Bolt PCR Cloning Kit

Bolt Cloning

ATUM SM (formerly DNA2.0) has developed a simple, fast, and efficient method for cloning PCR amplified products into a plasmid vector in a 5-minute bench-top reaction. This cloning kit contains an optimized cloning mix with a linearized vector that allows cloning of any PCR product amplified with proofreading DNA polymerases (not compatible with *Taq* polymerase).

Kit Components (20 Rx)

Linearized pJ201 (kan^R) in BKT-01 **or pJ204 (amp^R) vector** in BKT-02 is supplied as a 10X mix, to be diluted to 1X in the final reaction mix (10X mix contains 20ng/µl linearized vector DNA and topoisomerase).

Positive Control is a PCR amplicon of KringleYFP linked to a tet promoter, 25 ng/ µl, 864 base pairs, which can be cloned in either orientation.

Sequencing Primers: 1 µM stock**,** use 1 µl / sequencing reaction

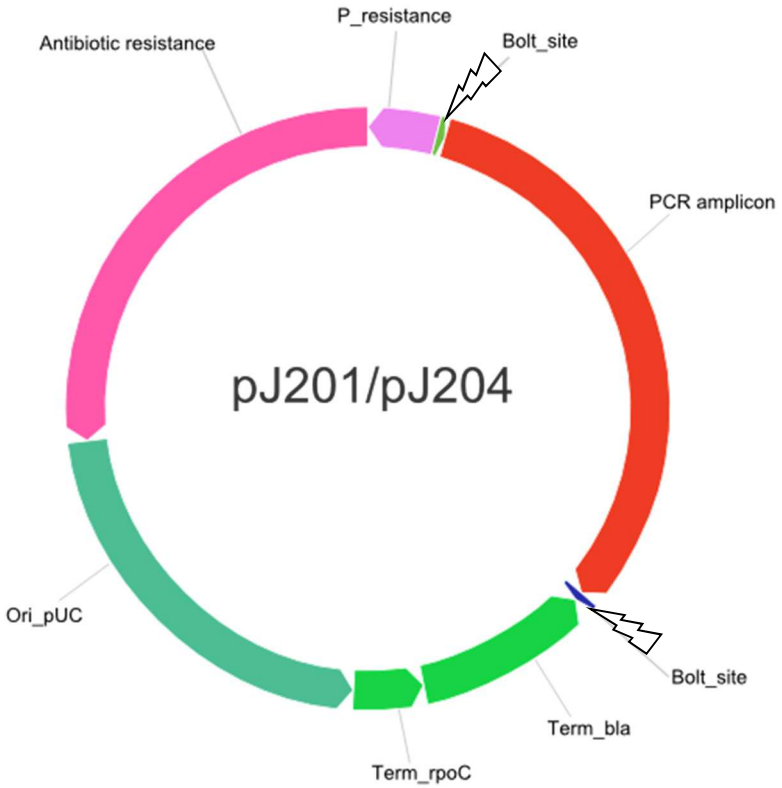
Forward primer: 5'- TGGTAGTGTGGGGACTC-3'

Reverse primer: 5'- TTGTCAGAATATTTAAGGGCG-3'

Storage

Store all kit components at -20°C.

Vector map



Cloning Information

To PCR your ORF: Bolt vectors do not contain multiple cloning sites (MCS), so flanking restriction sites must be incorporated in your PCR primers for easy subcloning into a vector of choice.

Note: Include 5 additional bases outside of your flanking restriction sites by adding 5 bp to each 5' end of the PCR primers. This will accommodate potential end deletions.

Bolt reaction is set up as follows:

COMPONENT	VOLUME (µl)
PCR product (~100ng)/Control	4
Linearized Bolt Vector (20ng) mix	1
Sterile ddH_2O	5
Total Volume	10

1. Combine components as listed above in single 1.5 ml tube. Incubate at room temperature for 5-20 minutes. For best results, pre-equilibrate PCR reaction to room temperature for 5 minutes prior to adding to vector.

2. Transform 2.5 µl of each reaction into 50 µl competent cells. Heat shock if appropriate, and then add 500 µl SOC Broth. Shake for 1 hour at 37C.

3. Plate 100 µl on LB + selection antibiotic – pJ201 on LB agar + 30 µg/ml kanamycin; pJ204 on LB agar + 100 µg/ml ampicillin or carbenicillin.

4. Incubate plates overnight at 37°C. Pick transformants.

Restriction enzymes that do not cut in pJ201:

AarI, AatII, Acc65I, AccI, AclI, AfeI, AgeI, AleI, AloI, ApaBI, AscI, AvrII, BaeI, BamHI, Bbr7I, BbsI, BbvCI, BcgI, BclI, BglII, BlpI, BmgBI, BmtI, BplI, BpmI, BsaAI, BsaBI, BsaI, BsaXI, BseRI, BsgI, BsiWI, BsmBI, BsmI, BspEI, BspMI, BsrDI, BsrGI, BstAPI, BstBI, BstEII, BstXI, BstZ17I, Bsu36I, BtgI, ClaI, DraIII, EagI, EarI, EcoICRI, EcoRI, FalI, FseI, FspAI, FspI, HindIII, HpaI, KpnI, MfeI, MluI, MscI, NaeI, NcoI, NdeI, NgoMIV, NheI, NotI, PacI, PfoI, PmeI, PmlI, PpiI, PpuMI, PshAI, PsrI, PstI, PvuII, RsrII, SacI, SacII, SalI, SanDI, SapI, SbfI, ScaI, SciI, SexAI, SfiI, SgrAI, SnaBI, SpeI, SphI, Sse232I, Sse8647I, StuI, StyI, SwaI, TatI, Tth111I, XcmI, XhoI, XmnI, ZraI

Restriction enzymes that do not cut in pJ204:

AarI, AatII, Acc65I, AccI, AgeI, AleI, AloI, ApaBI, AscI, AsiSI, AvrII, BaeI, BamHI, Bbr7I, BbsI, BbvCI, BclI, BglII, BlpI, BmgBI, BmtI, BplI, BpmI, Bpu10I, BsaAI, BsaBI, BsaI, BsaXI, BseRI, BsgI, BsiWI, BsmBI, BsmI, BspMI, BsrDI, BsrGI, BstAPI, BstBI, BstEII, BstXI, BstZ17I, Bsu36I, BtgI, ClaI, DraIII, EagI, EarI, EcoICRI, EcoNI, EcoRI, FalI, FseI, FspAI, HindIII, HpaI, KpnI, MfeI, MluI, MscI, NaeI, NcoI, NdeI, NgoMIV, NheI, NotI, PacI, PflMI, PfoI, PmeI, PmlI, PpuMI, PshAI, PsrI, PstI, PvuII, RsrII, SacI, SacII, SalI, SanDI, SapI, SbfI, SciI, SexAI, SfiI, SgrAI, SnaBI, SpeI, SphI, Sse232I, Sse8647I, StuI, StyI, SwaI, Tth111I, XcmI, XhoI, ZraI

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