Protocol for DNA Cleanup and Concentration Using the Monarch® PCR & DNA Cleanup Kit (5 µg) (NEB #T1030)

Date: 2025-10-06
Category: Protocols
Created by: Elisabeth Grönert

1.Dilute sample with DNA Cleanup Binding Buffer (ensure that isopropanol has been added, as indicated on the bottle label)* according to the table below. Mix well by pipetting up and down or flicking the tube. Do not vortex. A starting sample volume of 20–100 µl is recommended. For smaller samples, TE can be used to adjust the volume. For diluted samples larger than 800 µl, load a portion of the sample, proceed with Step 2, and then repeat as necessary.

**Beginning in April 2021, the DNA Cleanup Binding Buffer will be changed to a concentrated format which requires the addition of isopropanol by the user. Please refer to the instructions inside of the product that you receive.*

SAMPLE TYPE	RATIO OF BINDING BUFFER: SAMPLE	EXAMPLE
dsDNA > 2 kb (plasmids, gDNA)	2:1	200 µl:100 µl
dsDNA < 2 kb (some amplicons, fragments)	5:1	500 µl:100 µl
ssDNA > 200 nt**	7:1	700 µl:100 µl

** *Please note that recovery of ssDNA < 200 nts can be increased by using the Oligonucleotide Cleanup Protocol, but doing so will shift the cutoff size for DNA binding to 18 nt (versus 50 nt).*

2.Insert column into collection tube and load sample onto column and close the cap. Spin for 1 minute, then discard flow-through. (To save time, spin for 30 seconds, instead of 1 minute.)
3.Re-insert column into collection tube. Add 200 µl DNA Wash Buffer and spin for 1 minute. Discarding flow-through is optional.

4.Repeat wash (Step 3).

5.Transfer column to a clean 1.5 ml microfuge tube. Use care to ensure that the tip of the column does not come into contact with the flow-through. If in doubt, re-spin for 1 minute to ensure traces of salt and ethanol are not carried over to next step.

6.Add ≥ 6 µl of DNA Elution Buffer to the center of the matrix. Wait for 1 minute, then spin for 1 minute to elute DNA.

Attached file

T1030_Quick_Protocol_Card_Monarch_PCRDNA_Cleanup.pdf
sha256: e7472e3237a849cbd6f6297c850cbfe1dbfb80a6b5447655879f7dfaf399c9f5

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Monarch® PCR & DNA Cleanup Kit (5 µg) Protocol Card

NEB #T1030

For a detailed protocol or to download the full manual, visit www.neb.com/T1030.

BEFORE YOU BEGIN:

- **IMPORTANT UPDATE:** Add 0.36 volumes of isopropanol to one volume of DNA Cleanup Binding Buffer (e.g., 63.5 ml isopropanol to 175 ml buffer)
- Add 4 volumes of ≥ 95% ethanol to one volume of DNA Wash Buffer (e.g., 100 ml ethanol to 25 ml buffer)
- All centrifugation steps should be carried out at 16,000 x *g* (~13,000 RPM)
- If working with DNA fragments ≥ 10 kb, preheat the appropriate amount of DNA Elution Buffer to 50°C

THERE ARE TWO PROTOCOLS AVAILABLE FOR THIS PRODUCT:

- **DNA Cleanup and Concentration:** for the purification of up to 5 µg of DNA (ssDNA > 200 nt and dsDNA > 50 bp) from PCR and other enzymatic reactions
- **Oligonucleotide Cleanup:** for the purification of up to 5 µg of DNA fragments ≥ 15 bp (dsDNA) or ≥ 18 nt (ssDNA). Expected recovery is > 70%. When purifying ssDNA of any size, recovery can be increased by using this protocol; however, it is important to note that this protocol shifts the cutoff for smaller fragments to 18 nt (rather than 50 nt for the DNA Cleanup and Concentration Protocol).

DNA CLEANUP AND CONCENTRATION PROTOCOL STEPS:

1. **Dilute sample with DNA Cleanup Binding Buffer (ensure that isopropanol has been added, as indicated on the bottle label) according to the table below. Mix well by pipetting up and down or flicking the tube. Do not vortex.** We recommend a sample volume of 20-100 µl. For smaller samples, adjust the volume with TE. For diluted samples larger than 800 µl, load a portion of the sample, proceed with step 2, and repeat as necessary.

SAMPLE TYPE	RATIO OF BINDING BUFFER: SAMPLE	EXAMPLE
dsDNA > 2 kb (plasmids, gDNA)	2:1	200 µl: 100 µl
dsDNA < 2 kb (some amplicons, fragments)	5:1	500 µl: 100 µl
ssDNA* > 200 nt	7:1	700 µl: 100 µl

**Please note that recovery of ssDNA < 200 nts can be increased by using the Oligonucleotide Cleanup Protocol, but doing so will shift the cutoff size for DNA binding to 18 nt (versus 50 nt).*

2. **Insert column into collection tube and load sample onto column. Spin for 1 minute, then discard flow-through.**

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3. **Re-insert column into collection tube. Add 200 µl DNA Wash Buffer and spin for 1 minute.** Discarding flow-through is optional.

4. **Repeat step 3.**

5. **Transfer column to a clean 1.5 ml microfuge tube.** Use care to ensure that the tip of the column does not come into contact with the flow-through. If in doubt, re-spin for 1 minute.

6. **Add ≥ 6 µl of DNA Elution Buffer to the center of the matrix. Wait for 1 minute, then spin for 1 minute to elute DNA.** Typical elution volumes are 6-20 µl. Nuclease-free water (pH 7-8.5) can also be used to elute the DNA. Yield may slightly increase if a larger volume of DNA Elution Buffer is used, but the DNA will be less concentrated. For larger size DNA (≥ 10 kb), heating the elution buffer to 50°C prior to use can improve yield.

Want to use this kit to purify DNA from agarose gels?
Simply purchase the Monarch Gel Dissolving Buffer (NEB #T1021L) and use with this kit. Protocol available at **www.neb.com/T1020**

Questions?
Our tech support scientists would be happy to help. Email us at **info@neb.com**

V4.1 – 05.21 #101031

OLIGONUCLEOTIDE CLEANUP PROTOCOL STEPS:

1. **Add 100 µl DNA Cleanup Binding Buffer (ensure that isopropanol has been added, as indicated on the bottle label) to the 50 µl sample.** We recommend a sample volume of 50 µl. For smaller samples, adjust the volume with nuclease-free water.

2. **Add 300 µl ethanol (≥ 95%). Mix well by pipetting up and down or flicking the tube. Do not vortex.**

3. **Insert column into collection tube and load sample onto column. Spin for 1 minute, then discard flow-through.**

4. **Re-insert column into collection tube. Add 500 µl DNA Wash Buffer and spin for 1 minute.** Discard flow-through.

5. **(Optional) Repeat step 4.** This second wash step is optional, but recommended for removal of enzymes that may interfere with downstream applications (e.g., Proteinase K). Please note that if carrying out a second wash step, additional DNA Wash Buffer may be required.

6. **Transfer column to a clean 1.5 ml microfuge tube.** Use care to ensure that the tip of the column does not come into contact with the flow-through. If in doubt, re-spin for 1 minute.

7. **Add ≥ 6 µl of DNA Elution Buffer to the center of the matrix. Wait for 1 minute, then spin for 1 minute to elute DNA.** Typical elution volumes are 6-20 µl. Nuclease-free water (pH 7-8.5) can also be used to elute the DNA. Yield may slightly increase if a larger volume of DNA Elution Buffer is used, but the DNA will be less concentrated.

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Created by: Elisabeth Grönert

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Golden Gate Assembly Protocol for NEBridge® Ligase Master Mix (NEB #M1100)

Date: 2025-10-06
Category: Protocols
Created by: Elisabeth Grönert

1. Based on assembly complexity, determine reaction component volumes (Table 1). The volume of Type IIS restriction enzyme (x µl) can be found in Table 2.

Table 1: Assembly Component Amounts

COMPONENTS	2 FRAGMENT OR 3–6 FRAGMENT ASSEMBLY	7+ FRAGMENT
NEBridge Ligase Master Mix	5 µl	10 µl
DNA Fragments*	0.05 pmol each	0.05 pmol each
Type IIS Restriction Enzyme	x µl	x µl
Nuclease-free Water	y µl	y µl
Total Reaction Volume	**15 µl**	**30 µl**

** Use NEBiocalculator® to calculate the mass of each DNA fragment*

Table 2: Suggested Type IIS Restriction Enzyme Amounts

ENZYME	2 FRAGMENT ASSEMBLY	3–6 FRAGMENT ASSEMBLY	>7+ FRAGMENT ASSEMBLY
BbsI-HF	1 µl (20 U)	1 µl (20 U)	1 µl (50 U)*
BsaI-HFv2	1 µl (20 U)	1 µl (20 U)	1 µl (20 U)
BsmBI-v2	3 µl (30 U)	3 µl (30 U)	6 µl (60 U)
Esp3I	2 µl (20 U)	3 µl (30 U)	4 µl (40 U)
PaqCI**	1 µl (10 U)	1 µl (10 U)	2.5 µl (25 U)
SapI	1 µl (10 U)	1 µl (10 U)	2 µl (20 U)
BspQI	1 µl (10 U)	1 µl (10 U)	2 µl (20 U)
BspQI-HF	1 µl (10 U)	1 µl (10 U)	2 µl (20 U)

** Use BbsI-HF (NEB #R3539M) (50 U/µl).*

*** Requires PaqCI activator (20 µM), 0.5 µl for 2 and 3–6 fragment assembly; 1.25 µl for 7+ fragment assembly.*

2. Set up a reaction in a microcentrifuge tube on ice. Mix DNA fragments (0.05 pmol of each) with nuclease-free water (y µl).

3. Add NEBridge Ligase Master Mix (5 µl or 10 µl) to DNA fragments and water. Gently mix by pipetting 3 times.

4. Add Type IIS restriction enzyme (x µl). Gently mix by pipetting 5 times.

5. Incubate for the recommended time and temperature (see Table 3).

Table 3: Suggested cycle times

	2 FRAGMENT ASSEMBLY		3-6 FRAGMENT ASSEMBLY	7+ FRAGMENT ASSEMBLY	
	SINGLE GENE CLONING	LIBRARY CONSTRUCTION		7-13 FRAGMENT	14+ FRAGMENT
BbsI-HF, BsaI-HFv2, BspQI-HF, Esp3I, PaqCI, SapI	37ºC for 15 min.	37ºC for 60 min.	30 cycles at 37ºC for 1 min. and 16ºC for 1 min.	30 cycles at 37ºC for 5 min. and 16ºC for 5 min.	60 cycles at 37ºC for 5 min. and 16ºC for 5 min.
BsmBI-v2, BspQI	15 cycles at 42ºC for 1 min. and 16ºC for 1 min.	30 cycles at 42ºC for 1 min. and 16ºC for 1 min.	30 cycles at 42ºC for 1 min. and 16ºC for 1 min.	30 cycles at 42ºC for 5 min. and 16ºC for 5 min.	60 cycles at 42ºC for 5 min. and 16ºC for 5 min.

6. End Soak: Incubate at 60°C for 5 minutes, before transformation.

7. Chill on ice.

8. Use 2 µl of the reaction to transform 50 µl of competent cells. If reaction will not be used immediately for transformation, store at -20°C.

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Cryopreservation of E. coli

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Created by: Elisabeth Grönert

1. Under sterile conditions, add 500 uL of culture to a sterile microcentrifuge tube/cryo tube
2. Add 500 uL of ~50% (v/v) glycerol
3. Invert tube a few times
4. Freeze at -80°C

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Annealing Primers

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Created by: Elisabeth Grönert

In a 1.5 mL or 2 mL microcentrifuge tube, mix together

43 uL MilliQ water

1 uL forward primer

1 uL reverse primer

5 uL Ligase Buffer (10x)

Heat at 85°C for 10 mins and allow to cool down to room temperature before further use.

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Inoculating Liquid Bacterial Culture (E. coli)

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Created by: Elisabeth Grönert

All to be done under sterile conditions

1. Add 3-5 ml LB to a sterile inoculation tube or sterile 15 ml falcon tube.
2. Add appropriate antibiotic (See table below)
3. With a 200 ul pipette tip, pick colony of choice by gently tipping it.
4. Drop the tip into the LB + antibiotic.
5. Loosely screw the lid onto the tube.
6. Incubate bacterial culture at 37°C for 12-18 hours in a shaking incubator.
7. After incubation, check for growth, which is characterized by a milky haze in the media.

Antibiotic concentrations for E. coli

Ampicillin	100 µg/mL
Bleocin	5 µg/mL
Carbenicillin	100 µg/mL
Chloramphenicol	25 µg/mL
Coumermycin	25 µg/mL
Gentamycin	10 µg/mL
Kanamycin	50 µg/mL
Spectinomycin	50 µg/mL
Tetracycline	10 µg/mL

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