

Trouble shooting

Ligation efficiency is low:

- Extend ligation reaction up to 30 min.
- Improve quality of DNA, purify DNA.
- Try different ratios of insert to vector DNA.
- Try different restriction enzymes to generate the fragments to be ligated. Enzymes producing sticky ends are preferable.
- Use pure solvents (water, TE) and buffers (molecular biology grade, free of DNases).
- Minimize exposition of DNA to UV light during preparation of DNA fragments to be ligated.

Transformation efficiency is low:

- Extend incubation time of cells with ligation mix on ice up to 30 min.
- Be sure to perform heat shock according to manual.
- Do phenotypic expression with aeration.
- Check concentration of antibiotic in selection plate.
- Check storage of competent cells (-80 °C).
- Check thawing procedure of competent cells, keep them always on ice prior to heat shock.

Related Products

Product	Cat#	Pack size
Chemical competent cells TZ101 α ; 10 x 0.1 ml	M3434.0010	10 transformations
Chemical competent cells TZ101 α ; 20 x 0.1 ml	M3434.0020	20 transformations
Chemical competent cells for large / toxic plasmids; TZ102 α , 10 x 0.1 ml	M3435.0010	10 transformations

Genaxxon bioscience Alligator™ Ultra-fast Ligation Kit

Product	Cat#	Package size
Alligator Ligation kit	M3430.0030	30 reactions
Alligator Ligation kit	M3430.0060	60 reactions
Alligator Ligation kit with competent cells	M3431.0030	30 reactions
Alligator Ligation kit with competent cells	M3431.0060	60 reactions

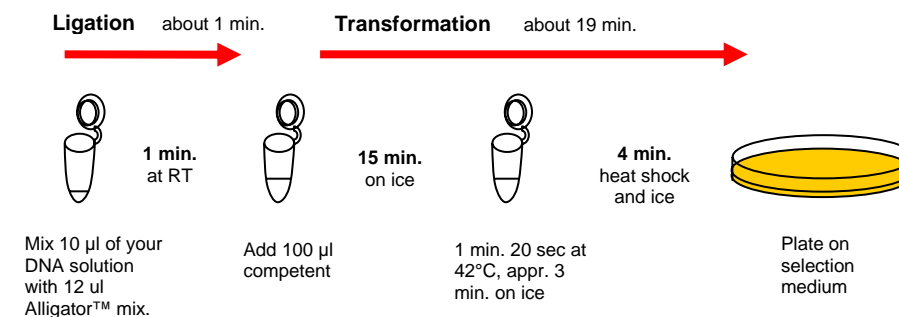
Description

The Genaxxon Alligator™ DNA ligation kit is a simple two component system that allows to perform DNA ligation reactions very rapidly. The kit uses T4 DNA ligase and an optimised propriety buffer system. Due to the extremely high efficiency of the ligation reaction, conventional overnight incubations are no longer necessary.

Various kinds of ligations with blunt or sticky end DNA fragments as well as T/A overhangs can be performed in a minimum time – in most cases, one minute reaction time leads to sufficient efficiency. The reaction can proceed directly to bacterial transformation.

By using the Genaxxon competent cells shipped with Alligator™ (Cat#: M3431) the whole ligation and transformation procedure can be done within 20 minutes.

Alligator™ can be purchased in package sizes to perform 30 reactions up to 120 reactions.



Kit components

Alligator™: 1 x 40 µl **Solution A**, 1 x 340 µl **Solution B** (per 30 reactions)

Alligator™: 1 x 40 µl **Solution A**, 1 x 340 µl **Solution B**
10 x 100 µl optimized competent cells (TZ101 alpha)

Storage

Solution A: +4 °C, protect from light

Solution B: -20 °C

Competent cells: -80 °C

Important notes

- **Solution A** might be unstable if frozen or if exposed to light for longer time. Also the mixture of Solution A with B might be instable if frozen and thawed.
- **Solution A and B** must be combined to obtain the final reaction mixture just before use. The final reaction mixture could be stored for 2 days at -20 °C (protected from light).
- **Solution B** contains T4 DNA ligase, therefore thaw Solution B on ice and mix gently before use. Keep Solution B on ice after thawing.
- Although **Solution B** can be thawed and frozen repeatedly, aliquotation of **Solution B** is recommended after the first thawing step.
- Protect **Solution A** and the final reaction mixture from light and repeated freezing.
- Use chemical competent cells for transformation. To use electro-competent cells, the compounds from the reaction mixture must be removed from the DNA by purification or precipitation (e.g. with CentriSpin columns from Genaxxon: S5301).

Only Alligator™ with competent cells:

- Thaw competent cells rapidly and keep them on ice only for a limited time. Prevent the cells from higher temperatures than 4 °C until the heat shock procedure.
- Perform the heat shock procedure exactly at 42 °C for 1 min 20 sec; use 1,5 ml reaction tubes.

Genotype TZ101 alpha: *F'/endA1 hsdR17 glnV44 thi-1recA1 gyrA recA1 Δ(lacIZYA-argF) U169 deoR(φ80dlacΔ(lacZ)M15)*

Transformation efficiency: >1 x10(8) cfu/µg
blue/white screening possible

Note: For research and in vitro use only. Not for use in diagnostics or therapeutics. Contains harmful chemicals.

Procedure for preparing ready-to-use Alligator™

1. Prepare the reaction mixture by combining **Solution A** and **Solution B**: (final reaction mixture could be stored for 2 days at -20 °C).

number of reactions	Volume: Solution A	Volume: Solution B
5	6 µl	54 µl
10	12 µl	108 µl

2. Combine the DNA fragments to be ligated in a total volume of 10 µl at room temperature. Use water or TE buffer (10 mM Tris-HCl,pH8,0,1 mM EDTA) as solvent. Recommended amounts for vector – insert ligations are the following ratios : vector to insert = 0,03 pmol to 0,1 – 0,3 pmol.
3. Add 12 µl of Alligator™ reaction mixture ((see step 1) and mix thoroughly.
4. Incubate at room temperature (about 20 °C) for 1 min. If desired, the incubation time can be extended to 30 min.
5. The ligation mixture can be stored on ice, at -20 °C or used directly for bacterial transformation.
IMPORTANT: Do not heat inactivate the T4 DNA ligase.

Only Alligator™ with competent cells:

6. Thaw competent cells rapidly just before use and keep them always on ice.
7. Add 5 -22 µl of the ligation mixture to 100 µl competent cells and mix carefully. Use 1,5 ml reaction tubes.
8. Incubate on ice for 15 min.
9. Transfer mixture with cells rapidly to 42 °C, incubate exactly for 1 min 20 sec.
IMPORTANT: Do not use higher temperatures or prolonged incubation times and use 1,5 ml reaction tubes.
10. Place mixture back on ice for 3 min.
11. Plate mixture on selection plate (only for ampicillin).

IMPORTANT: Only plasmid vectors with bla as selection marker (ampicillin resistance) can be plated without phenotypic expression. For optimal results, we recommend phenotypic expression for these plasmids as well.

12. **Alternatively to 11!** Add 300 µl SOC medium and perform phenotypic expression with aeration at 37 °C for 30 min to 1 h and plate on appropriate selection plate.

SOC Medium 2% Tryptone 10 mM MgCl₂
 0.5% Yeast Extract 10 mM MgSO₄
 10 mM NaCl 2.5 mM KCl
 20 mM Glucose