invitrogen

USER GUIDE

# Anza™ Restriction Enzyme Cloning System

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# Product Information

## Anza™ restriction enzyme cloning system

The Invitrogen™ Anza™ Restriction Enzyme Cloning System is a complete cloning system centered around a proprietary buffer system. The Anza™ buffer is formulated to minimize the need to accommodate the variable conditions that are required for optimal function of individual restriction enzymes, thus creating a unified system to simplify the restriction enzyme cloning process. All Anza™ restriction enzymes are fully functional in the Anza™ buffer.
The Anza™ cloning system also includes Anza™ Alkaline Phosphatase, the Anza™ T4 PNK (polynucleotide kinase) Kit, the Anza™ DNA Blunt End Kit, the Anza™ DNA End Repair Kit, and the Anza™ T4 DNA Ligase Master Mix.

## Anza™ Restriction Enzymes

The Anza™ cloning system utilizes a single protocol for digestion of all DNA, simplifying the overall procedure.
Anza™ restriction enzymes require no more than 15 minutes for complete digestion of any DNA substrate, but also permit flexibility for prolonged digestion up to 16 hours without deleterious star activity effects.

## Anza™ Alkaline Phosphatase

Anza™ Alkaline Phosphatase is used for removing the 5′-phosphate at the ends of DNA fragments.
Dephosphorylation is performed prior to 5′ end-labeling of DNA or RNA fragments prior to transfer of a labeled gamma-phosphate from ATP to that position.
In cloning, dephosphorylation prevents a vector from recircularizing as the ligase enzyme requires both a 5′-phosphate and a 3′-hydroxyl group to join the two ends of the vector.

## Anza™ T4 PNK Kit

The Anza™ T4 PNK (polynucleotide kinase) Kit is used to perform 5′-phosphorylation of DNA and oligonucleotides.
The T4 PNK enzyme catalyzes the transfer of the terminal phosphate of ATP to a 5′-hydroxyl group of a nucleic acid. It also exhibits 5′ polynucleotide kinase, and 3′-phosphatase activity.

## Anza™ DNA Blunt End Kit

The Anza™ DNA Blunt End Kit is used to convert DNA with overhanging ends to blunt ended DNA for blunt end ligation.

The Anza™ DNA Blunting Enzyme Mix contains T4 DNA polymerase and Klenow Fragment. The 3′→5′ exonuclease activity of T4 DNA polymerase acts to remove 3′ overhangs, while the 5′→3′ polymerase activity of the enzymes act to fill in 5′ overhangs. See Appendix D, page 21 for additional information on types of end repair.

The Anza™ 10X Blunting Buffer contains dNTPs to facilitate the synthesis of blunt ends.

## Anza™ DNA End Repair Kit

The Anza™ DNA End Repair Kit is used to convert DNA with overhanging ends to blunt ended DNA, while also performing 5′-phosphorylation, for blunt end ligation.
The Anza™ DNA End Repair Mix contains T4 DNA polymerase, Klenow Fragment, and T4 PNK. See Appendix D, page 21 for additional information on types of end repair.
The Anza™ 10X End Repair Buffer contains ATP and dNTPs to facilitate the activity of the enzyme mix.

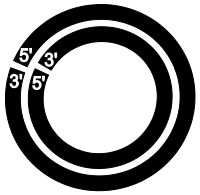
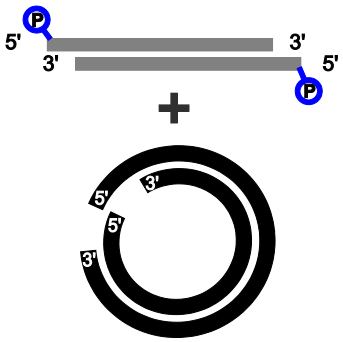
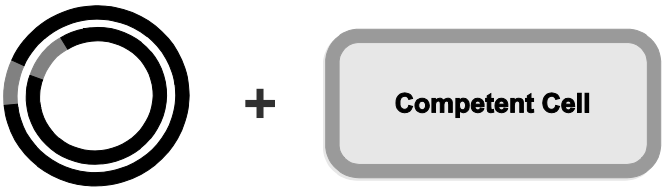
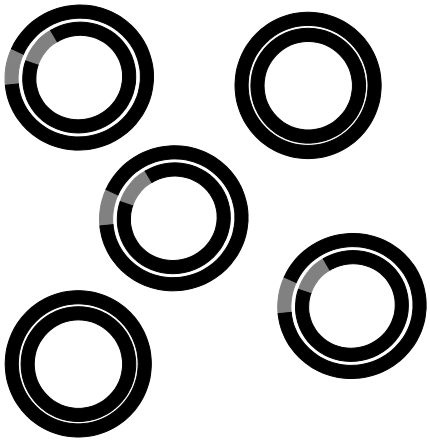
## Anza™ T4 DNA Ligase Master Mix

The Anza™ T4 DNA Ligase Master Mix is part of the overall Anza™ Restriction Enzyme Cloning System. The T4 DNA Ligase enzyme is formulated as a 4X concentrated master mix minimizing pipetting and facilitating ease of use.
Anza™ T4 DNA Ligase Master Mix can be used to join DNA fragments with overhanging ends or blunt ends, and repair nicks in double-stranded DNA having 3'-hydroxyl and 5'-phosphate ends.

# Anza™ Restriction Enzyme Cloning System Workflow

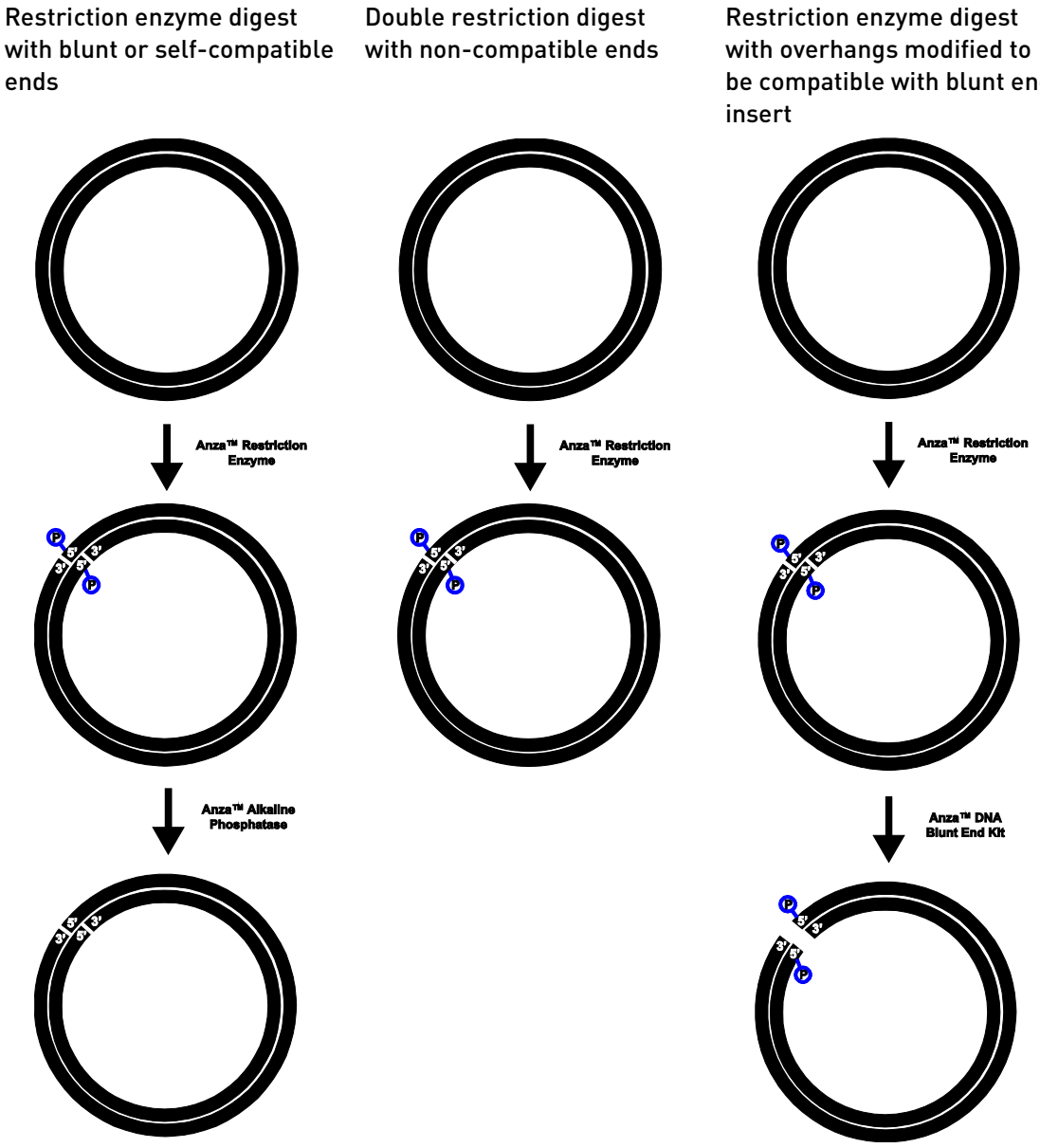
## Recombinant DNA technology

Traditional cloning is a recombinant DNA technique in which a vector and insert DNA are digested with restriction enzymes and joined by DNA ligase to form a new vector. A general workflow of traditional cloning includes the following steps:

| | | |
|---|---|---|
| 1 | Vector preparation (page 6) |   |
| 2 | Insert preparation (page 7) |   |
| 3 | Ligation (page 7) |   |
| 4 | Transformation (page 8) |   |
| 5 | Colony screening (page 8) |  |

## Vector preparation

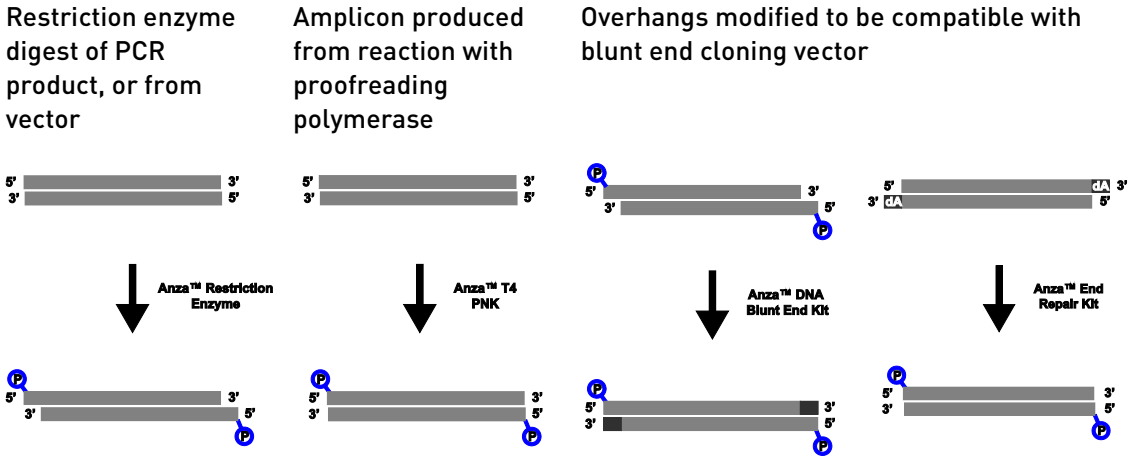
- Select a cloning vector with the desired restriction sites in the multiple cloning site (MCS).
- Digest the cloning vector with the appropriate restriction enzyme(s) to produce ends that are complementary to the insert. If necessary, modify the vector to create blunt ends compatible with blunt ended inserts.
- The option to select two restriction sites allows directional cloning of an insert, whereas using a single restriction site will result in inserts that can be in forward or reverse orientation.
- Directional cloning can be performed using two different restriction enzymes to generate non-compatible overhanging ends that allow the insert to be ligated in a specific orientation.
- Dephosphorylate blunt or self-compatible vector ends to prevent self-ligation. Dephosphorylation of the vector is important to reduce background and favor insertion of the desired fragment into the vector.



## Insert preparation

Considerations when preparing an insert for cloning:

- If using PCR to amplify an insert that does not contain the desired restriction sites, design PCR primers that incorporate the desired restriction sites in the appropriate location.
- Directional cloning can be performed using two different restriction enzymes to generate non-compatible overhanging ends that allow the insert to be ligated in a specific orientation.
- Digest PCR amplicon with appropriate Anza™ Restriction Enzyme(s) to produce fragments with phosphorylated ends complementary to vector.
- Phosphorylate amplicons from PCR reactions that do not require the ends to be digested. Reactions are typically performed with non-phosphorylated primers, resulting in in amplicons with non-phosphorylated 5' ends.
- If cloning an insert with ends that have overhangs into a vector with blunt ends, blunt the ends of the insert.
- Isolate inserts that are digested from a vector by gel electrophoresis and purify the fragment using a gel purification kit.




## Ligation

- Ligate the dephosphorylated DNA vector with the DNA insert.
  - Set up multiple reactions with varying insert to vector molar ratios (in the range of 1:1 to 5:1). Use the **CloningBench app** at **thermofisher.com/cloningbench** as a convenient tool to calculate ratios.
- The Anza™ T4 DNA Ligase Master Mix includes polyethylene glycol (PEG) to improve efficiency of blunt end ligation, because blunt end ligation can be less efficient than ligation of fragments with overhanging ends.

## Transformation

- Transform vector into competent cells to propagate cloned DNA.
  - The ligation mixture can be used directly in transformation of chemically competent cells, or may require purification prior to transformation of electrocompetent cells.
  - If a *dam* or *dcm* methylation-sensitive restriction enzyme to be used for downstream applications, transform the vector into INV110 Chemically Competent *E. coli*.
- Refer to the instruction manual of the competent cells being used to propagate your vector for details on how to perform the transformation protocol.

## Colony screening

- Determine the presence of colonies that carry your construct. All colonies that grow under antibiotic selection carry the vector, but not all of them will have the insert.
  - Use the selection markers on your cloning vector to identify bacteria colonies which contain your construct (e.g. blue/white screening of β-galactodiase activity).
  - Perform restriction enzyme digestion to determine the presence of your insert. Select the appropriate restriction enzymes to produce fragments that will indicate presence of the insert, and orientation.

    **Note**: Samples digested in Anza™ buffer need to be treated as high salt samples when used with E-gel™ agarose gels. Dilute the sample 2- to 5-fold with deionized water or TE before loading onto E-gel™ Agarose Gels, or at least 15-fold if with deionized water before loading onto E-gel™ EX Agarose Gels.
  - Perform colony PCR to identify colonies which carry your insert. Primer pairs can be designed to identify the presence of the insert, and the orientation.
  - Perform DNA sequencing to identify the orientation of your insert.

# Anza™ Restriction Enzyme digestion protocol

### General guidelines

Prepare vectors and inserts for cloning by restriction digestion. The choice of restriction enzymes depends upon the presence and location of compatible sequences on the vector and the insert.

Anza™ restriction enzymes require no more than 15 minutes for complete digestion of any DNA substrate. The Anza buffer also permits flexibility for prolonged digestions, up to 16 hours, without the deleterious star activity effects, when using 1 µL of restriction enzyme or enzymes in a digestion reaction following the Anza™ digestion protocol.

### Unit definition

One unit is the amount of enzyme required to completely digest 1 µg of substrate DNA in 50 µL of the reaction mixture in 1 hour at 37°C.

### Anza™ 10X Red Buffer

Anza™ 10X Red Buffer includes a density reagent containing red and yellow tracking dyes. The density reagent allows the reaction mixture to be loaded directly onto a gel for electrophoresis. The red dye of the Anza™ 10X Red Buffer migrates with 800 bp DNA fragments in a 1% agarose gel, while the yellow dye migrates significantly faster than 10 bp DNA fragments in a 1% agarose gel.

### Anza™ 10X Buffer

Use Anza™ 10X Buffer for applications that require analysis by fluorescence excitation, as the dyes in the Anza™ 10X Red Buffer may interfere with some fluorescence measurements. Add 1⁄10 volume of 10X Loading Buffer to samples digested in Anza™ Buffer before performing electrophoresis.

### Digestion protocol

1. Prepare a reaction mix by adding reagents in the order indicated in Table 1.

| Reagent | 1 enzyme reaction | 2 enzyme reaction | 3 enzyme reaction |
|---|---|---|---|
| Nuclease-Free Water | As required to make up final reaction volume | | |
| Anza™ 10X Buffer or Anza 10X Red Buffer | 2 µL | 2 µL | 3 µL |
| DNA | 0.2–1 µg | 0.2–1 µg | 0.2–1 µg |
| Anza™ restriction enzyme 1 | 1 µL | 1 µL | 1 µL |
| Anza™ restriction enzyme 2 | — | 1 µL | 1 µL |
| Anza™ restriction enzyme 3 | — | — | 1 µL |
| **Final reaction volume** | **20 µL** | **20 µL** | **30 µL** |

Volumes can be scaled up linearly to 5X.

2. Incubate at 37°C for 15 minutes.

# Anza™ Alkaline Phosphatase dephosphorylation protocols

## General guidelines

Anza™ Alkaline Phosphatase is used to perform dephosphorylation of DNA ends on cloning vectors prior to insert ligation. Dephosphorylation of blunt or self-compatible vector ends is recommended to prevent self-ligation, which results in high background when screening for clones containing the insert.

- Plasmid DNA should be free of RNA and genomic DNA for efficient dephosphorylation.
- The enzyme can be heat inactivated at 80°C in 5 minutes, eliminating the need for purification prior to ligation.
- The one step protocol is used to perform simultaneous digestion and dephosphorylation reactions.
- If the Anza™ restriction enzyme is incompatible with the one step protocol, use a two step protocol by performing restriction digestion prior to dephosphorylation.

## Anza™ 10X Red Buffer

Anza™ 10X Red Buffer includes a density reagent containing red and yellow tracking dyes. The density reagent allows the reaction mixture to be loaded directly onto a gel for electrophoresis. The red dye of the Anza™ 10X Red Buffer migrates with 800 bp DNA fragments in a 1% agarose gel, while the yellow dye migrates significantly faster than 10 bp DNA fragments in a 1% agarose gel.

## Anza™ 10X Buffer

Use Anza™ 10X Buffer for applications that require analysis by fluorescence excitation, as the dyes in the Anza™ 10X Red Buffer may interfere with some fluorescence measurements. Add 1⁄10 volume of 10X Loading Buffer to samples digested in Anza™ Buffer before performing electrophoresis.


### One step dephosphorylation protocol

The one step protocol is used to perform simultaneous digestion and dephosphorylation reactions. Refer to the product insert for the restriction enzyme used in the reaction or the Anza Enzyme Finder Tool at **thermofisher.com/anzafinder** to determine compatibility with the one step protocol.

1. Prepare a reaction mix by adding the reagents listed in the following table to a clean microcentrifuge tube:

| Reagent | Volume |
|---|---|
| Nuclease-free water | As required to reach final reaction volume |
| plasmid DNA | 0.2–1 µg |
| Anza™ 10X Buffer or<br>Anza 10X Red Buffer | 2 µL |
| Anza™ Restriction Enzyme | 1 µL |
| Anza™ Alkaline Phosphatase | 1 µL |
| **Final reaction volume** | **20 µL** |

2. Mix reagents by pipetting up and down.
3. Incubate at 37°C for 15 minutes.
4. Heat inactivate enzymes by incubating at 80°C for 20 minutes.
5. Ligate insert and vector using the Anza™ T4 DNA Ligase Master Mix.
6. Use 1–5 µL of the ligation reaction mixture to transform competent cells.

### Two step/heat dephosphorylation protocol

The two step/heat protocol is used to perform sequential digestion and dephosphorylation reactions. Refer to the product insert for the restriction enzyme used in the reaction or the Anza Enzyme Finder Tool at **thermofisher.com/anzafinder** to determine compatibility with the two step/heat protocol.

1. Perform restriction digestion reaction, and heat inactivate restriction enzyme.
2. Prepare a reaction mix by adding the reagents listed in the following table to a clean microcentrifuge tube:

| Reagent | Volume |
|---|---|
| Digested plasmid DNA (0.2–1 µg) (in 1x Anza Buffer) | 19 µL |
| Anza™ Alkaline Phosphatase | 1 µL |
| **Final reaction volume** | **20 µL** |

3. Mix reagents by pipetting up and down.
4. Incubate at 37°C for 15 minutes.
5. Heat inactivate enzyme by incubating at 80°C for 20 minutes.
6. Ligate insert and vector using the Anza™ T4 DNA Ligase Master Mix.
7. Use 1–5 µL of the ligation reaction mixture to transform competent cells.

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### Two step/column dephosphorylation protocol

The two step/column protocol is used to perform sequential digestion and dephosphorylation reactions. Refer to the product insert for the restriction enzyme used in the reaction or the Anza Enzyme Finder Tool at **thermofisher.com/anzafinder** to determine compatibility with the two step/column protocol.

1. Perform restriction digestion reaction, and column purify the insert.
2. Prepare a reaction mix by adding the reagents listed in the following table to a clean microcentrifuge tube:

| Reagent | Volume |
|---|---|
| Column Purified digested plasmid DNA (0.2–1 µg) | 17 µL |
| Anza 10X Buffer or Anza 10X Red Buffer | 2 µL |
| Anza™ Alkaline Phosphatase | 1 µL |
| **Final reaction volume** | **20 µL** |

3. Mix reagents by pipetting up and down.
4. Incubate at 37°C for 15 minutes.
5. Heat inactivate enzyme by incubating at 80°C for 5 minutes.
6. Ligate insert and vector using the Anza™ T4 DNA Ligase Master Mix.
7. Use 1–5 µL of the ligation reaction mixture to transform competent cells.

# Anza™ T4 PNK 5’ Phosphorylation Protocol

## General guidelines

The primers used in PCR amplification are usually non-phosphorylated, thereby resulting in PCR products that are not phosphorylated. Non-phosphorylated amplicons must be phosphorylated to allow ligation with a dephosphorylated vector to occur.

The Anza™ T4 PNK (polynucleotide kinase) Kit is used to perform 5’-phosphorylation of DNA and oligonucleotides.

- Perform protocol with linear double-stranded DNA (0.2–1 µg), or oligonucleotides (10–50 pmol).
- Phosphorylaton can be performed with DNA or oligonucleotides in water, TE, or 1X Anza™ buffers.

## DNA phosphorylation protocol

1. Prepare a reaction mix by adding the reagents listed in the following table to a clean microcentrifuge tube:

| Reagent | Volume |
|---|---|
| Nuclease-free water | As required to reach final reaction volume |
| DNA insert | 0.2–1 µg |
| Anza™ 10X PNK Buffer | 2 µL |
| Anza™ T4 PNK Enzyme | 1 µL |
| **Final reaction volume** | **20 µL** |

2. Mix reagents by pipetting up and down.
3. Incubate at 20°C for 15 minutes.
4. Heat inactivate enzyme by incubating at 80°C for 5 minutes.
5. Ligate insert and vector using the Anza™ T4 DNA Ligase Master Mix.
6. Use 1–5 µL of the ligation reaction mixture to transform competent cells.

# Anza™ DNA Blunt End Protocol

## General guidelines

The Anza™ DNA Blunt End Kit is used to convert DNA with overhanging ends generated by restriction enzyme digestion to blunt ended DNA for insertion into a dephosphorylated blunt ended vector.

## DNA blunting protocol

1. Prepare a reaction mix by adding the reagents listed in the following table to a clean microcentrifuge tube:

| Reagent | Volume |
|---|---|
| Nuclease-free water | As required to reach final reaction volume |
| Anza™ 10X Blunting Buffer | 2 µL |
| DNA insert | 0.2–1 µg |
| Anza™ DNA Blunting Enzyme Mix | 1 µL |
| **Final reaction volume** | **20 µL** |

2. Mix reagents by pipetting up and down.
3. Incubate at 20°C for 15 minutes.
4. Purify DNA insert from reaction mix using the PureLink™ PCR Purification Kit.
5. Ligate insert and vector (dephosphorylated and blunt ended) using the Anza™ T4 DNA Ligase Master Mix.
6. Use 1–5 µL of the ligation reaction mixture to transform competent cells.

# Anza™ DNA End Repair Protocol

## General guidelines

The Anza™ DNA End Repair Kit is used to convert non-phosphorylated DNA with overhanging ends (e.g. PCR products with a 3′ adenine residue added by *taq* polymerase) to blunt ended 5′-phosphorylated DNA for blunt end ligation.

- PCR products require clean up prior to performing the end repair protocol.
- DNA digested with Anza™ Restriction Enzymes can be used directly in the protocol following heat inactivation.

## DNA end repair protocol

Use this protocol to convert DNA with 3′ and 5′ overhangs to blunt-ended DNA for use in cloning.

1. Prepare a reaction mix by adding the reagents listed in the following table to a clean microcentrifuge tube:

| Reagent | Volume |
|---|---|
| Nuclease-free water | As required to reach final reaction volume |
| Anza™ 10X End Repair Buffer | 2 µL |
| DNA insert | 0.2–1 µg |
| Anza™ DNA End Repair Mix | 1 µL |
| **Final reaction volume** | **20 µL** |

2. Mix reagents by pipetting up and down.
3. Incubate at 20°C for 15 minutes.
4. Purify DNA insert from reaction mix using the PureLink™ PCR Purification Kit.
5. Ligate purified insert and vector using the Anza™ T4 DNA Ligase Master Mix.
6. Use 1–5 µL of the ligation reaction mixture to transform competent cells.

# Anza™ T4 DNA Ligase Master Mix

## General guidelines

The Anza™ T4 DNA Ligase Master Mix is used to perform 15-minute ligation of blunt-ended DNA or DNA with overhanging ends into a vector for cloning.

- Ligation can be performed with either phosphorylated or dephosphorylated DNA insert.

  Note: if using a dephosphorylated DNA insert, the linearized vector needs to be phosphorylated.
- Ligation can be performed with DNA in water, TE, elution buffer, or 1X Anza™ buffers.
- If using electrocompetent cells, perform column purification of ligated DNA prior to transformation.

## DNA ligation protocol

1. Prepare a reaction mix by adding the reagents listed in the following table to a clean microcentrifuge tube:

| Reagent | Volume |
|---|---|
| Nuclease-free water | As required to reach final reaction volume |
| Linearized vector DNA | 10–100 ng |
| DNA insert | 3:1 molar excess over vector DNA |
| Anza™ T4 DNA Ligase Master Mix | 5 µL |
| **Final reaction volume** | **20 µL** |

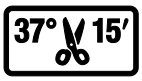
2. Mix reagents by pipetting up and down.
3. Incubate at room temperature for 15 minutes.
4. Use 1–5 µL of the ligation reaction mixture to transform competent cells.

   **Note**: the ligation reaction mixture can be stored at 0–4°C until required for transformation.

# Appendix A: Description of symbols

## Overview

Explanations of the symbols used to describe the characteristics of Anza™ enzymes are provided in the following table.

| Symbol | Explanation |
|---|---|
|   | **Digestion reaction protocol**<br>Anza™ universal digestion protocol: Digestion is performed at 37°C for 15 minutes. |
|   | **Heat inactivation**<br>Complete heat inactivation when heated to 80°C for 20 minutes. |
|  | **No Heat Inactivation**<br>Restriction enzyme is not heat sensitive and cannot be heat inactivated. |
| **Methylation Sensitivity** | |
|   | DNA cleavage by the restriction enzyme is *blocked or impaired* by Dam methylation within the target sequence. |
|   | DNA cleavage by the restriction enzyme is *blocked or impaired* by Dcm methylation within the target sequence. |
|   | DNA cleavage by the restriction enzyme is *blocked or impaired* by CpG methylation within the target sequence. |
|   | The target site *may have* overlapping Dam methylation which will result in *blocked or impaired* DNA cleavage. |
|   | The target site *may have* overlapping Dcm methylation which will result in *blocked or impaired* DNA cleavage. |
|   | The target site *may have* overlapping CpG methylation which will result in *blocked or impaired* DNA cleavage. |
| **Alkaline Phosphatase Protocol** | |
|   | **One Step**<br>Digestion and dephosphorylation can be performed simultaneously in Anza™ buffer in a one-step process. |
|   | **Two Step/Heat**<br>Digestion reaction requires heat inactivation prior to dephosphorylation with Anza Alkaline Phosphatase. |
|   | **Two Step/Column**<br>Digestion reaction requires column purification prior to dephosphorylation with Anza Alkaline Phosphatase. |
|   | Dithiothreitol (DTT) is an additive required to obtain the stated enzyme activity. |

# Appendix B: Additional bases required for complete digestion

## Overview

When designing PCR primers to produce amplicons with incorporated restriction sites, additional bases may be required in the regions flanking the restriction enzyme recognition site to allow complete digestion of the DNA fragment that is produced.

The following table is a list of Anza™ Restriction Enzymes, and the required number of additional bases that need to be added to either side of the recognition site to achieve complete digestion.

Complete digestion was tested with 0.2 µg of PCR product in a 20 µL reaction, incubated at 37°C for 15 minutes.

| Enzyme | Number of additional bases required for complete digestion |
|---|---|
| Anza 1 NotI | 2 |
| Anza 2 NcoI | 3 |
| Anza 3 BcuI | 1 |
| Anza 4 BpiI | 1 |
| Anza 5 BamHI | 2 |
| Anza 6 NheI | 5 |
| Anza 7 BshTI | 3 |
| Anza 8 XhoI | 4 |
| Anza 9 NdeI | 6 |
| Anza 10 DpnI | 1 |
| Anza 11 EcoRI | 3 |
| Anza 12 XbaI | 2 |
| Anza 13 Esp3I | 3 |
| Anza 14 SalI | 4 |
| Anza 15 XmaJI | 2 |
| Anza 16 HindIII | 6 |
| Anza 17 KpnI | 3 |
| Anza 18 PacI | 2 |
| Anza 19 BglII | 4 |
| Anza 20 SacI | 2 |
| Anza 21 SgsI | 2 |
| Anza 22 SmaI | 1 |
| Anza 23 PstI | 3 |
| Anza 24 MssI | 2 |
| Anza 25 PaeI | 5 |
| Anza 26 Eco32I | 2 |
| Anza 27 PvuI | 4 |
| Anza 28 MluI | 3 |
| Anza 29 KflI | 5 |
| Anza 30 Bsu15I | 2 |
| Anza 31 MunI | 3 |
| Anza 32 ApaI | 3 |

| Enzyme | Number of additional bases required for complete digestion |
|---|---|
| Anza 33 LguI | 2 |
| Anza 34 Pfl23II | 3 |
| Anza 35 Eco47III | 4 |
| Anza 36 Eco31I | 3 |
| Anza 37 Mph1103I | 3 |
| Anza 38 ScaI | 4 |
| Anza 39 Bsp1407I | 3 |
| Anza 40 SfaAI | 6 |
| Anza 41 HpyF3I | 3 |
| Anza 42 RsaI | 3 |
| Anza 43 Eco105I | 3 |
| Anza 44 AluI | 4 |
| Anza 45 PteI | 3 |
| Anza 46 AatII | 5 |
| Anza 47 Eco52I | 4 |
| Anza 48 MnlI | 2 |
| Anza 49 SmiI | 2 |
| Anza 50 KspAI | 2 |
| Anza 51 BspTI | 4 |
| Anza 52 PvuII | 2 |
| Anza 53 AanI | 5 |
| Anza 54 Eco147I | 3 |
| Anza 55 MboI | 1 |
| Anza 56 Hin1II | 4 |
| Anza 57 Bpu1102I | 2 |
| Anza 58 PagI | 3 |
| Anza 59 HhaI | 1 |
| Anza 60 Kpn2I | 3 |
| Anza 61 PfoI | 3 |
| Anza 62 MlsI | 4 |
| Anza 63 CpoI | 1 |
| Anza 64 SaqAI | 4 |

| Enzyme | Number of additional bases required for complete digestion |
|---|---|
| Anza 65 MspI | 3 |
| Anza 66 BstXI | 3 |
| Anza 67 RruI | 5 |
| Anza 68 BsuRI | 3 |
| Anza 69 BglI | 3 |
| Anza 70 NsbI | 3 |
| Anza 71 HinfI | 2 |
| Anza 72 HincII | 1 |
| Anza 73 BclI | 3 |
| Anza 74 CsiI | 4 |
| Anza 75 Alw44I | 4 |
| Anza 76 VspI | 2 |
| Anza 77 DraI | 4 |
| Anza 78 AdeI | 2 |
| Anza 79 PdiI | 3 |
| Anza 80 FspBI | 3 |
| Anza 81 Eco91I | 2 |
| Anza 82 Eco72I | 2 |
| Anza 83 Eco81I | 2 |
| Anza 84 FspAI | 4 |
| Anza 85 MreI | 3 |
| Anza 86 PdmI | 2 |
| Anza 87 Eco47I | 1 |
| Anza 88 Bsp119I | 1 |
| Anza 89 Mva1269I | 3 |
| Anza 90 Eco88I | 2 |
| Anza 91 Acc65I | 3 |
| Anza 92 EheI | 3 |
| Anza 93 HpaII | 3 |
| Anza 94 BfmI | 2 |
| Anza 95 MauBI | 5 |
| Anza 96 XmiI | 3 |

| Enzyme | Number of additional bases required for complete digestion |
|---|---|
| Anza 97 Bsp143I | 4 |
| Anza 98 XceI | 2 |
| Anza 99 XagI | 2 |
| Anza 100 Bsh1236I | 1 |
| Anza 101 BoxI | 3 |
| Anza 102 CaiI | 4 |
| Anza 103 Psp1406I | 3 |
| Anza 104 MboII | 2 |
| Anza 105 Hin1I | 1 |
| Anza 106 Van91I | 3 |
| Anza 107 BspLI | 2 |
| Anza 108 SatI | 3 |
| Anza 109 Alw26I | 1 |
| Anza 111 XapI | 4 |
| Anza 112 BseGI | 2 |
| Anza 113 BcnI | 3 |
| Anza 114 Hpy8I | 2 |
| Anza 115 MbiI | 3 |
| Anza 116 Cfr13I | 2 |
| Anza 117 EcoO109I | 3 |
| Anza 118 BseDI | 3 |
| Anza 119 BmsI | 1 |
| Anza 120 NmuCI | 2 |
| Anza 121 Bsp120I | 3 |
| Anza 122 Csp6I | 2 |
| Anza 123 Hin6I | 4 |
| Anza 124 PfeI | 2 |
| Anza 125 HpyF10VI | 2 |
| Anza 126 Alw21I | 1 |
| Anza 127 RseI | 4 |
| Anza 128 PspFI | 4 |
| Anza 129 BshNI | 2 |

# Appendix C: Restriction enzymes with compatible overhangs

## Overview

To join two DNA fragments, the ends need to be compatible. The ends of the two fragments can be generated by digestion with a single enzyme resulting in compatible overhangs, creation of blunt ended fragments, or digestion by two different enzymes that produce compatible ends.

The following table is a list of Anza™ Restriction Enzymes which can be used to generate DNA fragments with compatible overhanging ends.

| Enzyme | Anza™ Restriction Enzymes resulting in compatible overhangs |
|---|---|
| Anza 1 NotI | Anza 47 Eco52I, Anza 121 Bsp120I |
| Anza 2 NcoI | Anza 58 PagI |
| Anza 3 BcuI | Anza 6 NheI, Anza 12 XbaI, Anza 15 XmaJI |
| Anza 5 BamHI | Anza 19 BglII, Anza 55 MboI, Anza 73 BclI, Anza 97 Bsp143I |
| Anza 6 NheI | Anza 3 BcuI, Anza 12 XbaI, Anza 15 XmaJI |
| Anza 7 BshTI | Anza 60 Kpn2I, Anza 85 MreI, Anza 90 Eco88I* (*If recognized sequence is 5'-CCCGGG-3') |
| Anza 8 XhoI | Anza 14 SalI, Anza 90 Eco88I* (*if recognized sequence is 5'-CTCGAG-3') |
| Anza 9 NdeI | Anza 64 SaqAI, Anza 76 VspI, Anza 80 FspBI, Anza 122 Csp6I |
| Anza 11 EcoRI | Anza 31 MunI, Anza 111 XapI |
| Anza 12 XbaI | Anza 3 BcuI, Anza 6 NheI, Anza 15 XmaJI |
| Anza 14 SalI | Anza 8 XhoI, Anza 90 Eco88I* (*if recognized sequence is 5'-CTCGAG-3') |
| Anza15 XmaJI | Anza 3 BcuI, Anza 6 NheI, Anza 12 XbaI |
| Anza 18 PacI | Anza 27 PvuI, Anza 40 SfaAI |
| Anza 19 BglII | Anza 5 BamHI, Anza 55 MboI, Anza 73 BclI, Anza 97 Bsp143I |
| Anza 20 SacI | Anza 126 Alw21I* (*if recognized sequence is 5'-GAGCTC-3') |
| Anza 21 SgsI | Anza 28 MluI, Anza 45 PteI, Anza 95 MauBI |
| Anza 23 PstI | Anza 37 Mph1103I, Anza 126 Alw21I* (*if recognized sequence is 5'-GTGCAC-3') |
| Anza 25 PaeI | Anza 56 Hin1II, Anza 98 XceI |
| Anza 27 PvuI | Anza 18 PacI, Anza 40 SfaAI |
| Anza 28 MluI | Anza 21 SgsI, Anza 45 PteI, Anza 95 MauBI |
| Anza 29 KflI | Anza 63 CpoI, Anza 87 Eco47I |
| Anza 30 Bsu15I | Anza 65 MspI, Anza 88 Bsp119I, Anza 93 HpaII, Anza 103 Psp1406I, Anza 105 Hin1I, Anza 123 Hin6I, Anza 96 XmiI* (*if recognized sequence is 5'-GTCGAC-3') |
| Anza 31 MunI | Anza 11 EcoRI, Anza 111 XapI |
| Anza 34 Pfl23II | Anza 39 Bsp1407I, Anza 91 Acc65I, Anza 129 BshNI* (*if recognized sequence is 5'-GGTACC-3') |
| Anza 37 Mph1103I | Anza 23 PstI, Anza 126 Alw21I* (*if recognized sequence is 5'-GTGCAC-3') |
| Anza 39 Bsp1407I | Anza 34 Pfl23II, Anza 91 Acc65I, Anza 129 BshNI* (*if recognized sequence is 5'-GGTACC-3') |
| Anza 40 SfaAI | Anza 18 PacI, Anza 27 PvuI |
| Anza 45 PteI | Anza 21 SgsI, Anza 28 MluI, Anza 95 MauBI |
| Anza 47 Eco52I | Anza 1 NotI, Anza 121 Bsp120I |
| Anza 55 MboI | Anza 5 BamHI, Anza 19 BglII, Anza 73 BclI, Anza 97 Bsp143I |
| Anza 56 Hin1II | Anza 25 PaeI, Anza 98 XceI |
| Anza 58 PagI | Anza 2 NcoI |

| Enzyme | Anza™ Restriction Enzymes resulting in compatible overhangs |
|---|---|
| Anza 60 Kpn2I | Anza 7 BshTI, Anza 85 MreI, Anza 90 Eco88I* (*If recognized sequence is 5'-CCCGGG-3') |
| Anza 63 CpoI | Anza 28 KflI, Anza 87 Eco47I |
| Anza 64 SaqAI | Anza 9 NdeI, Anza 76 VspI, Anza 80 FspBI, Anza 122 Csp6I |
| Anza 65 MspI | Anza 30 Bsu15I, Anza 88 Bsp119I, Anza 93 HpaII, Anza 103 Psp1406I, Anza 105 Hin1I, Anza 123 Hin6I, Anza 96 XmiI* (*if recognized sequence is 5'-GTCGAC-3') |
| Anza 73 BclI | Anza 5 BamHI, Anza 19 BglII, Anza 55 MboI, Anza 97 Bsp143I |
| Anza 75 Alw44I | Anza 94 BfmI* (* if recognized sequence is 5'-CTGCAG-3') |
| Anza 76 VspI | Anza 9 NdeI, Anza 64 SaqAI, Anza 80 FspBI, Anza 122 Csp6I |
| Anza 80 FspBI | Anza 9 NdeI, Anza 64 SaqAI, Anza 76 VspI, Anza 122 Csp6I |
| Anza 85 MreI | Anza 7 BshTI, Anza 60 Kpn2I, Anza 90 Eco88I* (*If recognized sequence is 5'-CCCGGG-3') |
| Anza 87 Eco47I | Anza 28, KflI, Anza 63 CpoI |
| Anza 88 Bsp119I | Anza 30 Bsu15I, Anza 65 MspI, Anza 93 HpaII, Anza 103 Psp1406I, Anza 105 Hin1I, Anza 123 Hin6I, Anza 96 XmiI* (*if recognized sequence is 5'-GTCGAC-3') |
| Anza 90 Eco88I | If recognized sequence is 5'-CTCGAG-3', then generate compatible ends for Anza 8 XhoI, Anza 14 SalI<br>If recognized sequence is 5'-CCCGGG-3', then generate compatible ends for Anza 7 BshTI, Anza 60 Kpn2I, Anza 85 MreI |
| Anza 91 Acc65I | Anza 34 Pfl23II, Anza 39 Bsp1407I, Anza 129 BshNI* (*if recognized sequence is 5'-GGTACC-3') |
| Anza 93 HpaII | Anza 30 Bsu15I, Anza 65 MspI, Anza 88 Bsp119I, Anza 103 Psp1406I, Anza 105 Hin1I, Anza 123 Hin6I, Anza 96 XmiI* (*if recognized sequence is 5'-GTCGAC-3') |
| Anza 94 BfmI | If recognized sequnce is 5'-CTGCAG-3', then generate compatible ends for Anza 75 Alw44I |
| Anza 95 MauBI | Anza 21 SgsI, Anza 28 MluI, Anza 45 PteI |
| Anza 96 XmiI | If recognized sequence is 5'-GTCGAC-3', then generate compatible ends for Anza 30 Bsu15I, Anza 65 MspI, Anza 88 Bsp119I, Anza 93 HpaII, Anza 103 Psp1406I, Anza 105 Hin1I, Anza 123 Hin6I |
| Anza 97 Bsp143I | Anza 5 BamHI, Anza 19 BglII, Anza 55 MboI, Anza 73 BclI |
| Anza 98 XceI | Anza 25 PaeI, Anza 56 Hin1II |
| Anza 103 Psp1406I | Anza 30 Bsu15I, Anza 65 MspI, Anza 88 Bsp119I, Anza 93 HpaII, Anza 105 Hin1I, Anza 123 Hin6I, Anza 96 XmiI* (*if recognized sequence is 5'-GTCGAC-3') |
| Anza 105 Hin1I | Anza 30 Bsu15I, Anza 65 MspI, Anza 88 Bsp119I, Anza 93 HpaII, Anza 103 Psp1406I, Anza 123 Hin6I, Anza 96 XmiI* (*if recognized sequence is 5'-GTCGAC-3') |
| Anza 111 XapI | Anza 11 EcoRI, Anza 31 MunI |
| Anza 121 Bsp120I | Anza 1 NotI, Anza 47 Eco52I |
| Anza 122 Csp6I | Anza 9 NdeI, Anza 64 SaqAI, Anza 76 VspI, Anza 80 FspBI |
| Anza 123 Hin6I | Anza 30 Bsu15I, Anza 65 MspI, Anza 88 Bsp119I, Anza 93 HpaII, Anza 103 Psp1406I, Anza 105 Hin1I, Anza 96 XmiI* (*if recognized sequence is 5'-GTCGAC-3') |
| Anza 126 Alw21I | If recognized sequence is 5'-GAGCTC-3', then generate compatible ends for Anza 20 SacI<br>If recognized sequnece is 5'-GTGCAC-3', then generate compatible ends for Anza 23 PstI, Anza 37 Mph1103I |
| Anza 129 BshNI | If recognized sequence is 5'-GGTACC-3', then generate compatible ends for Anza 34 Pfl23II, Anza 39 Bsp1407I, Anza 91 Acc65I |

# Appendix D: Types of DNA end repair

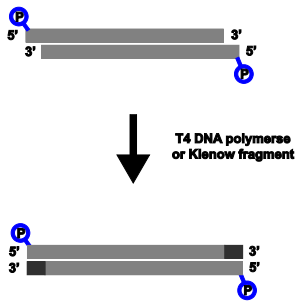
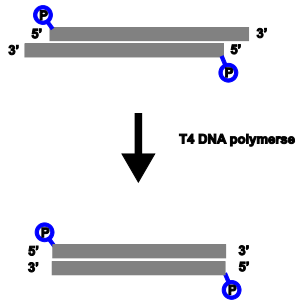
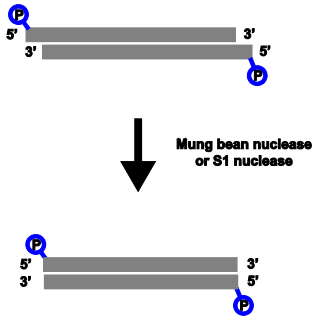
## Overview

Commonly used enzymes for modification of DNA ends are DNA Polymerase I, Large (Klenow) Fragment and T4 DNA Polymerase. The choice of enzyme depends on whether the restriction enzyme generates a 3′ or a 5′ overhang.

In the case of 3′ overhangs (e.g. those generated by KpnI), T4 DNA Polymerase is preferred because it has a stronger 3′→5′ exonuclease activity than Klenow. The 3′ overhang is removed by the exonuclease activity.

For 5′ overhangs (e.g. those generated by EcoRI), either Klenow or T4 DNA Polymerase can be used to fill in the overhangs through 5′→3′ polymerase activity.

In some instances, mung bean nuclease or S1 nuclease can be used to trim single-stranded DNA overhangs through 5′→3′ exonuclease activity on single-stranded DNA (ssDNA).

| 5'→3' polymerase activity | 3'→5' exonuclease activity | 5'→3' exonuclease activity |
|---|---|---|
|  |  |  |

# Appendix E

## Ordering information

### Additional products

The following products are also available from Thermo Fisher Scientific. For more details on these products, visit **thermofisher.com**, or contact **Technical Support** (page 23).

Use the Anza Enzyme Finder Tool at **thermofisher.com/anzafinder** to search for additional Anza™ Restriction Enzymes.

| Item | Amount | Cat. no. |
|---|---|---|
| Anza™ T4 DNA Ligase Master Mix | 50 reactions<br>200 reactions | IVGN210-4<br>IVGN210-8 |
| Anza™ Alkaline Phosphatase | 500 reactions<br>2000 reactions | IVGN220-4<br>IVGN220-8 |
| Anza™ T4 PNK Kit | 500 reactions | IVGN230-4 |
| Anza™ DNA Blunt End Kit | 100 reactions | IVGN240-4 |
| Anza™ DNA End Repair Kit | 20 reactions | IVGN250-4 |
| Anza™ 10X Buffer Set | 1 kit | IVGN200-8 |
| Anza™ Starter Kit | 5 pack | IVGN300-4 |
| Anza™ Starter Kit | 10 pack | IVGN300-6 |
| Platinum™ Taq Green Hot Start DNA Polymerase | 120 reactions | 11966-018 |
| Platinum™ Taq DNA Polymerase | 300 reactions | 10966-026 |
| PureLink™ PCR Purification Kit | 50 preps | K3100-01 |
| PureLink™ Quick Gel Extraction and PCR Purification Combo Kit | 50 preps | K2200-01 |
| PureLink™ Quick Gel Extraction Kit | 50 preps | K2100-12 |
| ReadyPouch™ 1% Agarose Gels | 10 pouches | A25647 |
| 1 Kb Plus DNA Ladder | 250 µg | 10787-018 |
| One Shot™ TOP10 Chemically Competent *E. coli* | 20 transformations | C4040-03 |
| One Shot™ INV110 Chemically Competent *E. coli* | 20 transformations | C7171-03 |
| One Shot™ OmniMAX™ 2 T1$^{R}$ Chemically Competent *E. coli* | 20 transformations | C8540-03 |
| One Shot™ Mach1™ T1 Phage Resistant Chemically Competent *E. coli* | 20 transformations | C8620-03 |
| Select Agar™, powder | 500 g | 30391-023 |
| S.O.C. medium | 10 x 10 mL | 15544-034 |
| LB Broth Base | 500 g | 12780-052 |
| LB Broth | 500 mL | 10855-021 |

# Technical Support

## Obtaining support

For the latest services and support information for all locations, go to **thermofisher.com**
At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com)**
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

## Safety Data Sheets (SDS)

Safety Data Sheets (SDSs) are available at **thermofisher.com/support**.

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**For support visit** thermofisher.com/support or email techsupport@lifetech.com

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