Transformation

Overview

This protocol covers transformation of chemically competent E.coli cells from NEB with plasmids from either miniprep or Gibson Assembly. See additional information for instructions about transforming homemade competent cells.

Background

Often, it is desired to place a given plasmid in *E. coli* cells. The two main reasons for giving *E. coli* plasmids are to perform experiments (placing circuits in E. coli for measurement)[i], or for cloning (obtaining more of that plasmid). Competent cells, cells which are capable of taking up plasmids come in two main forms, electrocompetent and chemically competent. Electrocompetent cells require more equipment and are somewhat more involved, but typically have better transformation efficiencies (number of successful transformants compared to DNA input), while chemically competent cells are easier and quicker to make and use. Although it is not difficult to make competent cells of either type, due to (fairly) cheap high quality chemically competent cells being commercially available, many labs no longer make their own[ii].

In general we use chemically competent cells from NEB, although we will transform homegrown cells when we know the transformation is likely to work (e.g. we have a confirmed miniprep and we want more of it).

Materials

- Chemically competent cells from NEB[iii]
    - Cells are stored at -80C and should be always be kept on ice. Note that once cells are thawed (removed from -80C), they cannot be refrozen and used again.[iv]
    - Tubes contain at least 50µl total volume. The minimum volume per transformation is 10µl.
    - We use 3 different cell types., 5-alpha, 10-Beta, BL21. 5-alpha is generally used for all cloning, while the latter two are used for experiments.[v]
- Temporary 1.7mL centrifuge tubes (1 per sample)
    - Each tube should be labeled with a key (typically numerical) that corresponds to a specific transformation
- Ice bucket (full)

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[i] There are other reasons why one might put a plasmid that expresses genes in a cell (e.g. protein purification).

[ii] The real main benefit to using commercial cells (besides ease of use), is that it eliminates a potential error source. Commercial cells will always work consistently well (likely better than any homemade ones), as such as long as you follow a consistent protocol, you can be assured that failed transformations are not because the competent cells go bad.

[iii] Theoretically most of this protocol should work for any chemically competent cells.

[iv] Technically they can be, but efficiency will be greatly reduced. (Hence it would not be worthwhile).

[v] 5-alpha is RecA deficient, which aids the maintenance of plasmids (recombination = bad). 10-Beta is incapable of metabolizing arabinose, which is useful when using the pBad promoter. 10-Beta has the RecA1 mutation, which reduces recombination. It is occasionally used as a cloning strain, particularly for large (>10 kb) plasmids. BL21 is a protein expression strain often used for creation and purification of protein, as such it is deficient in OmpT and Lon (proteases).

- Plasmid(s) for transformation
    - Gibson Assembly reactions can be directly transformed while plasmid minipreps should be diluted to ~1-2ng/1nM concentration.[vi]
- SOC/10-Beta media
    - 10-Beta media should only be used 10-Beta cells. SOC should not be used for 10-Beta cells[vii]
    - SOC contains: 2% w/v tryptone, .5% w/v yeast extract, 10mM NaCl, 10mM $MgCl_2$, 10mM $MgSO_4$, 20mM glucose[viii]
- Antibiotic resistant plates
    - Plates should obviously have the appropriate antibiotic(s) for the plasmids you are working with. Plates should be pre-warmed (lid side down) before plating, and before labeling (hard to label a foggy plate).

Procedure

1. Remove cells from -80C and **place onto ice**. Wait ~10 minutes for cells to thaw.
    - Note that at no point should you vortex or centrifuge these cells.
    - **Cells should be kept on ice** unless otherwise indicated
2. Add appropriate amount of **cells** to each keyed temporary transformation tube (minimum 10µl per tube).
    - If you are transforming less than 5 plasmids per tube, use slightly more cells.
    - For homemade competent cells, use 50µL per transformation
    - **Tubes should be on ice** before aliquoting cells
3. For each 50µL of cells add 2µl of each **plasmid** (diluted as above) **or Gibson** Assembly product to the appropriate tube. (i.e. add .5µL Gibson for 12.5µL of cells).
    - This should be done on ice
4. **Flick tube** 2-3 times to mix. Cells will be on the sides of tube, do not be alarmed. Do not centrifuge.
5. Preheat the heat block to 42C. Remember to both turn heat block on and to turn on heating element.

[vi] Efficiency is highest in the 1pg-1ng range (per 50µL cells), however the largest numbers of colonies will be gained by using 10ng per transformation (via NEB). Consider however that these statistics are for plasmids of a certain size, one would imagine that mols of DNA would be more important. See additional info for graph.

[vii] Strictly speaking 10-Beta cells will probably be fine in SOC. The same may not be true for the reverse. Also our transformation efficiencies are usually so high that a minor decrease would not prove problematic. Do not take this as an invitation to use the wrong media. I think NEB has info about this on their website.

[viii] SOC (Super Optimal broth with Catabolite repression) is a derivation of the rich media SOB (Super Optimal Broth), which contains the same components as SOB but with the addition of glucose.

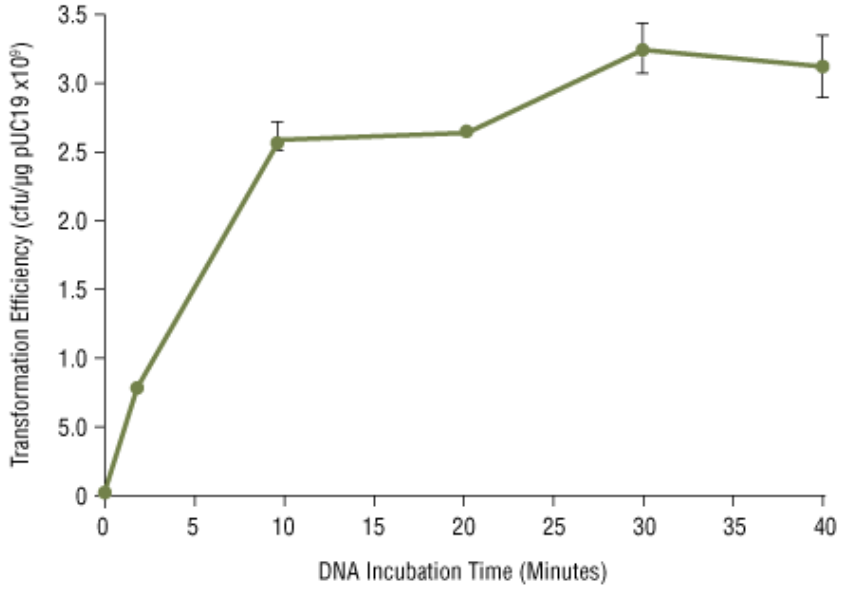
6. Allow cells to incubate on ice for 30 minutes[ix]
7. Heat shock (put in heat block) cells for **exactly[x] 30 seconds** (for 10-Beta, 5-alpha cells and JS006) **or exactly 10 seconds** (BL21 cells).[xi]
8. Immediately return cells to ice. Incubate **5 minutes**
9. Visually **inspect** SOC bottle to **ensure** it is not **contaminated**. Solution should be transparent, with no floating particles or cloudiness.
10. Add SOC (5-alpha and BL21 cells) or 10-Beta media (10-Beta cells) to tubes. For each 1µl of cells used, you should use 19µl of media (e.g. 10µl of 5-alpha cells, 190µl SOC).
11. Place tubes in shaking incubator (250 RPM, 37C) for 1 hour (non kanamycin antibiotics) or 2 hours (kanamycin)
12. At this point you should pre-warm and label your plates.
13. Remove tubes from incubator.
14. **Invert each tube at least 6 times** and add an appropriate amount (50µl for Gibson Assembly, 100µL for 3G Assembly, 30µl for experiment plates, 100µL for homegrown competent cells) to each plate.
15. Add ~3 sterilized glass beads to each plate and shake side to side (rotate 90 degrees after a few shakes).
16. Remove glass beads into used glass bead container. Place plates (lid side down) in incubator at 37C. Colonies should form within 16-18 hours.

[ix] Going slightly longer (~10 minutes) than 30 minutes will not harm the cells. See additional info section.
[x] Cells cannot actually tell time. 29 seconds is fine, as is 31 seconds. See additional information for graph.
[xi] An "easy" way to remember this is that **10**-Beta cells get 30 seconds and BL**21** cells get 10 seconds. /s/

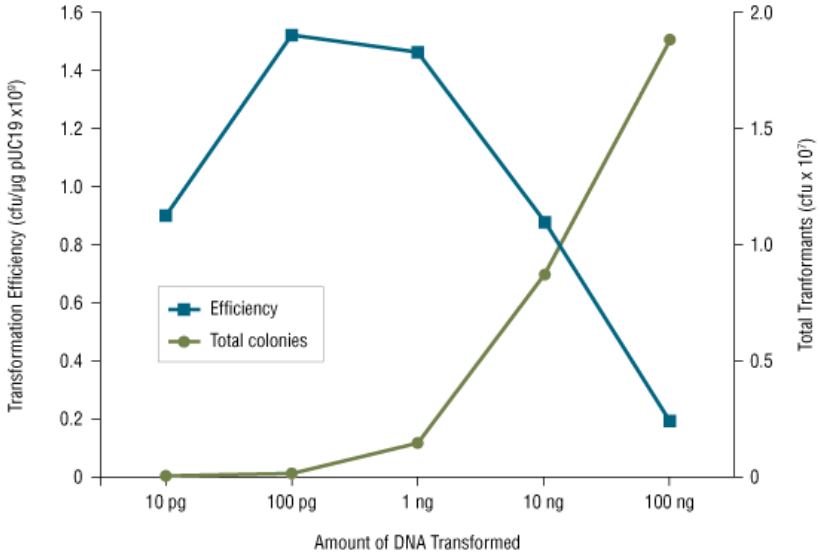
Additional Information

For homemade competent cells, follow the protocol as above, using a single tube (50µL of cells) for each transformation (950µL SOC, 30 second heat shock).

Effect of DNA incubation time on NEB 5-alpha competent E.coli transformation efficiency (via NEB):



DNA Effects on Transformation Efficiency and Colony Output (via NEB):



Effect of heat shock time on NEB 5-alpha competent E.coli transformation efficiency (via NEB):