

Genaxxon bioscience QuickClone Kit

**For fast cloning of DNA fragments from different origin,
incl. PCR DNA with or without TA overhangs**

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Product	Cat#	Package size
QuickClone Kit	M3457.0010	10 reactions

Abbreviations

L, mL, µL	Litres, milli litres, micro litres respectively
g, µg, ng	grams, micro grams, nano grams respectively
LB	Luria Bertani medium
X-Gal	5-Brom-4-Chlor-3-indolyl-β-D-galactopyranoside
TE	Tris-EDTA (10 mM Tris-HCl, pH8.1, 1 mM EDTA)
Amp	Ampicillin
RT	Room temperature (18°C – 24°C)

Related products / overview

• Genaxxon JustSpin Gel Extraction Columns	S5337
• Genaxxon SpinClean Spin Columns	S5304
• Genaxxon pMBL T/A Cloning Kit	M3164
• Genaxxon <i>Taq</i> DNA Polymerase	M3001
• Genaxxon Insert Inspector	M3458
• Genaxxon chemically competent cells <i>E. coli</i> TZ101α	M3434
• Genaxxon chemically competent cells <i>E. coli</i> TZ102α	M3435
• Genaxxon Alligator	M3430
• Genaxxon T4 DNA Ligase	M3027

Notes on warranties and disclaimer

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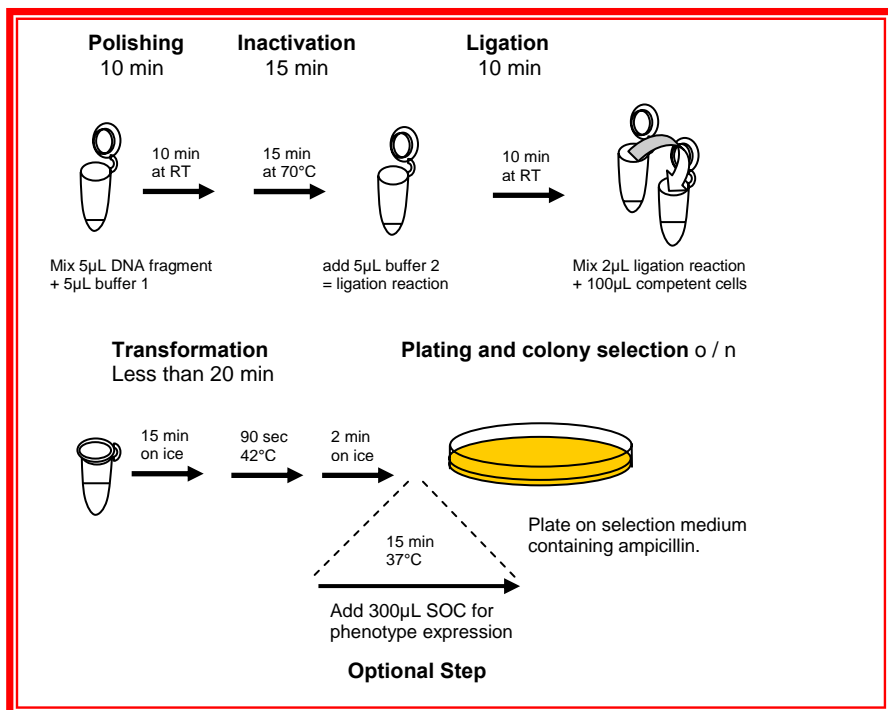
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Protocol overview



Kit Contents & Storage Information

Item	Amount	Storage
Buffer 1	50µL (10 reactions)	- 20°C
Buffer 2	50µL (10 reactions)	- 20°C
<i>E.coli</i> TZ101α competent cells	10x100µL (10 transformations)	- 80°C

Expected results

Depending on the quality and size of the DNA fragment, varying numbers of potentially positive white clones will grow, typically more than 50% of total transformants. They contain inserts of the fragment in clockwise or counter clockwise orientation.

Positive clones may be identified by isolation of the plasmid DNA followed by restriction analysis. For more convenient checking of your results, Genaxxon bioscience offers a colony screening kit:

- Genaxxon Insert Inspector (M3458)

Trouble shooting guide

No white colonies

- Use more DNA fragment (up to 300ng).
- Make sure to purify the insert DNA (PCR buffer inhibits buffer 1).
- Some cloned inserts might interfere with cellular functions of *E. coli*. To recover such clones, try the incubation of the plates at RT or 28°C rather than at 37°C.
- Plate out more cells to make sure inserts of both orientations are recovered since sometimes one orientation of the insert works better than the other.
- Increase ligation time to 30 min.
- Sometimes small inserts may not disrupt the reading frame of β-galactosidase and therefore clones remain blue or light blue.

White colonies do not contain (correct) inserts

- Since smaller fragments are inserted much easier than larger ones, make sure to purify the fragment by gel electrophoresis. Thus, short DNA molecules like primer dimers are removed.
- Make sure to inactivate reaction by heat before adding buffer 2.

Only few colonies

- Make sure to keep competent cells on ice all time prior to heat shock.
- Do not increase ampicillin concentration above 100µg/mL.
- Perform the phenotype expression step.
- Use a larger amount of DNA for transformation.

Applications

- Downstream mutagenesis
- Subcloning
- Sequencing
- "Back-up" for cloning experiments with "uncertain" results

Procedure

1. Purification: OPTIONAL STEP

Purify the DNA fragment of interest by agarose gel electrophoresis, excise the band of interest and extract DNA by means of a suitable kit.

2. Preparations

Thaw buffer 1 and buffer 2 on ice. Heat a heating block or water bath to 70°C.

3. Polishing reaction

Add 5µL purified DNA fragment (dissolved in TE) to 5µL buffer 1. Vortex briefly. Incubate for 10min at RT. **Avoid prolonged incubation.** Inactivate the reaction by placing tubes for 15min into a heating block at 70°C. Collect condensed droplets from the lid by brief centrifugation (10 sec).

4. Ligation reaction

Place tube on ice.
Add 5µL of buffer 2. Vortex briefly.
Incubate for 10 min at RT.

5. Preparation of competent cells

Thaw required amount of competent cells on ice. Keep them always on ice.

6. Set-up of transformation reaction

After 10min reaction time, transfer 2 µl of each ligation reaction to competent cells. Mix carefully by flicking the tube (the remaining of the ligation reaction may be stored at -20 °C for reference). Incubate mixture with cells for 15min on ice.

7. Transformation

Cool down the heating block to 42°C.
Transfer tubes from ice to heating block.
Incubate at 42°C exactly for 90 sec, then place back on ice for 2min.

8. Phenotype expression: OPTIONAL STEP

Add 300µL SOC to the transformation.
Incubate at 37°C for 30min (this incubation provokes expression of β-lactamase).
It may be omitted at the expense of reduced transformation efficiency (total colony number), however, in most cases a sufficient number of recombinant clones are recovered.

9. Growing recombinant bacteria

Plate 100µL of mixture (with or without phenotype expression) onto LB agar plates supplemented with 100mg/L ampicillin and 40mg/L X-Gal.
Grow overnight at 37°C.

10. Results

Pick putative recombinants (white colonies).
Analyse their plasmid DNA.
(See also "Expected results" on page 6.)

Description

The Genaxxon bioscience QuickClone Kit was developed to allow cloning of DNA fragments from various origins (sticky ends, PCR-products with or without TA overhangs, etc.) in a two-step reaction that takes less than 60min including transformation.

The kit comprises two buffers:

Buffer 1 renders all DNA termini blunt and phosphorylated.

Buffer 2 mediates the insertion of the fragment (now blunt ended) into a suitable vector, completely prepared and included in buffer 2.

Buffer 1 and buffer 2 are ready-to-use mixes.

This kit is supplied with optimized competent cells allowing high-efficiency transformation of the ligated DNA fragments.

Background information

Buffer 1 (Polishing Buffer)

Buffer 1 mediates the polishing of DNA fragments. Ends of PCR products (e. g. all 3' A-overhangs produced by *Taq* polymerase) are rendered blunt. Non-blunt restriction sites are polished as well. Polishing is achieved either by filling of 5' overhangs or by removal of 3' overhangs. 5' ends are phosphorylated. All reagents are premixed.

Buffer 2 (Ligation Buffer)

Buffer 2 mediates the insertion of the DNA fragment into vector pAlli10. All reagents are premixed.

E.coli TZ101α competent cells:

Genotype:

F/endA1 hsdR17 glnV44 thi-1recA1 gyrA recA1Δ(lacIZYA-argF) U169 deoR(Φ80dlacΔ(lacZ)M15)

E.coli TZ101α is made chemically competent to allow easy and reliable transformation of the ligation reaction at an efficiency of at least 10⁸ cfu / µg plasmid DNA. The strain allows blue/white screening.

The vector pAlli10

pAlli10 is supplied predigested and dephosphorylated. The *bla* gene encodes β-lactamase which confers resistance to ampicillin. A multiple cloning site (MCS) – into which the fragment is ligated - is located within a 5'-terminal fragment of the *lacZ*-gene (encoding β-galactosidase) downstream of the *lac* promoter. This allows α-complementation if a suitable strain like *E.coli* TZ101α (provided with the kit) is used.

Using the following standard pair of primers, sequencing of the fragment within the plasmid is easily possible:

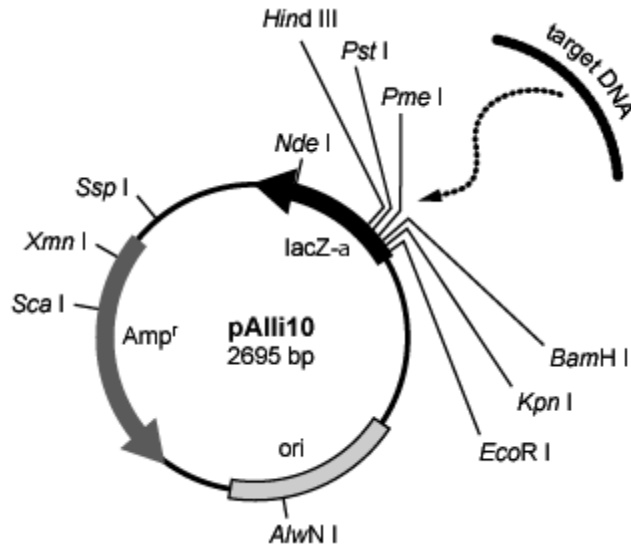
M13 fp: 5'-GTAAAACGACGGCCAG-3'

M13 rp: 5'-CAGGAAACAGCTATGAC-3'

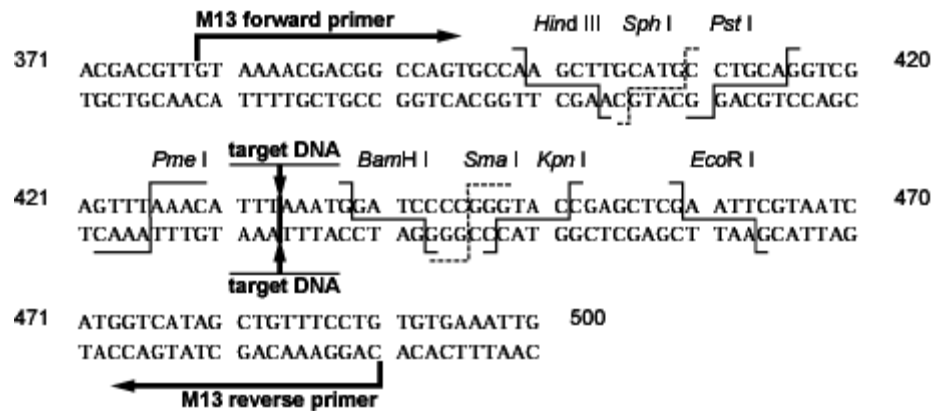
See the following page for a detailed map of pAlli10. The complete sequence of pAlli10 is provided for download on our homepage.

Map of vector pAlli10

The locations of genes are indicated by arrows. Selected unique restriction endonucleases sites are shown.



Multiple Cloning Site of vector pAlli10



Before you start: Helpful hints for optimum results

DNA fragment: Make sure that you use an ample amount of high-quality, purified DNA.

The outcome of the cloning procedure largely depends on the quality of the DNA fragment used. We recommend to perform size selection by purifying the DNA by agarose gel electrophoresis followed by extraction of the fragment using a suitable kit, e.g.

- Genaxxon DNA Gelextraction Kit S5344.0050
- Genaxxon JustSpin Gel Extraction Columns S5337.0050
- Genaxxon SpinClean Spin Columns S5304.0100

In the case of cloning a PCR fragment, the gel electrophoresis step may be omitted – but the PCR product should be purified using a suitable kit, e.g.

- Genaxxon PCR Clean-up Kit S5345.0050
- Genaxxon PSI Clone 96 HTS PCR purification Kit S5303.2196

Since many methods for clean-up of PCR fragments do not remove unspecific products like primer dimers, these small products could also be integrated in the vector pAlli10 during the cloning step and may produce false positive (white) clones with small inserts.

Make sure to use sufficient amounts of DNA (> 50 ng; if the size of the fragments is large, use more). The smaller the fragment, the easier it is inserted.

Buffers: Keep buffers on ice. Use buffer aliquots and avoid numerous freeze-thaw cycles.

To the best of our knowledge, both buffers are stable over many freeze-thaw cycles if the solutions are kept on ice. However, we recommend to limit the number of steps to as few as possible (up to 5).

Laboratory equipment

- heating block or water bath (range 37°C – 70°C)
- vortexer
- bench top centrifuge
- equipment for agarose gel electrophoresis
- gel extraction kit
- wet ice
- incubator for petri dishes (37°C)

Media

SOC (per L)

Dissolve 20g tryptone, 0.58g NaCl, 5 g yeast extract in desalted water and autoclave.

Cool down to 50°C.

Add sterile MgCl₂ and MgSO₄ solution to a final concentration of 10 mM each,

KCl to a final concentration of 2.5mM and

glucose to a final concentration of 20mM.

LB plates with ampicillin and X-Gal (per L)

Dissolve 10g tryptone, 5g NaCl, 5 g yeast extract, 18g agar in desalted water and autoclave.

Cool down to 50°C approximately.

Add 100 mg ampicillin (1000x stock solution in H₂O) and

40mg X-Gal (1000x stock solution in dimethyl-formamide).