DNA Resuspension and Storage Protocols

Twist Bioscience ships DNA products dried down or resuspended. This protocol provides guidance for the receipt, resuspension, and storage of single-stranded and double-stranded DNA that is supplied dried down. It also includes a rescue workflow for Clonal Genes that yield poorly in Twist's automated production environment (likely due to sequence toxicity to E. coli or metabolic burden), but which may perform differently under other laboratory conditions.

PROTOCOL COMPONENTS

STABILITY OF DRIED DOWN AND RESUSPENDED DNA			
PRODUCT	**PHYSICAL STATE**	**SHORT-TERM STORAGE**	**LONG-TERM STORAGE**
Clonal Genes Gene Fragments Multiplexed Gene Fragments Gene Pools Oligo Pools Cloned Oligo Pools Combinatorial Variant Libraries Combinatorial Assembly Libraries Site Variant Libraries SOLD Libraries	Dried down	3 months @ room temperature 12 months @ 4°C	24 months @ -20°C 24+ months @ -80°C
Clonal Genes Gene Fragments	Resuspended	3 months @ 4°C	12 months @ -20°C 24 months @ -20°C 24+ months @ -80°C

NOTES:

- Room temperature storage in water for more than one week is not recommended.
- Avoid repeated freeze/thaw cycles, which lead to the formation of precipitates.

HAVE QUESTIONS?
Email **customersupport@twistbioscience.com**

MATERIALS SUPPLIED BY USER

The following materials or their equivalent are required for DNA resuspension.

MATERIALS
REAGENTS AND CONSUMABLES
Resuspension solution (i.e. Elution buffer, TE buffer, Nuclease-free water, etc.) Consult your downstream application protocol for guidance
Protocol B only: LB agar plate and liquid LB with appropriate antibiotic resistance (see General Notes and Precautions section)
Protocol B only: Competent cells such as LacIq strains or CopyCutter cells
EQUIPMENT
Pipette(s) and pipette tips
Microcentrifuge or plate centrifuge, as applicable
Water bath or heat block set to 50 °C

PROTOCOL OVERVIEW

This document provides instructions for two use cases: Protocol A is for routine DNA resuspension, while Protocol B is for the resuspension and transformation of Clonal Genes with a yield of <10 ng.

PROTOCOL	HANDLING TWIST DNA
A	**DNA Resuspension**
B	**Low Yield Clonal Gene Resuspension & Transformation**

GENERAL NOTES AND PRECAUTIONS

Wear appropriate protective equipment (lab coat, gloves, and protective glasses or goggles) at all times when performing this protocol.

For best results, read this document before performing the protocol, and follow the instructions provided. Twist Bioscience cannot guarantee the performance of this workflow if modifications are made to the protocol.

Store light-sensitive antibiotic agar plates in foil to avoid loss of activity.

Recommended antibiotic concentrations for LB plates can be found in the table below:

ANTIBIOTIC	RECOMMENDED CONCENTRATION (mg/ml)	LIGHT SENSITIVE
Tetracycline	10	**YES**
Chloramphenicol	25	NO
Apramycin	50	NO
Kanamycin	50	NO
Zeocin	50	**YES**
Ampicillin	100	NO
Blasticidin	100	**YES**
Carbenicillin	100	NO
Spectinomycin	100	NO
Erythromycin	500	NO

PROTOCOL A: DNA RESUSPENSION

Reagents Required

- Dried down Twist DNA product
- Resuspension buffer or nuclease-free water
- Additional tubes or plates for aliquots (if desired)

A.1 Before opening, briefly spin down the tube or plate at 400 × g to collect material at the bottom.

A.2 Add resuspension buffer or nuclease-free water to each tube or well to achieve the desired final DNA concentration (see Appendix A for dilution calculation formula). Ensure that the buffer and final DNA concentration chosen are appropriate for your downstream application.
NOTE: Optimal buffer and concentration depend on downstream requirements.

A.3 Gently pipette the buffer along the tube or well walls to dislodge and resuspend any DNA that may be adhered to the surface.

A.4 **(Optional)** If the DNA does not fully dissolve after pipetting, or if you see visible material or particles in the tube or well, incubate in water bath or on heat block at 50 °C for 20 minutes to promote complete reconstitution.

A.5 If DNA will be used repeatedly over long periods of time, prepare separate aliquots to reduce the number of freeze/thaw cycles.
NOTE: Use or store working aliquots as soon as possible after preparation, and minimize exposure to temperatures above room temperature.

PROTOCOL B: LOW YIELD CLONAL GENE RESUSPENSION AND TRANSFORMATION

Lower than expected yields are often the result of sequences that encode for proteins toxic to our bacterial cell strains. After resuspension, the addition of competent cells followed by growth at lower temperatures can improve the likelihood of successfully transforming and recovering these challenging constructs.

Reagents Required

- Dried down Twist DNA product
- Resuspension buffer or nuclease-free water
- Competent cells for Transformation
- LB agar plate and liquid LB with appropriate antibiotic

DNA RESUSPENSION

B.1 Before opening, briefly spin down the tube or plate at 400 × g to collect material at the bottom.

B.2 Add 5 µL of resuspension buffer or nuclease-free water to each tube or well.

B.3 Gently pipette the buffer along the tube or well walls to dislodge and resuspend any DNA that may be adhered to the surface.

B.4 Incubate in water bath or on heat block at 50 °C for 20 minutes to promote complete reconstitution.

TRANSFORMATION

B.5 Add 2 µL of resuspended DNA to 50 µL of competent cells.
NOTE: If the protein is known to be toxic to E. coli, use a strain or cell line that regulates gene expression or plasmid copy number (e.g. LacIq strains or CopyCutter cells) and grow transformed cells at room temperature or 30 °C overnight.

B.6 For detailed transformation steps, refer to your competent cell transformation protocol.

APPENDIX A: DILUTION CALCULATION

The formula to calculate a dilution is (C1)(V1) = (C2)(V2)

C1 = concentration of the starting solution

V1 = volume of the starting solution

C2 = concentration of the final solution

V2 = volume of the final solution

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