PCR Amplification Protocol and Record

Verification PCR for pNX-303 Insert in *Escherichia coli* DH5α Transformants

Protocol ID: PCR-SOP-114
Revision: 3
Effective Date: 2026-07-29

Facility: Molecular Cloning Core
Location: Building 4, Room 214
Instrument: Nimbus GT-96 Thermocycler

Operator: Priya Nair
Reviewer: Dr. Marcus Webb
Run ID: TC-2026-0729-C

Sample Manifest 11 reactions (8 test, 1 NTC, 1 positive control, 1 spare)

Source plate: pNX-303 transformation plate TXP-0729-A, colonies picked 2026-07-29 09:40
Template prep: Single colony resuspended in 30 µL nuclease-free water, 5 µL used as boiled lysate template
Target amplicon: Insert-junction fragment, 612 bp (empty-vector religant control band 348 bp)

1 Purpose and Scope

This protocol defines the standard colony-PCR workflow used by the Molecular Cloning Core to verify insert presence in *Escherichia coli* DH5α colonies following transformation with the pNX-303 expression construct. The assay distinguishes recombinant colonies carrying the 612 bp insert-junction fragment from empty-vector religants, which instead yield a 348 bp product from the unoccupied multiple cloning site. It applies to all transformation screens processed under Nexa Biosciences cloning workflows NX-CL-01 through NX-CL-07 and is executed by trained laboratory staff following the equipment qualification described in NX-EQ-014.

This document serves a dual purpose: it is both the controlled procedure (Sections 2–5) and the executed record for Run TC-2026-0729-C (Sections 5–7), completed contemporaneously by the operator and countersigned by the reviewer in Section 7.

1.1 Principle

The polymerase chain reaction amplifies a defined DNA segment through repeated cycles of thermal denaturation, primer annealing, and polymerase-mediated extension, first described by Mullis and Faloona [1987] and established as a routine molecular biology tool following Saiki et al. [1988]. Two oligonucleotide primers (NX-303-F and NX-303-R) flank the insert–vector junction; their relative positions produce a diagnostic size shift between recombinant and non-recombinant templates, allowing colony genotype to be read directly from an agarose gel without plasmid extraction. Reaction assembly and thermocycling parameters below follow the general practice outlined in Green and Sambrook [2019] and Sambrook and Russell [2001], adapted to the Nimbus GT-96 thermocycler and the Vertex Taq 2X Master Mix used in this facility.

Record Note

Colony PCR uses whole cells (or a boiled lysate) directly as template. Cell debris and residual LB medium are tolerated by the Vertex Taq 2X Master Mix at the input volumes specified in Section 3, but template volume must not exceed 15% of the final reaction volume or amplification efficiency degrades measurably.

2 Materials, Reagents, and Equipment

2.1 Reagents

Reagent	Description	Lot No.	Expiry
Vertex Taq 2X Master Mix	Hot-start *Taq* polymerase, dNTPs, reaction buffer, loading dye	VMX-2291	2027-02-01
Primer NX-303-F (10 µM)	Forward primer, vector-side, binds pNX-303 backbone at nt 1180	OLG-6604-F	2027-04-15
Primer NX-303-R (10 µM)	Reverse primer, insert-side, binds target ORF at nt 402	OLG-6604-R	2027-04-15
Nuclease-free water	Molecular-biology grade, DNase/RNase-free	NFW-0157	2027-06-30
Positive control plasmid	pNX-303, verified by Sanger sequencing, 5 ng/µL	PLS-0303-V2	2026-12-01
100 bp DNA ladder	Nimbus LowRange, 100–1500 bp, 10 fragments	LDR-1004	2027-01-20
Agarose (molecular grade)	Standard-melt, for 1.5% gel	AGR-3390	2028-03-01
1X TAE running buffer	Prepared in-house from 50X stock, batch-dated	TAE-0729	2026-08-05
6X gel loading dye	Glycerol / bromophenol blue / xylene cyanol	GLD-2217	2027-05-11

2.2 Equipment

- Nimbus GT-96 thermocycler (calibrated 2026-06-02, cert. CAL-4471)
- Apex horizontal gel electrophoresis tank, 10-well comb
- Meridian PowerBasic 300V power supply
- Blue-light transilluminator with amber filter
- Calibrated single-channel pipettes: 0.5–10 µL, 2–20 µL, 20–200 µL
- PCR strip tubes, 0.2 mL, thin-wall, optically clear cap
- Sterile filter tips (aerosol-barrier)
- Benchtop microcentrifuge, PCR-workstation UV hood

Caution

Prepare PCR master mix in the dedicated pre-PCR workstation only. Template addition and all post-amplification handling (gel loading, imaging) occur at physically separate benches to prevent amplicon carryover contamination. Never carry gloves, pipettes, or tube racks between zones.

3 Master Mix Calculation

Reaction volume is 20 µL per tube. Eleven reactions are run (8 test colonies, 1 no-template control, 1 positive control, 1 pipetting-loss spare); the master mix below is scaled for $n = 13$ (11 reactions + 2 overage) and excludes template, which is added individually to each tube after aliquoting.

Component	Stock / Final Conc.	Vol./rxn (µL)	Vol. ×13 (µL)
Vertex Taq 2X Master Mix	2X → 1X	10.0	130.0
Primer NX-303-F	10 µM → 0.4 µM	0.8	10.4
Primer NX-303-R	10 µM → 0.4 µM	0.8	10.4
Nuclease-free water	—	6.4	83.2
Master mix subtotal		**18.0**	**234.0**
Template (colony lysate / plasmid)	added individually per tube	2.0	—
Total reaction volume		**20.0**	

Dispensing order

1. Water
2. 2X Master Mix
3. Primer NX-303-F
4. Primer NX-303-R
5. Vortex 3 s, brief spin
6. Aliquot 18 µL per tube
7. Add 2 µL template last, per tube

Template inputs, this run

- Colonies 1–8: boiled lysate, 2 µL each
- NTC: nuclease-free water, 2 µL
- Positive control: pNX-303 plasmid @ 5 ng/µL, 2 µL
- Spare: reserved, no template added

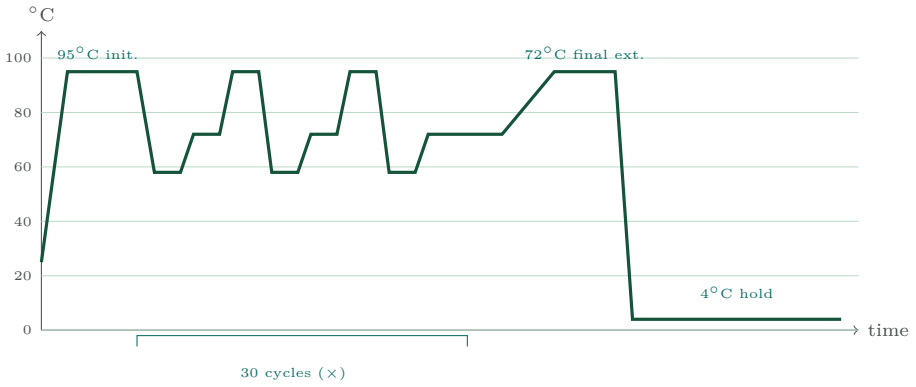
Record Note

Prepared master mix volume: 234 µL, dispensed as 18 µL into thirteen 0.2 mL strip tubes at 09:52. Two tubes (spare) held on ice, unused. Actual pipetted subtotal verified against target by visual meniscus check against a calibrated 250 µL reference tube — no deviation observed.

4 Thermocycler Program

Program `NX303_COLPCR` was loaded on the Nimbus GT-96, heated-lid set to 105°C, reaction volume 20 µL, ramp rate standard (3.0°C/s).

Step	Stage	Temp.	Time
1	Initial denaturation	95°C	5:00
2–4	Denaturation	95°C	0:30
	Annealing	58°C	0:30
	Extension	72°C	0:45
	Repeat steps 2–4 for 30 cycles		
5	Final extension	72°C	5:00
6	Hold	4°C	∞



Schematic temperature trace (not to time scale) — generated from run log `TC-2026-0729-C.log`.

Record Note

Run started 10:04, estimated duration 1 h 52 min. Lid temperature confirmed reached 105°C at 00:38 before block ramp began. Actual completion logged 11:58; block held at 4°C until retrieval at 12:15 (2:17 hold, within the ≤4 h post-run hold limit for this chemistry).

5 Procedure

5.1 Colony Pick and Template Preparation

1. Using a sterile 10 µL pipette tip, touch 8 well-isolated, single colonies from transformation plate TXP-0729-A. Streak each tip briefly onto a numbered master patch plate for archival, then eject the tip into 30 µL nuclease-free water in a labelled 0.2 mL tube.

2. Vortex briefly and heat lysates at 95°C for 8 minutes in a benchtop heat block to release template DNA and inactivate nucleases. Cool on ice for 2 minutes before use.

3. Label 13 PCR strip tubes: `1-8` (colonies), `NTC`, `PC` (positive control), `S1-S2` (spares). Keep on a chilled rack throughout setup.

5.2 Reaction Assembly

4. Prepare master mix per Section 3 in the pre-PCR workstation. Vortex 3 s and centrifuge briefly to collect contents.

5. Aliquot 18 µL master mix into each of the 13 tubes.

6. Move tubes to the template-addition bench. Add 2 µL of the appropriate template (boiled lysate, water for NTC, or diluted plasmid for PC) to each tube, changing tips between every sample. Cap tubes immediately after each addition.

7. Centrifuge the strip briefly (5 s pulse-spin) to collect contents at the tube base and eliminate air bubbles.

5.3 Amplification and Post-Run Handling

8 Load the strip into the Nimbus GT-96, close the heated lid, and start program `NX303_COLPCR` (Section 4). Record start time in the run log.

9 At completion, retrieve tubes promptly from the 4°C hold (within 4 hours) and proceed directly to gel loading, or store at 4°C short-term / −20°C for longer storage.

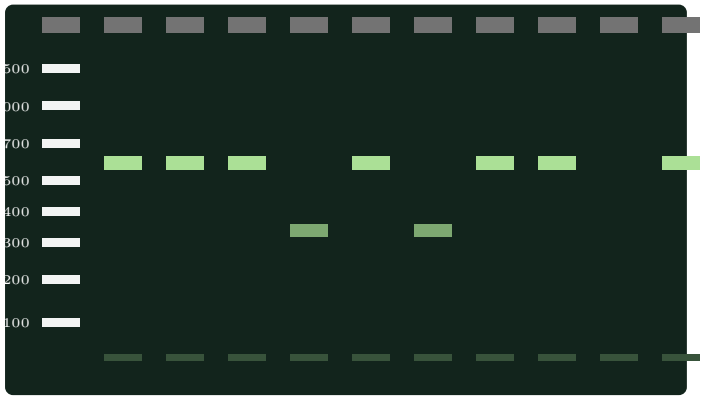
Caution

Do not leave amplified product at room temperature after the hold step — prolonged exposure promotes non-specific re-annealing artefacts on the gel that can be mistaken for additional bands.

6 Gel Electrophoresis and Results

6.1 Electrophoresis Conditions

Amplicons were resolved on a 1.5% agarose gel (AGR-3390) cast in 1X TAE, stained with intercalating dye pre-cast in the gel. 5 µL of each PCR product was mixed with 1 µL 6X loading dye and loaded alongside 4 µL Nimbus LowRange 100 bp ladder. Electrophoresis was run at 100 V (constant voltage) for 40 minutes on the Apex horizontal tank, then imaged on the blue-light transilluminator with an amber filter, following standard practice for nucleic-acid gel documentation [Lee et al., 2012].



Lanes: L = 100 bp ladder; 1–8 = colonies; N = no-template control; P = positive control. Image `GEL-2026-0729-C.tif`, contrast-normalised for print.

6.2 Lane Interpretation

Lane	Sample	Expected	Observed	Call
1	Colony 1	612 bp	612 bp	Insert present
2	Colony 2	612 bp	612 bp	Insert present
3	Colony 3	612 bp	612 bp	Insert present
4	Colony 4	612 bp	348 bp	Empty-vector religant
5	Colony 5	612 bp	612 bp	Insert present
6	Colony 6	612 bp	348 bp	Empty-vector religant
7	Colony 7	612 bp	612 bp	Insert present
8	Colony 8	612 bp	612 bp	Insert present
N	No-template control	no band	no band	Clean — no contamination
P	Positive control (pNX-303)	612 bp	612 bp	Assay valid

Record Note

6 of 8 screened colonies (75%) carry the pNX-303 insert junction. The no-template control shows no amplification, confirming absence of reagent contamination. The positive control reproduces the expected 612 bp product, satisfying the run-validity criterion in Section 7.1. Colonies 1, 2, 3, 5, 7, and 8 are approved for expansion and glycerol-stock banking under NX-CL-05.

7 QC Review, Deviations, and Approval

7.1 Run Validity Criteria

Criterion	Result
Positive control yields 612 bp product	Pass
No-template control shows no amplification	Pass
Ladder resolves all 8 fragments (100–1500 bp)	Pass
Thermocycler lid reached 105 °C before ramp start	Pass
Gel run completed within 40–50 min at 100 V	Pass (40 min)

7.2 Deviations

Record Note

One minor deviation: master mix preparation began at 09:52, 7 min behind the 09:45 schedule — the Vertex Taq 2X Master Mix needed extra thaw time from −20 °C storage. Fully thawed and mixed before use; no impact on results. Logged per NX-CL-01 §4.2.

7.3 Sample Disposition

Colonies 1, 2, 3, 5, 7, and 8 released to strain-banking (request GSR-0729-11). Colonies 4 and 6 discarded per NX-WD-02. Remaining PCR product and gel retained at 4 °C for 14 days, then discarded.

7.4 Approval

Role	Name	Signature	Date
Operator	Priya Nair, Research Associate II	_____	2026-07-29
Reviewer	Dr. Marcus Webb, Lab Manager	_____	_____
QA Sign-off	Dr. Elena Castillo, QA Officer	_____	_____

This record becomes part of the permanent laboratory notebook for project NX-CL-05 upon countersignature and may not be altered thereafter; corrections require a dated addendum per NX-QM-01.

References

Michael R. Green and Joseph Sambrook. Polymerase chain reaction. *Cold Spring Harbor Protocols*, 2019(6): pdb.top095117, 2019.

Pui Yan Lee, John Costumbrado, Chih-Yuan Hsu, and Yong Hoon Kim. Agarose gel electrophoresis for the separation of DNA fragments. *Journal of Visualized Experiments*, (62):e3923, 2012.

Kary B. Mullis and Fred A. Faloona. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Methods in Enzymology*, 155:335–350, 1987.

Randall K. Saiki, David H. Gelfand, Susanne Stoffel, Stephen J. Scharf, Russell Higuchi, Glenn T. Horn, Kary B. Mullis, and Henry A. Erlich. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*, 239(4839):487–491, 1988.

Joseph Sambrook and David W. Russell. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 3rd edition, 2001.