USER GUIDE

TaqMan®
life technologies™

TaqMan® 3C Chromosome Conformation Kits

FOR EARLY ACCESS SITES USE ONLY

Catalog Number 4466151 (EcoRI) and 4466152 (HindIII)

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life technologies™

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About This Guide

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Purpose

The TaqMan® 3C Chromosome Conformation Kits User Guide — Early Access provides:

- Detailed procedures for using the 3C Library Reagents to prepare 3C libraries and for using the 3C Human Control Assays to evaluate the quality of the 3C libraries
- Guidelines for designing 3C-qPCR assays and instructions for performing 3C-qPCR assays to validate DNA sequences that interact with the region of interest and to quantify the rates of interaction
- Troubleshooting information to help you resolve low digestion or ligation efficiencies or problems with 3C-qPCR assays

User attention words

Five user attention words may appear in this document. Each word implies a particular level of observation or action as described below:

Note: Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! Provides information that is necessary for proper instrument operation or accurate chemistry kit use.

CAUTION! Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING! Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

DANGER! Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

Product Information

Purpose of the product

Use the TaqMan® 3C Chromosome Conformation Kits (Catalog nos. 4466151 [EcoRI] and 4466152 [HindIII]) to perform chromosome conformation capture (3C) method to analyze the three-dimensional organization of chromosomes *in vivo* and to better understand how chromatin structure and DNA interactions regulate gene expression within cells. The TaqMan® 3C Chromosome Conformation Kits contain:

- Optimized reagents to prepare 3C DNA libraries
- Control qPCR assays to evaluate the quality of your 3C DNA libraries and to normalize input quantity

You can perform standard PCR or qPCR with the 3C DNA libraries to detect chromosomal interactions.

About 3C

Gene expression in cells is often regulated by the chromosomal structure, which is dynamic. The chromosomal structure can be changed by many factors, including the formation of protein-DNA complexes between loci on the same chromosome and between loci on different chromosomes. To analyze the frequency of interaction and proximity between any two genomic loci and their impact on gene expression, Dekker and colleagues developed the chromosome conformation capture (3C) method (Dekker *et al.*, 2002). This method "captures" or freezes the complexes to allow you to purify them and analyze the DNA elements that are in close proximity to better understand the structure and function of these complexes.

The 3C method involves the following steps:

1. Chromosomal proteins are crosslinked to interacting proteins and DNA to capture chromatin associations.
2. The crosslinked chromosomes are digested with a restriction enzyme that cuts within the DNA loci of interest.
3. The DNA strands for the two interacting loci are ligated together under conditions that favor the ligation of the intramolecular, cross-linked DNA fragments.
4. The protein-DNA and protein-protein crosslinks are reversed and the 3C DNA library is purified.

   The resulting 3C DNA library contains DNA fragments with interacting DNA sequences ligated together. The concentration of an individual ligation product is correlated to the frequency of looping between the two genomic regions.
5. Chromosomal interactions are detected by PCR using primers that are designed to amplify a possible interacting DNA target.

Types of studies possible using 3C

Studies using the 3C method have provided insights into gene expression control, cell differentiation, immune response, and association marker validation. Using this technique, researchers have been able to examine long-range chromatin interactions between promoters and regulatory elements at the β-globin locus (Tolhuis *et al.*, 2002; Palstra *et al.*, 2003; Vakoc *et al.*, 2005), the T-helper 2 cytokine locus (Spilianakis and Flavell, 2004), and the Igf2/H19 imprinted locus (Murrell *et al.*, 2004). Also, trans-interactions between functional regulatory elements on different chromosomes have been identified with the 3C method (Spilianakis *et al.*, 2005; Xu *et al.*, 2006).

Kit advantages

The TaqMan® 3C Chromosome Conformation Kits were developed to simplify and optimize the 3C workflow and to perform process controls:

- Quantitative controls – Easy-to-use qPCR control assays to measure the efficiencies of the restriction digestion and ligation steps
- Standardized conditions – Reproducible conditions and reagents translate to more efficient and controlled crosslinking, lysis, and restriction digestion
- Improved ligation – Optimized conditions allow for a reduced reaction volume that is easy to handle and promotes higher throughput
- Increased purity and yield – Rapid, nontoxic purification process improves DNA quality, increases DNA recovery by an order of magnitude, and improves reproducibility
- Time savings – Workflow time is reduced by ~50% compared to published protocols

These process improvements combined with our streamlined workflow mean that you can obtain more reliable chromatin interaction frequency data in less time.

Kit contents and storage

The TaqMan® 3C Chromosome Conformation Kits contain the following materials sufficient to prepare a total of ten 3C sample and/or control libraries:

- 3C Library Reagents – To generate 3C libraries of DNA fragments that contain interacting DNA sequences
- 3C Human Control Assays – To measure the efficiencies of the critical steps during the 3C library preparation and to normalize interaction frequencies

Table 1 Kit contents and storage for the TaqMan® 3C Chromosome Conformation Kit (EcoRI) (Catalog no. 4466151)

Box	Contents	Storage Conditions
3C Library Reagents (Ambient)	3C Quenching Solution 3C 1× PBS 3C Lysis Solution 3C RE Stop Solution 3C Neutralization Solution 3C DNA Binding Solution 3C DNA Wash Solution 3C Elution Solution 1 (ES1) 3C Elution Solution 2 (ES2)	Room temperature (RT, 18°C to 25°C)
	3C Magnetic Beads	4°C to 8°C
3C Library Reagents (–20°C)	3C Protease Inhibitor 3C 5× Ligation Buffer 3C DNA Ligase 3C Proteinase K 3C RNase A	–15°C to –25°C
3C EcoRI Human Control Assays	3C EcoRI Hu Assay 1 3C EcoRI Hu Assay 2 3C Hu Input Normalization Assay	–15°C to –25°C

Table 2 Kit contents and storage for the TaqMan® 3C Chromosome Conformation Kit (HindIII) (Catalog no. 4466152)

Box	Contents	Storage Conditions
3C Library Reagents (Ambient)	3C Quenching Solution 3C 1× PBS 3C Lysis Solution 3C RE Stop Solution 3C Neutralization Solution 3C DNA Binding Solution 3C DNA Wash Solution 3C Elution Solution 1 (ES1) 3C Elution Solution 2 (ES2)	Room temperature (RT, 18°C to 25°C)
	3C Magnetic Beads	4°C to 8°C
3C Library Reagents (–20°C)	3C Protease Inhibitor 3C 5× Ligation Buffer 3C DNA Ligase 3C Proteinase K 3C RNase A	–15°C to –25°C
3C HindIII Human Control Assays	3C HindIII Hu Assay 1 3C HindIII Hu Assay 2 3C Hu Input Normalization Assay	–15°C to –25°C

Materials and equipment required but not included

For the Safety Data Sheet (SDS) of any chemical not distributed by Life Technologies, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

Item	Source
Ambion® Non-Stick RNase-free Microfuge Tubes (2.0 mL)	Life Technologies (Cat. no. AM12475)
Applied Biosystems Real-Time PCR System	Life Technologies
Culture medium	Life Technologies
Magnet and rack: • DynaMag™-2 magnet (holds 16 tubes) • MagnaRack™ (holds 12 tubes)	Life Technologies • Cat. no. 123-21D • Cat. no. CS15000
MicroAmp® Optical Reaction Plates	Life Technologies
MicroAmp® Optical Adhesive Film	Life Technologies
TaqMan® Gene Expression Master Mix, 1 Mini-Pack (1 × 1 mL)	Life Technologies Cat. no. 4370048

Item	Source
Nuclease-free Water (not DEPC-treated) (1 × 100 mL)	Life Technologies Cat. no. AM9938
PBS, pH 7.4 (500 mL) **Note:** Additional 1X PBS is required if you are using adherent cells. You can also use the 1X PBS you are using to grow the cells.	Life Technologies Cat. no. 10010-023
Primer Express® Software v3.0, 1-user License	Life Technologies Cat. no. 4363991
Sequence Detection Primers 10,000 picomoles	Life Technologies Cat. no. 4304970
TaqMan® MGB Probe 6,000 pmol	Life Technologies Cat. no. 4316034
Centrifuge, refrigerated	Major laboratory suppliers (MLS)
Ethanol, 100%, molecular biology grade	MLS
Formaldehyde, 37%, molecular biology grade	MLS
Isopropanol, 100%, molecular biology grade	MLS
Microcentrifuge	MLS
Microcentrifuge tubes	MLS
Pipettors and pipette tips	MLS
Restriction enzyme of choice (100 U/µL recommended)	MLS
Platform shaker	MLS
Vortexer	MLS
Water baths at 16°C, 37°C, and 65°C	MLS

Methods

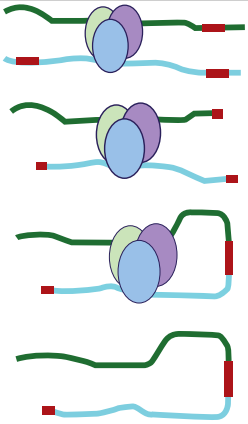
3C workflow

Design the 3C-qPCR assays (page 14)

Design the primers and probe (page 16)

Prepare 3C libraries (page 17)

1. Crosslink and lyse suspension cells (page 17)
   or
   Crosslink and lyse adherent cells (page 19)
2. Digest the DNA (page 21)
3. Ligate the DNA (page 22)
4. Decrosslink the DNA (page 22)
5. Purify the 3C library (page 23)



Evaluate the quality of the 3C libraries (page 26)

1. Perform qPCR using the 3C Human Control Assays (page 27)
2. Calculate digestion efficiency (page 28)
3. Calculate ligation efficiency (page 28)

Detect the interacting DNA target (page 28)

1. Perform 3C-qPCR (page 29)
2. Review the results (page 29)

Before you begin

PCR good laboratory practices

When preparing samples for PCR amplification:

- Wear clean gloves and a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation).
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation
  - PCR setup
  - PCR amplification
  - Analysis of PCR products
- Never bring amplified PCR products into the PCR setup area.
- Open and close all sample tubes carefully. Try not to splash or spray PCR samples.
- Keep reactions and components capped whenever possible.
- Use a positive-displacement pipettor or aerosol-resistant pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution.

Design the 3C-qPCR assays

About 3C-qPCR assays

Each 3C-qPCR assay contains:

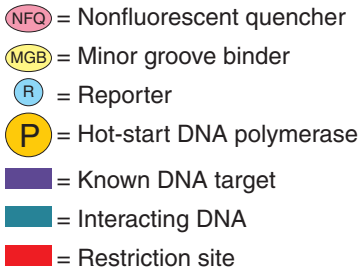
- Forward PCR primer complementary to the sequence for the known DNA target.
- TaqMan® MGB probe complementary to the sequence for the known DNA target. The sequence is downstream of the forward PCR primer. The TaqMan® MGB probe contains:
  - A reporter dye (for example, FAM™ dye) linked to the 5′ end of the probe.
  - A minor groove binder (MGB) at the 3′ end of the probe.

    The MGB increases the melting temperature (Tm) of the probe without increasing the length; it also allows for the design of shorter probes.
  - A nonfluorescent quencher (NFQ) at the 3′ end of the probe.

    Because the quencher does not fluoresce, Applied Biosystems real-time PCR systems can measure reporter dye contributions more accurately.
- Reverse PCR primer complementary to a possible interacting DNA target.

During PCR amplification, the 5′ nuclease assay process takes place. The 5′ nuclease assay process occurs in every cycle and does not interfere with the exponential accumulation of product.

Figure 1 Legend for 5′ nuclease assay figures



During PCR, the TaqMan MGB probe anneals specifically to a complementary sequence within the known DNA target sequence (Figure 2). When the probe is intact (Figure 2 and Figure 3), the proximity of the reporter dye to the quencher dye results in suppression of the reporter fluorescence, primarily by Förster-type energy transfer.

Figure 2 Polymerization

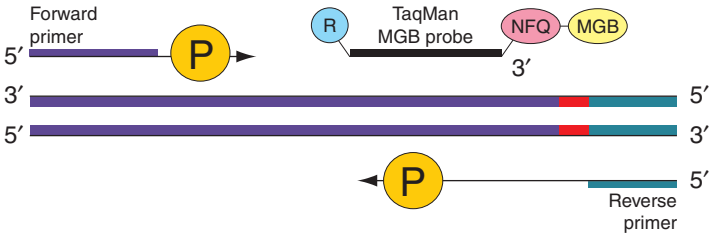
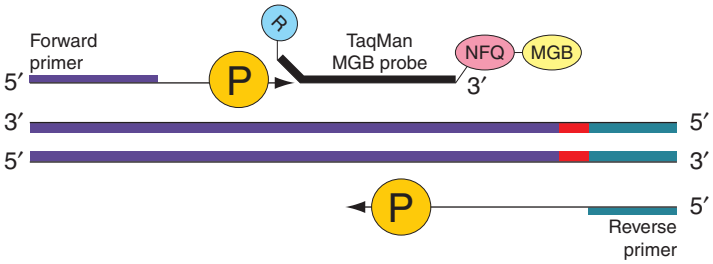
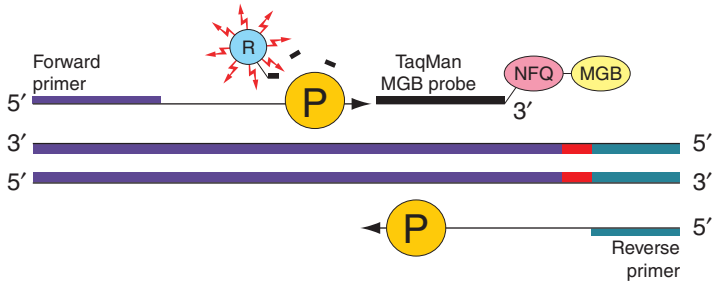


Figure 3 Strand displacement



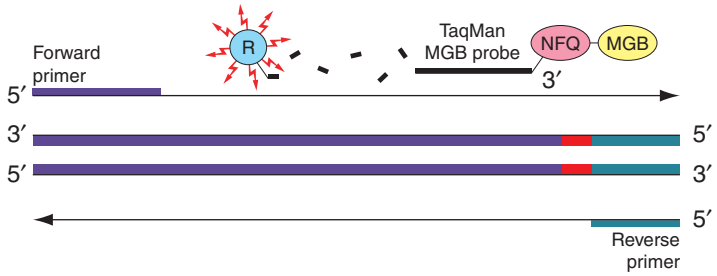
The DNA polymerase cleaves only probes that are hybridized to the known DNA target and if the reverse PCR primer binds to the same 3C library fragment. Cleavage separates the reporter dye from the quencher dye; the separation of the reporter dye from the quencher dye results in increased fluorescence by the reporter. The increase in fluorescence occurs only if the target is complementary to the probe and is amplified during PCR. Because of these requirements, nonspecific amplification is not detected.

Figure 4 Cleavage



Polymerization of the strand continues, but because the 3′ end of the probe is blocked, no extension of the probe occurs during PCR (Figure 5).

Figure 5 Completion of polymerization



Design the primers and probe

Using Primer Express® Software v3.0 or later, design forward and reverse PCR primers and a TaqMan probe to detect the 3C library fragments of interest. For instructions on how to order custom primers and TaqMan probes, go to **www.lifetechnologies.com**.

1. Design these assay components:

Assay component	Sequence guidelines
Forward PCR primer	Design one forward PCR primer for the known DNA target sequence. The primer should: • Hybridize to the known DNA target sequence • Be located close to the restriction site
TaqMan® MGB Probe	For 3C-qPCR reactions, design one probe for the known DNA target sequence. The probe should: • Hybridize to the known DNA target sequence, between the forward PCR primer and the restriction site • Be located close to the restriction site
Reverse PCR primers	Design one reverse PCR primer for each potential interacting DNA sequence. The primer should: • Hybridize to a potentially interacting DNA target sequence • Be located close to the restriction site

2. Design the primers so that they meet these guidelines:

Assay component	Sequence guidelines
Amplicon	Length is 60–150 basepairs
Primer length	~20 bases each
Tm	60–61°C (Optimal Tm is 60°C)
% GC content	30–80% GC content in each primer
3′ end	No more than 2 G+C residues in the last 5 nucleotides at the 3′ end
Repeating oligonucleotides	Avoid consecutive identical nucleotides. If you are unable to avoid consecutive identical nucleotides, make sure that each primer contains fewer than 4 consecutive Gs.

3. Go to **www.lifetechnologies.com**, then follow the instructions on the website to place your order for custom primers and TaqMan® probes.

Prepare 3C libraries

Use the 3C Library Reagents in the TaqMan® 3C Chromosome Conformation Kit to perform the Chromosome Conformation Capture (3C) method and generate 3C libraries of DNA fragments that interact with each other in the three-dimensional chromatin structure.

Starting cell input

The TaqMan® 3C Chromosome Conformation Kits can process samples containing 1×10^6 to 1×10^7 viable cells. Samples must contain the same starting cell input to perform direct comparisons between them.

You can crosslink and lyse suspension cells (page 17) or adherent cells (page 19).

Crosslink and lyse suspension cells

Crosslink suspension cells

Use formaldehyde (not included) on live cells to crosslink proteins to interacting proteins and DNA.

1. Pre-warm fresh culture media at 37°C, and pre-chill the 3C 1X PBS at 4°C.
2. Prepare the cells in the pre-warmed fresh culture medium:
   a. Transfer the cell suspension (1×10^6 to 1×10^7 cells) to a 15-mL conical tube.
   b. Spin at 200 × *g* for 5 minutes at room temperature.

      Note: Do not spin at speeds that exceed 200 × *g*.
   c. Remove the supernatant by aspirating or pipetting.

      Note: Do not pour off the supernatant.
   d. Add 500 µL of the pre-warmed 37°C fresh culture medium, then gently pipet up and down a few times to resuspend.
   e. Transfer the cell suspension to a 2-mL nonstick microcentrifuge tube.

3. In a fume hood, perform the crosslinking procedures:
    a. Add 14–28 µL of 37% formaldehyde (1–2% final concentration) to the 500 µL of prepared sample, invert to mix, then incubate on a shaker for 10 minutes at room temperature.

       Note: Most cells are crosslinked efficiently with 1–2% formaldehyde. For the cells that you are studying, you can further optimize crosslinking conditions if necessary.

    b. Add 200 µL of 3C Quenching Solution, invert to mix, then incubate for 5 minutes at room temperature.
    c. Place the sample on ice for 5 minutes.
4. Wash the cells at 4°C with the pre-chilled 3C 1✕ PBS:
    a. Spin the crosslinked cells at 500 × *g* for 3 minutes at 4°C.
    b. Place the tube on ice, then carefully remove the supernatant without disturbing the pellet.
    c. Add 500 µL of the pre-chilled 3C 1✕ PBS, then pipet up and down a few times to resuspend.
    d. Spin the cells at 500 × *g* for 3 minutes at 4°C.
    e. Place the tube on ice, then carefully remove the supernatant without disturbing the pellet.

STOPPING POINT (*Optional*) Store the pellet at –80°C before proceeding to cell lysis.

Lyse suspension cells

Lyse the cells using 3C Lysis Solution and add 3C Protease Inhibitor to keep the protein-DNA complexes intact.

1. Immediately before use, prepare the lysis cocktail (add 5% excess):

Component	Volume per sample
3C Lysis Solution (swirl gently to mix before use; do not vortex)	985 µL
3C Protease Inhibitor	15 µL
Total volume per sample	1 mL

   Note: The 3C Lysis Solution may appear cloudy when stored at the higher end of the room temperature range.

2. Add 1 mL of the lysis cocktail to the cell pellet, then vortex lightly to resuspend. Pipet up and down if necessary to ensure the pellet is thoroughly resuspended.
3. Incubate the sample for 5 minutes in a 65°C water bath.
4. Spin the lysate at 2000 × *g* for 5 minutes at room temperature.

5. Carefully remove all of the supernatant without disturbing the pellet.

   IMPORTANT! Pipet a second time or use finer pipette tips as needed to remove all of the supernatant. Repeat the spin if the pellet is disturbed or becomes resuspended.

6. Immediately before use, prepare for each sample 1.05–1.10 mL of 1X digestion buffer recommended for the restriction enzyme you are using.
7. Wash the cell lysate two times with 1X digestion buffer. For each wash:
   a. Add 500 µL of the freshly prepared 1X digestion buffer, then pipet up and down a few times to resuspend.
   b. Spin at 2000 × *g* for 5 minutes at room temperature.
   c. Carefully remove all of the supernatant without disturbing the pellet.

      IMPORTANT! Pipet a second time or use finer pipette tips as needed to remove all of the supernatant. Repeat the spin if the pellet is disturbed or becomes resuspended.

After the cells are crosslinked and lysed, digest the DNA (page 21).

Crosslink and lyse adherent cells

Volumes are provided for use with adherent cells in T-175, T-75, and T-25 flasks.

Crosslink adherent cells

Use formaldehyde (not included) on live cells to crosslink proteins to interacting proteins and DNA.

1. Pre-warm fresh culture media at 37°C, and pre-chill the 3C 1X PBS and/or 1X Culture PBS at 4°C.
2. When cells reach ~80% confluency, remove the medium and replace with pre-warmed culture medium:

Component	Volume per T-175 flask	Volume per T-75 flask	Volume per T-25 flask
Pre-warmed culture medium	20 mL	10 mL	5 mL

3. In a fume hood, add 37% formaldehyde (1–2% final concentration) to the flask, swirl to mix, then incubate 10 minutes at room temperature. Gently rock the flask a few times during the incubation.

Component	Volume per T-175 flask	Volume per T-75 flask	Volume per T-25 flask
37% formaldehyde (not included)	560 µL	280 µL	140 µL

4. Immediately before use, prepare the quenching cocktail:

Component	Volume per T-175 flask	Volume per T-75 flask	Volume per T-25 flask
3C Quenching Solution	200 µL	200 µL	200 µL
3C 1X PBS and/or additional 1X Culture PBS (not included)	10 mL	10 mL	5 mL
Total volume of quenching cocktail per flask	10.2 mL	10.2 mL	5.2 mL

5. Carefully aspirate to remove the media and formaldehyde without disturbing the cell layer.

6. Wash the cells with the quenching cocktail:

   a. Gently pipet the quenching cocktail over the cells, then gently swirl the cocktail over the cells a few times to wash.

   b. Carefully aspirate to remove the quenching cocktail without disturbing the cell layer.

Lyse adherent cells

Lyse the cells using 3C Lysis Solution and add 3C Protease Inhibitor to keep the protein-DNA complexes intact.

1. Add the lysis reagents to the crosslinked cells:

   a. Immediately before use, prepare the lysis cocktail (add 5% excess):

Component	Volume per T-175 flask	Volume per T-75 flask	Volume per T-25 flask
3C Lysis Solution (swirl gently to mix before use)	1970 µL	985 µL	985 µL
3C Protease Inhibitor	30 µL	15 µL	15 µL
Total volume of lysis cocktail per flask	2 mL	1 mL	1 mL
Total volume of sample per flask	2 × 1 mL	1 × 1 mL	1 × 1 mL

   Note: The 3C Lysis Solution may appear cloudy when stored at the higher end of the room temperature range.

   b. Add the lysis cocktail to each flask, then gently swirl the cocktail over the cells.

   c. Use a cell scraper to scrape cells from the flask, then transfer 1 mL of cells to a nonstick 2-mL tube.

   Note: If you are using a T-175 flask, prepare two 2-mL tubes with 1 mL of cells in each tube. You can use one tube to prepare the non-ligated control.

2. Lyse the cells:
   a. Incubate the sample for 5 minutes in a 65°C water bath.
   b. Spin the lysate at 2000 × *g* for 5 minutes at room temperature.
   c. Carefully remove all of the supernatant without disturbing the pellet.

   IMPORTANT! Pipet a second time or use finer pipette tips as needed to remove all of the supernatant. Repeat the spin if the pellet is disturbed or becomes resuspended.

3. Immediately before use, prepare for each sample 1.05 –1.10 mL of 1X digestion buffer recommended for the restriction enzyme you are using.
4. Wash the cell lysate two times with 1X digestion buffer. For each wash:
   a. Add 500 µL of the freshly prepared 1X digestion buffer, then pipet up and down a few times to resuspend.
   b. Spin at 2000 × *g* for 5 minutes at room temperature.
   c. Carefully remove all of the supernatant without disturbing the pellet.

   IMPORTANT! Pipet a second time or use finer pipette tips as needed to remove all of the supernatant. Repeat the spin if the pellet is disturbed or becomes resuspended.

STOPPING POINT (*Optional*) Store the pellet at –80°C before proceeding to DNA digestion.

After the cells are crosslinked and lysed, digest the DNA (page 21).

Guidelines for restriction enzyme selection

Select a restriction enzyme with a restriction site within the DNA target of interest.

However, to use the 3C Human Control Assays and to determine the digestion and ligation efficiencies, you must use the restriction enzyme that corresponds to the control assay in your kit: *Eco*RI or *Hind*III. The 3C Human Control Assays are designed to measure the digestion and ligation efficiencies for a particular restriction enzyme.

Digest the DNA

Digest the DNA with the restriction enzyme of choice (not included). Use 3C RE Stop Solution and 3C Neutralization Solution to stop the digestion.

1. Immediately before use, prepare a fresh dilution of 1X digestion buffer and digestion cocktail. For each sample, prepare:
   - 1X digestion buffer: 140 µL + 5–10% excess
   - Digestion cocktail (add 5% excess):
     – 140 µL of freshly diluted 1X digestion buffer
     – 400 U of the restriction enzyme of choice

   Note: Use the digestion buffer recommended for the restriction enzyme you are using.

2. Add the digestion cocktail to the cell pellet, pipet up and down a few times to resuspend, then incubate the sample in a water bath at 37°C for 2 hours to overnight.
3. Invert the tube to collect small droplets in the cap, spin the tube briefly, add 3 µL of 3C RE Stop Solution, vortex gently, then incubate at 65°C for 15 minutes.

   Note: If any sample sticks to the cap, spin down before proceeding.
4. Add 40 µL of 3C Neutralization Solution, then vortex gently.

Ligate the DNA

Ligate the digested DNA using 3C DNA Ligase. The ligation conditions must favor the ligation of the intramolecular, cross-linked DNA fragments to produce a high quality 3C library.

For non-ligated control samples, substitute 3C DNA Ligase with water.

1. Prepare the ligation cocktail (add 5% excess):

Component	Volume per sample
3C 5✕ Ligation Buffer	120 µL
3C DNA Ligase or water for non-ligated control samples	22.5 µL
Water	270 µL
Total volume per sample	412.5 µL

2. Add 412.5 µL of ligation cocktail to each sample, vortex gently, then incubate at 16°C for 1 hour.

Decrosslink the DNA

Add 3C Proteinase K and perform a 65°C incubation to degrade the proteins and to reverse the protein-protein and protein-DNA crosslinks.

1. Add 30 µL of 3C Proteinase K, vortex, then incubate at 65°C for 4 hours to overnight, until the solution is completely clear.
2. Incubate at 95°C for 5 minutes in a wet heat block.
3. Allow the sample to cool to room temperature, spin the tube briefly, add 5 µL of 3C RNase A, vortex gently, then incubate for 30 minutes in a 37°C water bath.

STOPPING POINT (*Optional*) Store the sample at 4°C overnight before purifying the DNA.

Purify the 3C library

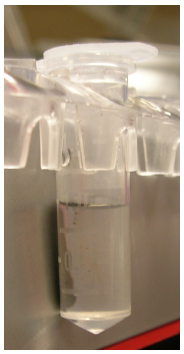
Use 3C Magnetic Beads, 3C DNA Wash Solution, 3C Elution Solution 1 (ES1), and 3C Elution Solution 2 (ES2) to purify the 3C library.

1. Before the first use, add 20.7 mL of 100% ethanol to the 3C DNA Wash Solution, mix well, then mark the bottle to indicate that you added the ethanol.
2. Bind the DNA to the 3C Magnetic Beads:
   a. Add 650 µL of 3C DNA Binding Solution, then invert to mix.
   b. Vortex gently and invert or swirl the tube of 3C Magnetic Beads until all beads are resuspended.
   c. Add 100 µL of 3C Magnetic Beads, make sure all beads are transferred to the sample, invert to mix, then place the tube in the rack for the magnetic stand (without the magnet).

      Note: Alternatively, place the tubes in a standard rack for microcentrifuge tubes.

   d. Place the rack on its side on a room temperature shaker, then incubate for 5 minutes.

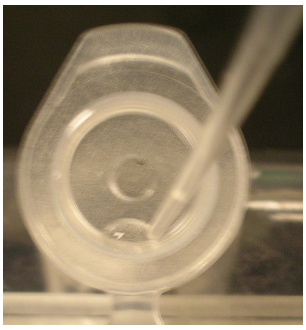
   e. Add 500 µL of 100% isopropanol, invert to mix, return the rack on its side on the room temperature shaker, then incubate for 5 minutes.
   f. Place the rack on the magnetic stand, then allow to stand for 15 minutes, or until the solution is clear.



g. Invert the tube and magnetic stand to collect sample from the tube cap, then allow to stand upright for a few minutes, or until the solution is clear.

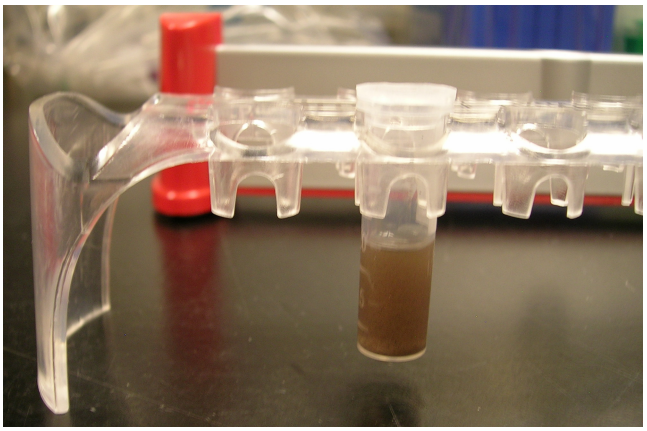
Sample in the tube cap	Invert the tube and magnetic stand to collect sample from the tube cap	Solution is clear

h. With the tube on the magnetic stand, carefully remove the supernatant without touching the pellet, and use a small pipette tip to remove droplets of liquid remaining in the cap.



3. Wash the beads two times with 3C DNA Wash Solution. For each wash:

   a. Move the tube away from the magnet, add 1 mL of 3C DNA Wash Solution, then mix the beads using a 1-mL pipettor until the beads are dissociated and there are no clumps.



b. Cap the tube, then invert up and down to expose the entire tube, including the cap, to the 3C DNA Wash Solution.

c. Return the tube to the magnetic stand, then allow to stand for 2 minutes, or until the solution is clear.

d. Invert the tube and magnetic stand to collect sample from the tube cap, then allow to stand upright for a few minutes, or until the solution is clear.

e. With the tube on the magnetic stand, carefully remove the supernatant without touching the pellet.

4. Remove all traces of 3C DNA Wash Solution from the cap using a small-volume pipettor, then air-dry for at least 30 minutes, or until the beads are completely dry.

5. Elute the DNA from the beads:

   Note: You may decrease the elution volume to increase the concentration of the purified 3C library. Use equal volumes of ES1 and ES2.

   a. Pre-warm the 3C Elution Solution 1 (ES1) at 65°C.

      Note: Keep the cap on the bottle when not in use to prevent evaporation.

   b. Add 200 µL of 65°C 3C Elution Solution 1 (ES1) to the pellet, pipet up and down to resuspend completely, then incubate at 65°C for 3 minutes.

   c. Add 200 µL of 3C Elution Solution 2 (ES2), then vortex gently.

   d. Place the tube on a magnetic stand, then allow to stand for 10 minutes to 1 hour, or until the solution is completely colorless.

6. With the tube on the magnetic stand, carefully transfer the DNA solution to a new tube, then store at 4°C.

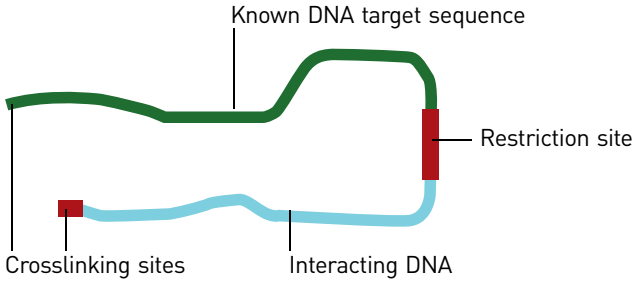
7. Discard the used magnetic beads.

8. Measure DNA concentration with a UV-Vis or NanoDrop® spectrophotometer.

Store the DNA at 4°C for short-term storage (less than 1 month) or –20°C for long-term storage.

About the resulting 3C libraries

The 3C libraries should contain DNA fragments that contain sequences from two different DNA targets that interact within the cell nucleus. The two DNA targets are ligated together at a restriction site for the enzyme you used to digest the DNA.



Evaluate the quality of the 3C libraries

Perform quantitative PCR (qPCR) using the 3C Human Control Assays in your TaqMan® 3C Chromosome Conformation Kit, then calculate the efficiency of the digestion and ligation steps to evaluate the quality of the 3C libraries.

Refer to the user documentation for your real-time PCR instrument for instructions on how to set up a qPCR run, run the instrument, and analyze the data.

Quantitative PCR guidelines

- Quantify and normalize sample concentrations with a spectrophotometer before performing quantitative PCR.
- We recommend the use of electronic repetitive dispensing pipettors to minimize variability.
- For each sample and non-ligated control sample, prepare 3 sets of control assays, two replicates for each set.
- Use the same amount of DNA template (in the range of 50 ng to 100 ng) in the Assay 1 and Assay 2 reactions.

Control assay reactions to set up

To calculate the digestion and ligation efficiencies, set up control assay reactions with non-ligated control 3C libraries and with the ligated sample 3C libraries.

Assay	Reactions to set up
3C Hu Assay 1	Non-ligated control
3C Hu Assay 2	• Non-ligated control • Ligated sample
3C Hu Input Normalization Assay	• Non-ligated control • Ligated sample

Perform qPCR using the 3C Human Control Assays

The 3C Human Control Assays are designed to measure efficiencies of the critical digestion and ligation steps when using the EcoRI or HindIII restriction enzyme. Use the 3C Human Control Assays designed for the restriction enzyme you used to digest the DNA.

1. Prepare the qPCR reactions using either the EcoRI or HindIII Human Control Assays:

Component	Volume per reaction
2X TaqMan® Gene Expression Master Mix	5 µL
One 20X Assay Mix: • 3C Hu Assay 1 • 3C Hu Assay 2 *or* • 3C Hu Input Normalization Assay	0.5 µL
50 ng to 100 ng DNA sample (1 µL to 4 µL) + Water (0.5 µL to 3.5 µL)	4.5 µL
Total volume per reaction	10.0 µL

2. Load and run the qPCR reactions on a real-time PCR instrument:

Stage	Step	Temperature	Time
Holding	Enzyme activation	95°C	10 minutes
Cycling (40 cycles)	Denature	95°C	15 seconds
	Anneal/extend	60°C	1 minute

3. Using the system software for your real-time PCR instrument, review the results:
   a. Review the amplification plots.
   b. Set the baseline and threshold values.
   c. Obtain the Ct values.

Calculate digestion efficiency

Calculate digestion efficiency using the Ct values from the Assay 1 and Assay 2 reactions with the non-ligated control.

1. Calculate the ΔCt value:

   ΔCt = (Ct from Assay 2 with non-ligated control) – (Ct from Assay 1 with non-ligated control)

2. Calculate the digestion efficiency using the ΔCt value:

Restriction enzyme	Formula
EcoRI	$\%\text{Digestion} = \frac{1}{1 + 10^{\left(\frac{\Delta Ct - 4.404}{3.28}\right)}}$
HindIII	$\%\text{Digestion} = \frac{1}{1 + 10^{\left(\frac{\Delta Ct - 5.66}{3.015}\right)}}$

To troubleshoot low digestion efficiency, see page 30.

Calculate ligation efficiency

Calculate ligation efficiency using the Ct values from Assay 2 and the 3C Hu Input Normalization Assay.

1. Calculate the ΔΔCt value:

   Ct_2 = Ct from Assay 2

   Ct_3 = Ct from 3C Hu Input Normalization Assay

   ΔΔCt = (Ct_2 – Ct_3 with ligated sample) – (Ct_2 – Ct_3 with non-ligated control)

2. Calculate the ligation efficiency using the ΔΔCt value:

$$\%\text{Ligation} = 1 - \frac{1}{2^{\Delta\Delta Ct}}$$

To troubleshoot low ligation efficiency, see page 30.

Detect the interacting DNA target

After you evaluate the quality of the 3C library, perform 3C-qPCR assays to identify the DNA targets that interact with the DNA region of interest.

Guidelines for the 3C-qPCR assays

Start with the reaction conditions and thermal cycling conditions that are provided, but if you do not detect amplification in any of your reactions, you can modify the conditions.

- Start with 50–100 ng of DNA sample, but you can add up to 400 ng of DNA sample to the reaction.
- Start with 40 cycles for qPCR, but you can run up to 50 cycles.

Perform 3C-qPCR

1. Prepare the qPCR reactions:

Component	Quantity per reaction
2X TaqMan® Gene Expression Master Mix	5 µL
Forward PCR primer (known DNA target)	900 nM
Reverse PCR primer (potential DNA target)	900 nM
TaqMan® MGB probe	250 nM
50 ng to 100 ng DNA sample (1 µL to 4 µL) + Water (0.5 µL to 3.5 µL)	4.5 µL
Total volume per reaction	10.0 µL

2. Load and run the qPCR reactions on a real-time PCR instrument:

Stage	Step	Temperature	Time
Holding	Enzyme activation	95°C	10 minutes
Cycling (40-50 cycles)	Denature	95°C	15 seconds
	Anneal/extend	60°C	1 minute

Review the results

Using the system software for your real-time PCR instrument, review the results for reactions that produced amplification:

1. Review the amplification plots.
2. Set the baseline and threshold values.
3. Obtain the Ct values.

About the 3C-qPCR results

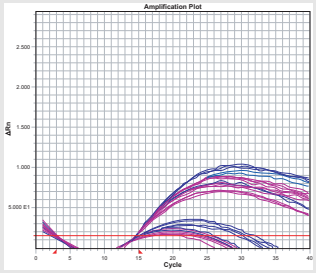
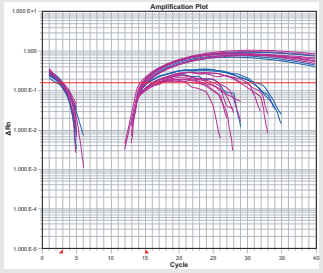
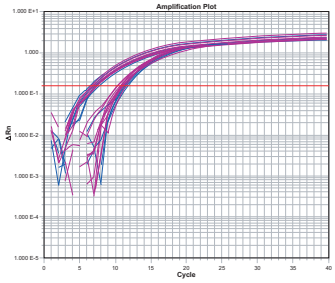
If there is no interaction between the known DNA target and the DNA target for the reverse primer, there should be no ligated DNA fragments that contain both sequences and therefore, no amplification.

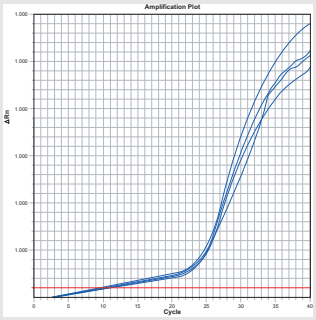
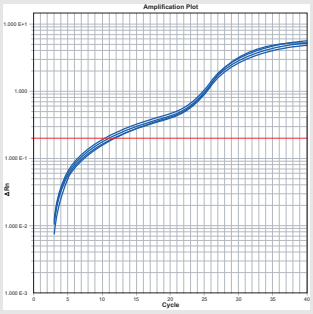
The presence of amplification and the Ct values indicate the frequency of interaction between the known DNA target and the DNA target for the reverse primer. Lower Ct values indicate higher frequencies of interaction, and higher Ct values indicate lower frequencies of interaction.

Troubleshooting

Observation	Possible causes	Recommended action
Low digestion efficiency, with higher than expected yield.	The samples are contaminated with Mycoplasma, and the restriction enzyme was used up by digestion of Mycoplasma.	• Take preventative measures, including using good aseptic techniques, using primary cells when possible, and wearing protective clothing. • Confirm Mycoplasma contamination using established methods. • Use mycoplasmacidal reagents in the cell culture. • Start over with new medium and cell stocks.
Low digestion efficiency.	The reactions contained insufficient restriction enzyme and/or the digestion time was too short.	The definition of restriction enzyme units may differ according to the vendor. Optimize the amount of restriction enzyme and the digestion time for your enzyme and cell lines.
	Cells were not lysed efficiently.	• Increase the incubation time at 65°C. • Optimize crosslinking conditions suitable for your cell lines.
	The Ct for Assay 2 is shifted up by an increased amount of carryover in the control PCR.	• Check PCR inhibition. • Reduce the amount of carryover in qPCR by using 50–100 ng of DNA in the reactions.
	The reactions for Assay 2 contained less DNA template than the reactions for Assay 1.	• Use the same amount of DNA template for the Assay 1 and Assay 2 reactions. • Make sure that you are pipetting accurately. • Pre-mix the DNA template with PCR master mix.
	Crosslinking was too extensive.	Use a lower concentration of formaldehyde.
	The diluted digestion buffer was not freshly prepared.	Use freshly diluted digestion buffer.
	Lysis buffer carried over into the digestion reaction.	Wash the cells according to the procedures provided. Make sure that you completely remove the supernatant at the lysis and wash steps.

Observation	Possible causes	Recommended action
Non-reproducible digestion efficiency.	The conditions for tissue culture, crosslinking, lysis, and/or digestion were inconsistent.	• Use consistent tissue culture conditions. • Follow the procedures consistently. • Run replicates in parallel. • Make sure that you are pipetting accurately. • Make sure that you mix the formaldehyde, lysis buffer, or restriction enzyme thoroughly at each step.
	The conditions for qPCR were inconsistent.	• Make sure that you are pipetting accurately. • Mix the PCR reactions and spin down before loading into the instrument. • Run all of the qPCR reactions on the same plate, on the same instrument. • Use the same threshold in the analysis.
Low ligation efficiency.	Cell input was too high.	• Recheck cell counts. • Reduce cell input. • Make sure the cell culture is not contaminated with Mycoplasma.
	Ligation buffer was expired.	Use fresh ligation buffer within the expiration date.
	The DNA was degraded by DNase.	• Check the length of the DNA on a gel. • Reduce the length of time that you store the cells after crosslinking, before proceeding to digestion. • Inactivate DNase by incubating at 65°C for 30 minutes.
Non-reproducible ligation efficiency.	The temperature for cell lysis was inconsistent.	Use a water bath pre-heated to 65°C for the incubation.

Observation	Possible causes	Recommended action
Missing or reduced interaction.	Cross-linked cells were stored for too long before digestion.	Process freshly cross-linked cells if possible.
	The DNA was degraded by DNase.	• Check the length of the DNA on a gel. • Reduce the length of time that you store the cells after crosslinking, before proceeding to digestion. • Inactivate DNase by incubating at 65°C for 30 minutes.
	The amount of DNA template in the qPCR reactions was too low.	Increase the amount of template to 400 ng per reaction if necessary. **Note:** Interaction is not present if there is no amplification at 400 ng.
	The 3C-qPCR assay design is flawed.	• Verify that the sequence that you used to design the 3C-qPCR assay is correct. • Design and run locus-specific PCR with undigested genomic DNA. Successful amplification indicates that the primers and probe are effective in amplifying the target sequence.
Amplification curve shows abnormal plot and/or low ΔR_n values. Linear view:  Log view:  	The baseline was set improperly: some samples have Ct values lower than the baseline stop value.	Switch from manual to automatic baselining, or move the baseline stop value to a lower Ct (2 cycles before the amplification curve for the sample crosses the threshold). **Note:** Refer to your real-time PCR system user guide for procedures on setting the baseline. Log view corrected: 
	An amplification signal is detected in the early cycles (no baseline can be set because the signal is detected too early).	Dilute the sample to increase the Ct value.

Observation	Possible causes	Recommended action
Amplification curve shows a rising baseline. Linear view:  Log view:  	There is interaction between the primer and probe.	• Adjust the threshold manually. • Select another assay from the same gene, if available. Try a probe from the complementary strand.

Observation	Possible causes	Recommended action
Amplification curve shows weak amplification.	Sequence used for the assay design contains mismatches with sample sequences due to natural variations.	Perform bioinformatics analysis.
	Reagents and/or probe are degraded.	• Check the expiration date of the reagents. • Verify that you follow the correct handling and storage conditions. • Avoid excessive freeze-thaw cycles.
	Template is contaminated or degraded.	• Improve the sample integrity. • Check template preparation by agarose gel electrophoresis or bioanalyzer to determine the: – Purity (only one product should be formed) – Level of degradation
	PCR inhibitors are present in the reaction.	Verify the presence of PCR inhibitors: 1. Create a serial dilution of your sample. 2. Run the serial dilution with a 3C-qPCR assay. If an inhibitor is present, high sample concentrations yield higher-than-expected C_T values. (High sample concentrations result in more inhibition because the sample is not diluted.) 3. Rerun the assay with purified template. 4. Improve the sample integrity (extraction methods).
Amplification curve shows low ROX™ dye (passive reference dye).	Inaccurate pipetting: The reaction contained little or no TaqMan® Master Mix.	Follow accurate pipetting practices.

Observation	Possible causes	Recommended action
Amplification curve shows no amplification of the sample (Ct = 40) across all assays or in an unusually large number of assays.	One or more of the reaction components was not added.	Verify that the cDNA and TaqMan Gene Expression Master Mix were added to the reaction plate. (If the master mix is missing, the passive reference fails.)
	Incorrect dye components were selected.	Check the dye components settings and reanalyze the data.
	The annealing temperature on the thermal cycler was too high for the primers and/or probe.	Verify that the thermal cycler is set to the correct annealing and extension temperatures. Ensure that the thermal cycler is calibrated and maintained regularly.
	The template is degraded.	• Determine the quality of the template. • Rerun the assay with fresh template.
	Inhibitors are present in the reaction.	Verify the presence of an inhibitor: 1. Create a serial dilution of your sample. 2. Run the serial dilution with an expressing assay (for example, an endogenous control). If an inhibitor is present, high sample concentrations yield higher-than-expected C_T values. (High sample concentrations result in more inhibition because the sample is not diluted.) 3. Rerun the assay with purified template.
	The baseline and/or threshold was improperly set.	Refer to the user guide for your real-time PCR system for procedures on setting the baseline and threshold: • Switch from automatic to manual baselining, or from manual to automatic. • Lower the threshold value to within the appropriate range.
	Assay design or synthesis failure: The wrong sequence was used.	Verify that the sequence that you used to design the 3C-qPCR assay is correct.

Observation	Possible causes	Recommended action
Amplification curve shows samples targeted by the same assay that have differently shaped curves.	The baseline was set improperly.	Refer to your real-time PCR system user guide for procedures on setting the baseline: • Switch from automatic to manual baselining, or from manual to automatic. • Increase the upper or lower value of the baseline range.
	Sample quality is poor.	1. Perform a quality check on the sample. 2. If necessary, reextract the sample.
	Different concentrations caused by imprecise pipetting.	Follow accurate pipetting practices.
	A reagent or equipment is contaminated.	Be sure that your workspace and equipment are properly cleaned.
Amplification curve shows no amplification of the sample (C_T = 40) in the target assay.	The DNA targets are not interacting.	Verify the known interaction in the sample type: • Rerun the sample using the same assay. • Rerun the assay using more sample. Avoid preparing the PCR reaction mix with more than 40% DNA template. • Run the sample using an alternative assay, if available, that detects a different sequence from the same gene. **Note:** If the recommended actions do not resolve the problem, the result may be correct.
	The sample may not have enough copies of the target template.	Verify the quantity of target template: • Rerun the sample using the same assay. • Rerun the assay using more sample. Avoid preparing the PCR reaction mix with more than 40% DNA template. **Note:** If the recommended actions do not resolve the problem, the result may be correct.
	One or more of the reaction components was not added.	Check your pipetting equipment and/or technique.
	Incorrect dye components were selected.	Check the settings of the dye components before data analysis.

Observation	Possible causes	Recommended action
Decrease in ROX™ dye fluorescence (passive reference dye).	The TaqMan® reagents contain precipitates.	Be sure to mix the solutions thoroughly to produce a homogenous solution.
	Reagents are degraded.	Verify that kits and reagents have been stored according to the instructions on the packaging and have not expired.
Simultaneous increase in fluorescence from both the: • Passive reference (ROX™) dye. • Reporter dye(s).	The sample evaporated.	Check the seal of the optical adhesive cover for leaks.
Multicomponent signal for ROX™ dye is not linear.	Pure dye components spectra are incorrect.	Rerun the pure dye spectra.
	Incorrect dye components were selected.	Select the correct dyes for the data analysis.
R_n in R_n-vs.-Cycle plot is very high.	ROX™ dye was not selected as the passive reference when the experiment run file was set up.	Select the ROX™ dye as the passive reference, then reanalyze the data.
No template control (NTC) shows amplification.	One or more reagents is contaminated with gDNA, amplicon, or plasmid clones.	• Rerun the assay using new reagents. • Be sure your workspace and equipment are cleaned properly. • Use AmpErase® UNG. • Run non-ligated controls to rule out non-specific amplification.
The 3C Input Normalization Assay Ct values vary for the same amount of DNA input.	Sample purity varies widely.	If desired, quantify samples by running the 3C Hu Input Normalization Assay with purified genomic DNA standards and re-normalize samples before running them in 3C-qPCR assays.
	Pipetting was inaccurate.	• Check the calibration of the pipettors. • Premix the master mix, assay, sample, and water for replicates in a reaction mix, then aliquot into wells using volumes ≥5 µL.

Observation	Possible causes	Recommended action
High standard deviation of replicates (inconsistent data, C_T varies).	Inefficient mixing of reagents.	• Increase the length of time that you mix the reagents. • Validate your mixing process by running a replicate plate.
	Pipetting was inaccurate.	• Check the calibration of the pipettors. • Premix the master mix, assay, sample, and water for replicates in a reaction mix, then aliquot into wells using volumes ≥5 µL.
	Threshold was set improperly.	Set the threshold above the noise and where the replicates are tightest. Refer to your real-time PCR system user documentation for procedures on setting the threshold.
	Low concentration of target.	Rerun the assay using more template.
	Template absorption occurred (adhering to the tube).	Add a carrier (for example, yeast tRNA).
C_T value is lower than expected.	Nonspecific amplification of gDNA occurred.	Redesign the 3C-qPCR assay and verify specificity before use.
	More sample added than expected.	• Reduce the amount of sample. • Quantitate and adjust the concentration of the sample.
	Template or amplicon contamination.	Follow established PCR good laboratory practices.
Amplification occurs in the non-ligated controls.	Amplification of gDNA occurred.	Redesign the 3C-qPCR assay and verify specificity before use.
	Template or amplicon is contaminated.	Follow established PCR good laboratory practices.
Shifting Rn value during the early cycles of the PCR (cycles 0 to 5).	Fluorescence did not stabilize to the buffer conditions of the reaction mix. **Note:** This condition does not affect PCR or the final results.	• Reset the lower value of the baseline range. • Use automatic baselining.
Small ΔRn.	PCR efficiency is poor.	Recheck the concentration of the reagents.
	Quantity of starting target is low (low copy number of target).	Increase the quantity of the starting target.

Observation	Possible causes	Recommended action
Noisy signal above the threshold.	The sample evaporated.	Check the seal of the optical adhesive cover for leaks.
	The well is empty because of inaccurate pipetting.	• Check the calibration of the pipettors. • Premix the master mix, assay, sample, and water for replicates in a reaction mix, then aliquot into wells using volumes ≥5 µL.
	The well is labeled with a target in the experiment file, but the well is empty.	• Be sure that your experiment file is set up correctly. • Exclude the well and reanalyze the data.

A Ordering Information

Optical reaction plates and adhesive films

Item	Source
MicroAmp® Optical 48-Well Reaction Plate, 20 plates	Life Technologies (Cat. no. 4375816)
MicroAmp® 48-Well Optical Adhesive Film: • 100 films • 25 films	Life Technologies • Cat. no. 4375323 • Cat. no. 4375928
MicroAmp® Optical 96-Well Reaction Plate: • 10 plates • 500 plates	Life Technologies • Cat. no. N8010560 • Cat. no. 4316813
MicroAmp® Optical 96-Well Reaction Plate with Barcode: • 20 plates • 500 plates	Life Technologies • Cat. no. 4306737 • Cat. no. 4326659
MicroAmp® Optical 96-Well Reaction Plate with Barcode and Optical Adhesive Films, 100 plates with covers	Life Technologies (Cat. no. 4314320)
MicroAmp® 96- and 384-Well Optical Adhesive Film: • 100 films • 25 films	Life Technologies • Cat. no. 4311971 • Cat. no. 4360954

B

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: **www.cdc.gov/biosafety**
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: **www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: **www.cdc.gov**

In the EU:

Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at: **www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/**

Documentation and Support

Related documentation

The following related documents are available at **www.lifetechnologies.com/support**:

Document	Part no.	Description
TaqMan® 3C Chromosome Conformation Kits Early Access Quick Reference	4471872	Provides brief procedures for advanced users who are familiar with the procedures in the *TaqMan® 3C Chromosome Conformation Kits Early Access User Guide*.

Note: To open the user documentation, use the Adobe® Reader® software available from **www.adobe.com**.

Note: For additional documentation, see "Obtaining support" on page 47.

Obtaining SDSs

Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

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Obtaining support

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