Certificate of Analysis

Project ID: U8141BJ070-10

Construct Information:

Gene Name: Cyclin D3；2_pDEST 22

Clone ID: M66564 **Gene Length:** 1185 bp

Cloning Vector: pDEST 22 **Cloning Strategy:** Gateway

QC Items	Specifications	Results	
Sequencing Alignment	Sequencing results are consistent with the targeted insert sequence.	Pass	Consistent
Vector Sequence	The flanking sequences of the cloning site are correct.	Pass	Correct Shown in the SQD file
Restriction Digests	The size of inserted fragment is correct and free of unexpected bands suggesting contamination.	Pass	Correct Shown in attachment 1
DNA Quality	Miniprep: 4 μg OD260/280=1.8~2.0 Free of contamination	Pass	⩾ 4 μg OD260/280=1.82 Pure
Quality grade	Research Grade	Pass	Research Grade
Appearance	Clear and free of foreign particles.	Pass	Clear Free of foreign particles
Additional Test		N/A	

NOTE

Shipping at	Plasmid Storing at	Bacstab Storing at	Glycerol Stock Storing at
Room Temperature	-20℃	4℃	-20℃/-80℃

Morgan

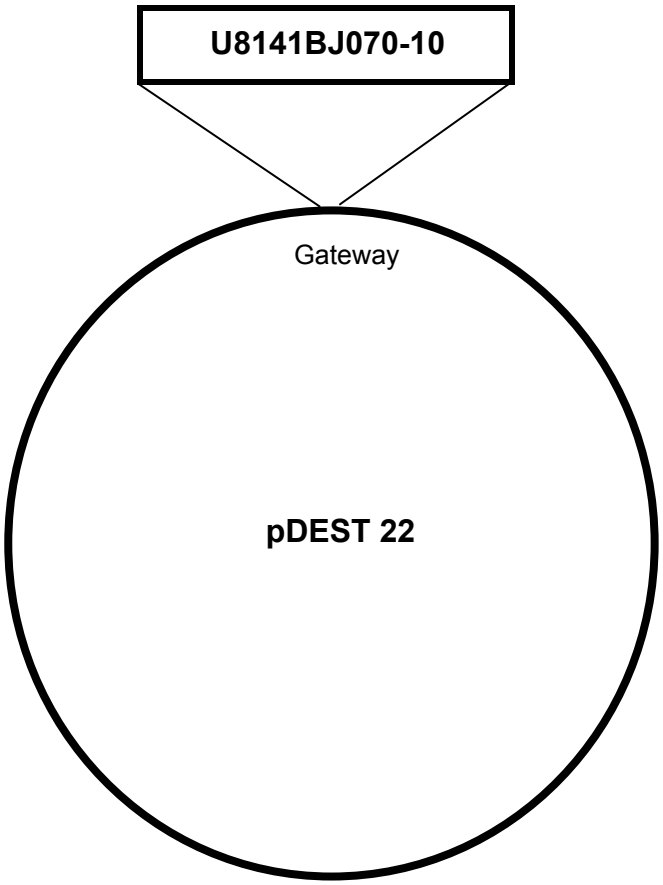
Certified by: **Date: 11-11-2016**

Thank you for your patronage to our Gene services! To maintain this working relationship, we shall be grateful if you can add our webpage URL into your lab website. As a token of appreciation, you will be rewarded by 1,000 EZcoupon™ points. For more information, please contact us by e-mail at web@genscript.com.

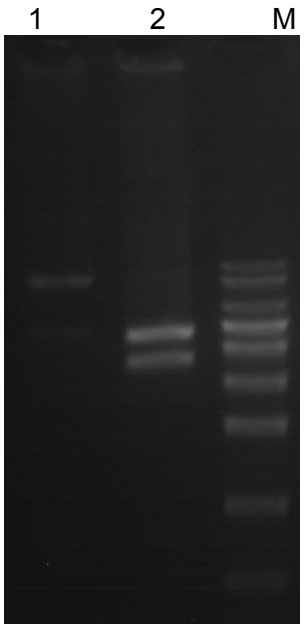
Attachment 1

Plasmid Construct Map

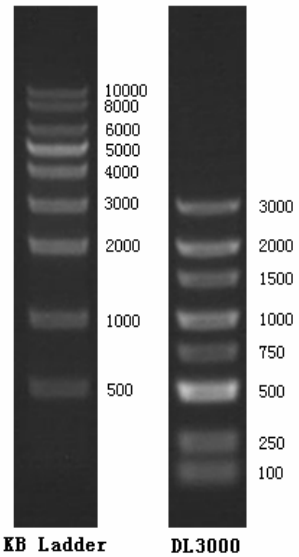
The gene was cloned in pDEST 22 by Gateway.



Enzyme Digestion



Lane M: KB Ladder
Lane 1:U8141BJ070-10 plasmid
Lane 2:U8141BJ070-10 plasmid digested by KpnI and BamHI



Digestion Conditions:
About 300ng plasmid digested
Digestion in water-bath,37℃ for 40 minutes
1% Agarose Gel

Attachment 2

FAQ

1. Q: What options does GenScript offer for gene deliverables?

A: Our standard deliverable is 4 µg lyophilized plasmid containing the gene of interest. An extra cost will apply if a bacstab or glycerol stock is requested. It is not recommended to directly use the 4 µg plasmid for cell transfection or mammalian protein expression. However, we can work with you on an additional plasmid preparation to fulfill such needs. We offer different grade of plasmid, eg. research grade, transfection grade, to suit your project.

2. Q: How to store gene deliverables, including lyophilized plasmid, bacstab and glycerol stock?

A: Lyophilized plasmid is stable under -20°C for at least one year, bacstab stable at 4°C for one week and glycerol stock at -80°C for several years.
For some of our gene library deliverables, liquid plasmid and/or PCR products need to be stored at -20°C and avoid repeated freezing and thawing.

3. Q: What kind of bacterial strain is used in gene synthesis service, and how should it be handled?

A: Generally, we use E.coli TOP10 strain for most of our gene synthesis services. Whilst for special cases, we may apply Stbl3, EPI400 or EPI300 strains. The information of what competent cell type used in the service can be shared with our customer along with the corresponding bacstab or glycerol stock.
Growth conditions to handle different cell type:

Top10, growth in LB overnight at 37°C
Stbl3, growth in LB for 20~24h at 30°C
EPI400/EPI300, the protocol is as below.

a) Add 4 ml LB media into each test tube. Inoculate each tube with bacterial culture with antibiotic at the proper concentration.
b) Incubate the tubes at 37°C, shaking overnight.
c) Dilute the starting culture (from step b)1:10 into antibiotic supplemented fresh media.
d) Supplement induction solution with a ratio of 1:1000, grow the culture at 37°C for 4h with vigorous shaking (approx. 250 rpm).
e) Isolate DNA from the induced culture cells as per protocol provided.

4. Q: How should I amplify plasmid DNA from a bacstab or glycerol stock received from GenScript?

A:For bacstab or glycerol stock, we suggest the following steps:
a)streak bacteria on the plate
b) isolate a single colony
c) inoculate it in LB containing suitable antibiotic
d) plasmid isolation as usual

5. Q: Is it possible to share some helpful tips on handling the plasmid?

A: In order to avoid any loss from the cap of the vial, the following is recommended:

a) Briefly centrifuge the tube
b) Open the cap and add 40µl of TE buffer/ddH_2O, tap gently
c) Do a second round of centrifugation to bring down contents

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Attachment 2

FAQ

6. Q: How do you typically transform plasmids?

A: Our plasmid transformation protocol is as follows:

a) Fetch one tube (size of 1.5ml) containing 50µl competent cells (stored at -80°C) and thaw it on ice for 20-30 min.
b) In the meanwhile dissolve the lyophilized 4µg plasmid with sterilized water to make 100ng/µl .
c) Mix 2~3µl of plasmid with the competent cells by tapping the tube.
d) Place the tube on ice for 12-15 min.
e) Heat shock the tube by placing two-thirds of it into a 42°C water bath for 120 seconds.
f) Return the tube back on ice for 2 min.
g) Add 800µl LB and shake in a 37°C incubator (200rpm) for 40min.
h) Plate some or all of the transformation mixture onto a LB agar plate containing antibiotic.
i) Incubate plates at 37°C overnight.

7. Q: Do you have any suggestion on enzyme digestion upon receiving the plasmid?

A: The restriction enzymes we used in the COA file may not be present at the two ends of your insert. Basically, we use one or two unique and/or common restriction enzymes to identify and confirm that the insert has been successfully cloned in the vector of choice. Customer can specify the restriction enzymes to be used during QC while placing the order.

8. Q: Sometimes the enzymes I choose do not digest the plasmid. Why?

A: A: If the recognition site is preceded by GA and followed by TC, or constitutes a CCWGG sequence (methylation site), you may not be able to digest the plasmid. Hence, it is recommended to avoid, if possible, enzymes such as: XbaI(TCTAGA), ClaI(ATCGAT), ApaI(GGGCCC), AvaII(GGWCC), SfiI(GGCCNNNNNGGCC), StuI(AGGCCT).
However, if you are unable to avoid using these enzymes on the methylated cloning sites in your plasmid, you may use a dam deficient strain such as GM2163 (E4105S), ER2925 or JM110 to amplify the DNA before digestion. You are requested to inform us of this while placing the order.

9. Q: Why are some extra bases are added at cloning sites? Do these bases interfere with any downstream applications?

A: Protective bases are added outside the insert to facilitate cloning and normally should not interfere with your subcloning projects. For any special cloning manipulations,customers are requested to inform us, so we shall be able to work together to determine an ideal strategy.

10. Q: If I have any questions about your service (product/data), how can I contact you?

A: You may contact our account managers, sales managers or project managers by phone, fax or email with any questions or concerns you have.Our customer representatives are available 24 hours, Monday to Friday, to assist you.

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