

ExactORF cDNA Clones

Application Guide

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Package Contents and Storage Conditions

The following components are included:

One (1) vial containing the cDNA clone as 10 ug lyophilized plasmid DNA. Store at -20°C.
One (1) USB-Flash drive containing .ab1 DNA sequence chromatograms, consensus sequence file and vector map.
Certificate of Analysis
Application Guide

The cDNA clone is shipped at room temperature, but should be kept at -20°C for long-term storage. If properly stored, clones are guaranteed to be stable for 12 months.

Optional Reagents

Transfection Reagent (Turbofectin 8.0; OriGene)
Restriction enzymes and buffer
Nuclease free water
T4 ligase and buffer
Competent *E. coli* cells
LB agar + ampicillin plates
LB broth (10 g/L Tryptone, 5 g/L Yeast Extract, 10 g/L NaCl Adjust pH to 7.0 with 1 N NaOH and autoclave)
Antibiotic selection media (Ampicillin or Kanamycin)
DNA purification reagents

Related Products

HuSH™ shRNA Plasmids	http://www.origene.com/rna/
Validated Antibodies	http://www.origene.com/antibody/
Functional Proteins	http://www.origene.com/protein/
Transfection Reagents	http://www.origene.com/cdna/turbofectin.msp
GFC-Transfection Arrays	http://www.origene.com/cdna/gfc-array/default.msp
Precision Shuttle System**	http://www.origene.com/cdna/trueorf/destinationvector.msp

** If the clone you received was in an OriGene Precision Shuttle vector and you wish to move the open reading frame into a new destination vector, please follow the instructions in the TrueORF manual, which is provided in the technical support section of OriGene's website: http://www.origene.com/support/product/product_manuals.msp

Protocols for handling the DNA

OriGene provides 10ug of circular plasmid DNA purified via ion-exchange methods to give you high quality DNA for use in transfections, PCR amplification and most other molecular and cell biology procedures. However, should you require more DNA, you can always call OriGene to order more or you can transform the DNA into an appropriate host bacterium (DH5alpha) and grow as much culture as necessary to obtain the final desired amount of DNA.

Protocol for Plasmid DNA Recovery from 2D Barcode Tube

Carefully open the tube, and add 100 uL of sterile, deionized H₂O to the bottom of a tube containing 10 ug DNA. This volume will result in a DNA concentration of 100 ng/uL. Dissolving the DNA in a lower volume is not recommended as an increased final EDTA concentration may affect some downstream applications.

Close the tube and incubate for 10 minutes at room temperature, or 4°C overnight. After vortexing for 10 seconds, quickly spin down the contents of the tube.

For short-term storage (<1wk) place the DNA at 4°C, for any longer term place at -20°C.

Protocol for Plasmid DNA Transformation into E. coli

Add a portion of the DNA solution (~0.5 uL) to a mixture of competent cells and proceed with DNA transformation according to the manufacturer's directions.

Note: We recommend using competent cells with a minimum efficiency rating of 1×10^6 CFU/ug. When one-tenth of the transformation solution (0.5 uL plasmid DNA, 50 uL competent cells, and 950 uL SOC) is plated on an appropriate LB-agar/ampicillin (100 ug/mL) plate, one can obtain between 100 to 1000 colonies. Please note that due to the inherent nature of some plasmids, special handling may be required to obtain colonies (see Trouble-Shooting Guide).

After the transformed cells have been incubated overnight at 37°C on an LB-agar/ampicillin (100 ug/mL) plate, we recommend the evaluation of 2-5 independent colonies of varying sizes to avoid the isolation of a rare contaminant.

A single colony can be used to inoculate a growth culture for DNA purification/sequencing.

A single colony can be used to inoculate a growth culture for the creation of a glycerol stock.

A single colony can also be used for PCR amplification.

How do I use the cDNA clone?

The open reading frame cDNA fragment is present in the expression vector that was specified at the time of order. For more information on the vector's functionality and consequently the use of this plasmid, please read the application guide pertaining to the original vector, or if an OriGene vector, the information is available on the OriGene website at:

http://www.origene.com/support/product/product_manuals.msp

Production and Quality Assurance

The plasmid-based construct was created to the exact specification that was received by OriGene on the original submission form and was described on the confirmation quotation. Should there be a problem with the service, please contact our technical support department at techsupport@origene.com for resolution.

This project was an agreement for services indicated. There are no implied warranties beyond the specification of the end product. Cancellations of an order will result in a pro-rated partial payment invoice.

Troubleshooting (Transformation)

For questions not addressed here, please contact OriGene's Technical Support professionals. You may dial 888-267-4436 from any US location, or 301-340-3188 outside the US. E-mail inquiries to techsupport@origene.com are also invited.

No colonies or low number of colonies from transformation

Cause	Remedy
The competent cells used in the transformation were not as efficient as necessary.	Obtain a fresh batch of competent cells and ensure that the efficiency is $\geq 1 \times 10^8$ CFU/ μ g DNA by performing a separate transformation reaction with a transformation-qualified control (usually a fixed amount of supercoiled plasmid such as pUC19). In some extreme cases, especially for larger inserts (>5 kb), higher efficiency cells or electroporation may be required. Should a gene prove to be toxic to the cells, transforming into strains that reduce the copy number can increase the odds of obtaining colonies (i.e. ABLE-C or ABLE-K strains; Stratagene, La Jolla CA).
Too little DNA was used in the transformation reaction.	Add more DNA (but not more than 10% of the volume of competent cells used).

The purified DNA is not the correct clone

Cause	Remedy
A contaminant colony was picked into the media to grow a liquid culture for purification. On occasion, even the smallest amount of contaminant DNA can be amplified preferentially during this process due to a favorable growth bias.	Pick and analyze 2-5 colonies by mini-prep prior to committing to larger scale growth cultures. Sometimes the intended clone causes slower growth in E. coli and would produce a smaller colony in comparison. Choose colonies of various sizes.
Restriction Digest mapping results can be hard to interpret	Make sure there is complete cutting. Always run an uncut DNA control to assess this. Be sure all internal restriction sites have been accounted for, and make sure that the sites are also unique in the host vector. OriGene always recommends a 5'-end sequence read for confirmation as well.
The wrong antibiotic selection plate was used.	Make sure to use an LB-agar plate containing 100 ug/mL ampicillin or 35 ug/mL Kanamycin as appropriate for the host vector. A few clones are designated as "slow-growers" and will be given special growth conditions in the COA.

Frequently Asked Questions

Am I assured that the clone I receive will match the nucleotide sequence I entered?

Answer: Absolutely. We will use every technology at OriGene to make sure that the exact nucleotide sequence is created and verified.

What quality of sequence do you provide on these clones?

Answer: OriGene uses quality ABI reagents and the newest ABI3730 DNA sequencer. Both nucleotide strands across the insert and cloning junctions are covered by reads whose quality is checked using the PHRED algorithm.

What will I receive in terms of sequence confirmation?

Answer: All chromatograms, a consensus sequence file and a vector map will be included on a USB-Flash drive for easy upload to your computer.

How much DNA will I receive?

Answer: 10ug; lyophilized into the bottom of a Matrix-brand 2D bar-coded screw-cap tube. This should be plenty for most applications without the need to re-transform, select a colony and re-purify. You can also ask OriGene to provide you with more DNA at the time of order.

How much more does OriGene charge per bp for larger sizes or GC-richness?

Answer: There are no hidden surcharges with the ExactORF service. Many other providers need to charge for this to offset the costs they have using their technology. This is not the case at OriGene.

Why does it take so long to deliver?

Answer: As a custom service, specialized care in project design and quality assurance takes time. All clones are different and it is quite possible that you receive your clone ahead of what was predicted. However, we wish to over-deliver, and not over-sell. Please contact custsupport@origene.com for updates.

What vector will be used to clone my ORF?

Answer: You may select from any vector that OriGene offers from our PrecisionShuttle vector system. OriGene offers vectors to over-express your protein or domain in the native form or tagged with a host of fusion partners.

What will I receive in the box?

Answer: 10ug of the final plasmid DNA (ion-exchange grade for immediate transfection); all chromatograms in the .ab1 file format; a vector map; a certificate of analysis and Application Guide.

Can I dictate which restriction sites to use?

Answer: To maintain reading frames, OriGene PrecisionShuttle vectors are specially designed to use rare cutters. Your additional sites can be added but may result in additional coding amino acids or may introduce a frameshift. A trained consultant (contact OriGene technical support) will be happy to discuss your options.

Does OriGene guarantee the protein expression level from this construct?

Answer: No. There are too many variables with respect to transfection efficiency, host cells and growth conditions. However, our techsupport team will help offer suggestions. OriGene guarantees the correct sequence in the correct vector and cloned appropriately into the specified restriction sites.

Why is OriGene a preferred provider for synthetic clones?

Answer: OriGene remains "Your Gene Company" and as such it recognizes that the researcher's need for synthetic constructs is complementary to its world-leading business in supplying naturally occurring TrueClone cDNA transcripts. Its decade long immersion in the genetics field and its laboratories fully stocked with seasoned PhD-level molecular biologists, together make OriGene the right choice for experience and know-how.