

## Genewiz Unpurified PCR Product Submission Protocol

To prepare samples for DNA sequencing, please follow these easy steps:

1. For orders with <48 samples, please use 8-strip PCR tubes to streamline preparation and processing. Label your tubes on the side with your initials and sample number. For orders with  $\geq 48$  samples, you can receive a discount by using a 96-well PCR plate and arranging the samples vertically (A1 to H1). See the Tubes and Plates section for more details.
2. Dilute your sequencing primer to 5  $\mu\text{M}$  (pmol/ $\mu\text{l}$ ) using water. You will need 5  $\mu\text{l}$  for each sequencing reaction. If you want to use a GENEWIZ Universal Primer, we will add it for you at no charge. Remember that only one primer is used in a sequencing reaction. See the Technical Notes section for tips on designing primers for sequencing.
3. For the amount of template needed in each of our DNA Sequencing Services (Pre-Mixed, Pre-Defined, and Custom), please refer to the tables below. Prepare template in 10  $\mu\text{l}$  for each sequencing reaction. Please make dilutions in water or Tris. For best results, do not use Tris-EDTA (TE) because EDTA will inhibit the sequencing reaction.

DNA Type	DNA Length (include vector)	Template Concentration in 10 $\mu\text{l}$	Template Total Mass (recommended)	Template Volume Per Reaction	Your Primer Concentration $\mu\text{M}$ (pmol/ $\mu\text{l}$ )	Your Primer Volume Per Reaction
Plasmids	Any size	Unknown	At least 500 ng	10 $\mu\text{l}$	5 $\mu\text{M}$	5 $\mu\text{l}$
PCR Products*	Any size	Unknown	At least 500 ng	10 $\mu\text{l}$	5 $\mu\text{M}$	5 $\mu\text{l}$

\*(US) If you are sending unpurified PCR products, please send the same amount of DNA that is required for purified PCR products (minimum volume of 10  $\mu\text{l}$ ).

1. Dilute your sequencing primer to 5  $\mu\text{M}$  (pmol/ $\mu\text{l}$ ) using water. You will need 1  $\mu\text{l}$  (minimum volume of 10  $\mu\text{l}$ ) for each sequencing reaction. If you want to use a GENEWIZ Universal Primer, we will add it for you at no charge. Remember that only one primer is used in a sequencing reaction. See the Technical Notes section for tips on designing primers for sequencing.
2. For the amount of template needed in each of our DNA Sequencing Services (Pre-Mixed, Pre-Defined, and Custom), please refer to the tables below. Prepare template in 10  $\mu\text{l}$  for each sequencing reaction. Please make dilutions in water or Tris. For best results, do not use Tris-EDTA (TE) because EDTA will inhibit the sequencing reaction.

DNA Type	DNA Length (include vector)	Template Concentration in 10 $\mu$ l	Template Total Mass	Template Volume Per Reaction	Your Primer Concentration $\mu$ M (pmol/ $\mu$ l)	Your Primer Volume Per Reaction
Plasmids	<6 kb	~50 ng / $\mu$ l	~500 ng	10 $\mu$ l	5 $\mu$ M	5 $\mu$ l
	6 - 10 kb	~80 ng / $\mu$ l	~800 ng			
	> 10 kb	~100 ng / $\mu$ l	~1000 ng			
Purified PCR Products	<500 bp	~1 ng / $\mu$ l	~10 ng	10 $\mu$ l	5 $\mu$ M	5 $\mu$ l
	500 - 1000 bp	~2 ng / $\mu$ l	~20 ng			
	1000 - 2000 bp	~4 ng / $\mu$ l	~40 ng			
	2000 - 4000 bp	~6 ng / $\mu$ l	~60 ng			
	> 4000 bp	Treat as plasmid	Treat as plasmid			

All direct shipments should be sent to:

GENEWIZ  
 Attn: Primer Walking  
 733 Concord Ave.  
 Cambridge, MA 02138  
 USA  
 617-300-0184

You will need to prepare a gel image and save as a .jpg to upload for the order. If sending multiple products, label each amplicon. on the image.

