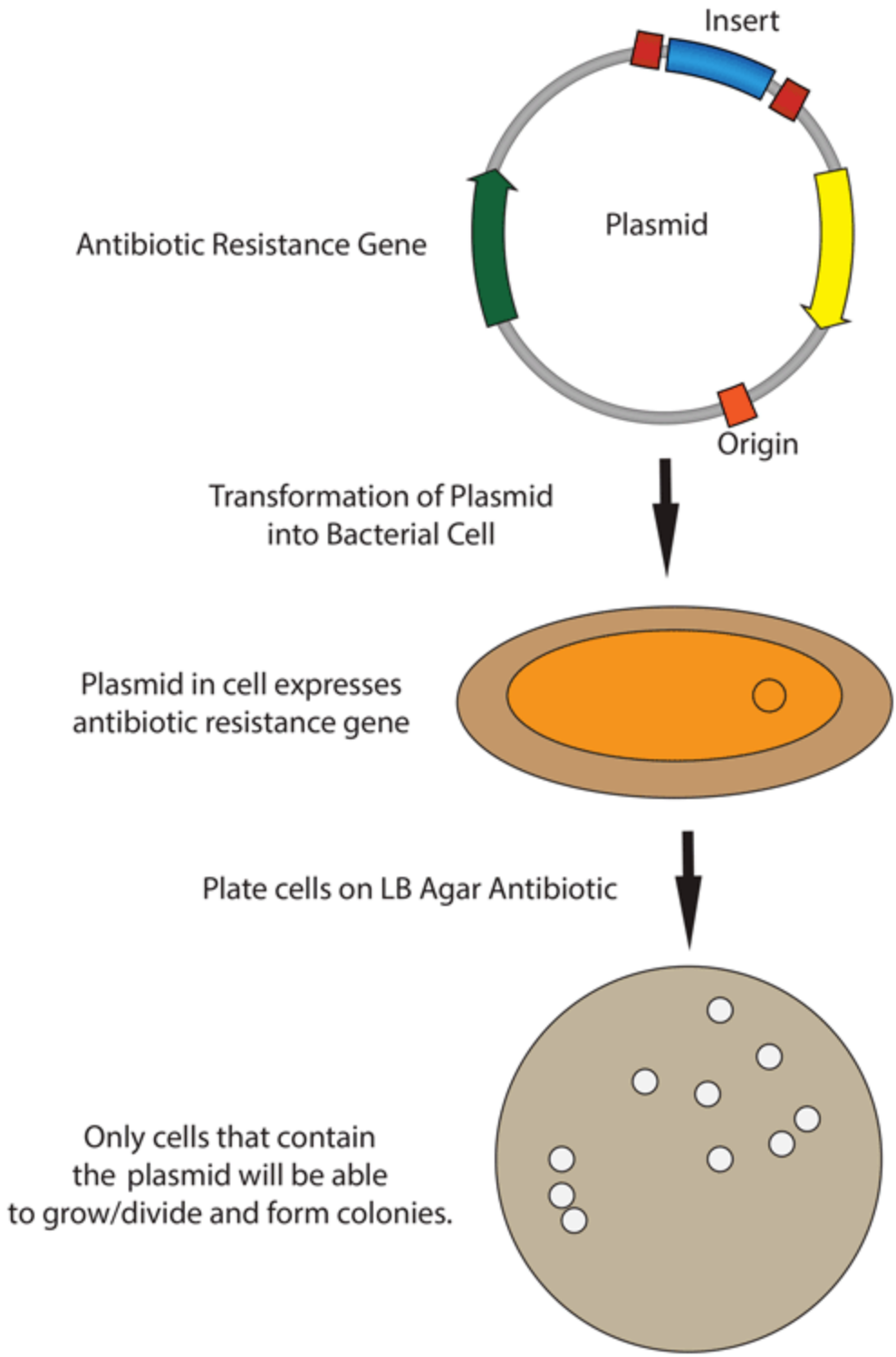
Created: December 31, 2019

Last Modified: August 17, 2020

Protocol Integer ID: 31400

Abstract

The following protocol is for bacterial transformation. To see the full abstract and additional resources, visit the **Addgene protocol page**.



Guidelines

Tips and FAQ

How can I save time when carrying out transformations?
If you are not concerned with transformation efficiency (such as when you have a tube of plasmid DNA and just need to transform bacteria so that you can grow up more of the plasmid) you can save a lot of time by shortening or skipping many steps and will still get enough colonies for your next step. Remember that each of these shortcuts will reduce the efficiency of the transformation, so when higher efficiency is needed follow the complete protocol.

- Thaw the competent cells in your hand instead of on ice
- Reduce step 4 from 20 - 30 mins to 2 mins on ice before heat-shock
- Shorten or skip the outgrowth (for Ampicillin resistance it is ok to completely skip the outgrowth, for the other antibiotics it is a good idea to outgrow for at least 20-30 mins)

I get very few if any transformants when transforming large plasmids (>10 kb) or BACs, what can I do?
Chemically competent cells are fast and easy to use, but are less efficient at taking up larger plasmids. If you need to transform large plasmids, it is a good idea to use electro-competent cells. Instead of relying on the heat-shock to cause the cells to take up the DNA, an electro-magnetic field is applied to the cell/DNA mixture to induce membrane permeability. To do this you will need to have access to an electroporator and the appropriate cuvettes. Follow the manufacturer's instructions for each.

I got no transformants. What went wrong?

Check that you are plating on an LB Agar plate containing the correct antibiotic. The resistance gene on your plasmid must match the antibiotic on the plate. You should also add a positive control (many companies include a positive control plasmid with their competent cells) to ensure that your transformation procedure is working.

TIP: Sometimes less is more.

Although it may be counter-intuitive, you will often get higher transformation efficiencies with less DNA, especially when using highly competent cells. If you used 100-1000 ng of total DNA in a ligation you will often get more colonies if you use 1 µl of a 1:5 or 1:10 dilution rather than 1 µl directly.

Note

Pro-Tips

- Commercial competent cells range significantly in their transformation efficiency. The lowest efficiency cells (usually the least expensive) are fine for transforming plasmid DNA for the purposes of storage and amplification. Higher efficiency cells are more important if you will be transforming with very small amounts of DNA or if you're multiple plasmids at once.
- To save money, many labs also make their own competent cells. This is a relatively simple **procedure** and is useful for performing low efficiency transformations.

Materials

Equipment

- Shaking incubator at 37 °C
- Stationary incubator at 37 °C
- Water bath at 42 °C
- Ice bucket filled with ice
- Microcentrifuge tubes
- Sterile spreading device

Reagents

- LB agar plate (with appropriate antibiotic)
- LB or SOC media
- Competent cells
- DNA you'd like to transform

1 Take competent cells out of -80 °C and thaw on ice (approximately 00:20:00 - 00:30:00).

2 Remove agar plates (containing the appropriate antibiotic) from storage at 4 °C and let warm up to room temperature and then (optional) incubate in 37 °C incubator.

3 Mix 1 µL - 5 µL of DNA (usually 10 pg - 100 ng) into 20 µL - 50 µL of competent cells in a microcentrifuge or falcon tube. GENTLY mix by flicking the bottom of the tube with your finger a few times.

Note

Pro-Tip
Transformation efficiencies will be approximately 10-fold lower for ligation of inserts to vectors than for an intact control plasmid.

4 Incubate the competent cell/DNA mixture on ice for 00:20:00 - 00:30:00 .

5 Heat shock each transformation tube by placing the bottom 1/2 to 2/3 of the tube into a 42 °C water bath for 00:00:30 - 00:01:00 (00:00:45 is usually ideal, but this varies depending on the competent cells you are using).

6 Put the tubes back on ice for 00:02:00 .

7 Add 250 µL - 1000 µL LB or SOC media (without antibiotic) to the bacteria and grow in 37 °C shaking incubator for 00:45:00 .

Note

Pro-Tip
This outgrowth step allows the bacteria time to generate the antibiotic resistance proteins encoded in the plasmid backbone so that they will be able to grow once plated on the antibiotic containing agar plate. This step is not critical for Ampicillin resistance but is much more important for other antibiotic resistances.

8 Plate some or all of the transformation onto a 10 cm **LB agar plate** containing the appropriate antibiotic.

Note

Pro-Tip
We recommend that you plate 50 µL on one plate and the rest on a second plate.
This gives the best chance of getting single colonies, while allowing you to recover all transformants.

9 Incubate plates at 37 °C overnight.

Note

Pro-Tip
If the culture volume is too big, gently collect the cells by centrifugation and resuspend in a smaller volume of LB so that there isn't too much liquid media on the agar plates. If the agar plate doesn't dry adequately before the cells begin dividing, the bacteria diffuse through the liquid and won't grow in colonies.