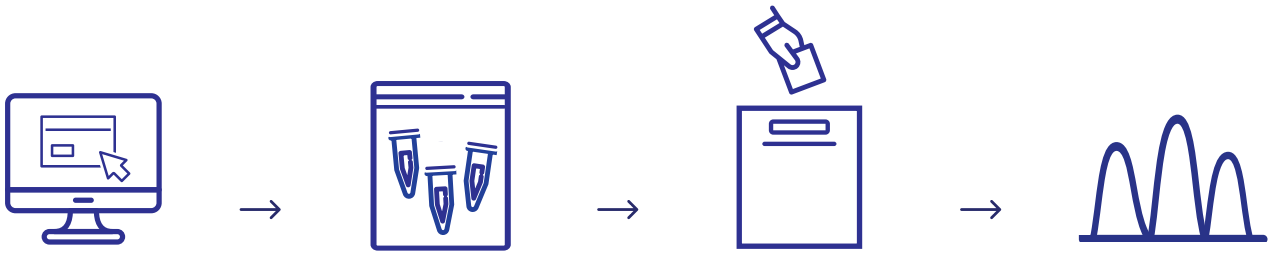
Sample Preparation Guidelines



Order Online
Log in to your account at etonbio.com to place an order in just a few minutes.

Prepare Samples
Label your tubes/plates and make sure they are properly sealed to prevent leakage or evaporation. Place samples in a bag with your order number.

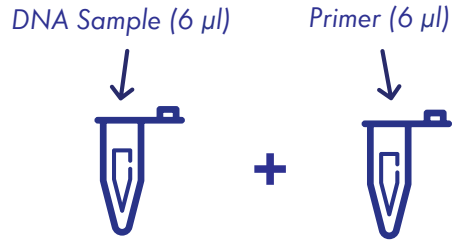
Submit your Samples
Customers with local pickup should place samples in their Eton dropbox by the cutoff time. Mail-In customers should mail samples to thier regional Eton branch.

Get Results
Results will be emailed to you as soon as they become available. You will also have access to all your orders through your account.

- Label each tube individually instead of using tape to physically group multiple samples.
- Include name and email in addition to the order number in case the order number is mislabeled.
- If possible use ethanol resistant markers when labeling tubes to prevent writing from rubbing off.
- You may use parafilm to secure tubes to reduce the risk of cracking and sample evaporation.
- Place primers in order of usage if using a 96-well plate for submitting a large volume of primers.
- Choose the *"To Be Saved"* option, if you would like us to store primers for 3 months. *(default is 2 weeks)*
- Submit *"To Be Saved"* primers in 1.5 mL microcentrifuge tubes instead of PCR/strip tubes

Plasmid & PCR Samples - Regular Sequencing *(Per Reaction)*

Sample Type	Concentration
Plasmid < 6 kb	50-150 ng/µl
Plasmid > 6 kb	75-150 ng/µl
Unpurified PCR Product*	N/A
Purified PCR Product < 1 kb	5-15 ng/µl
Purified PCR Product > 1 kb	10-30 ng/µl
ExoSAP-it Treated PCR Product*	N/A



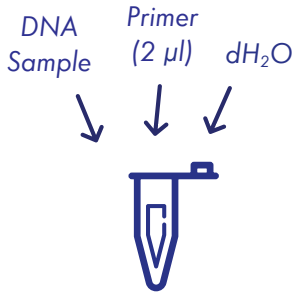
Primer concentration should be 5 uM or 5 pmol/µl. (40 ng/µl)

- **Please provide 6 µl of sample for each reaction.**
- Please submit samples & primers in separate tubes that are clearly labeled.

Concentrations not required for ExoSAP-it treated or unpurified PCR products. Please DO NOT dilute these type of samples.

Premixed Samples - Regular Sequencing *(Per Reaction)*

Sample Type	Template Concentration (ng/µl)	Template Volume Needed (µl)	dH_2O Volume Needed (µl)
Plasmid < 6 kb	~ 40 - 70	4.5	5.5
	~ 70 - 100	3	7
	~ 100-140	2	8
	~ 140 - 180	1.6	8.4
	~ 180 - 220	1.2	8.8
Plasmid 6 - 10 kb	~ 50 - 80	4.6	5.4
	~ 80 - 120	3	7
	~ 120 - 160	2	8
	~ 160 - 200	1.6	8.4
	~ 200 - 240	1.4	8.6
Plasmid >10 kb	~ 90 - 110	3.5	6.5
Purified PCR Products < 1 kb	~ 5 - 15	3	7
	~ 20 - 30	1.2	8.8
Purified PCR Products > 1 kb	~ 15 - 25	4	6
	~ 35 - 45	2	8



Total Volume = 12 µl

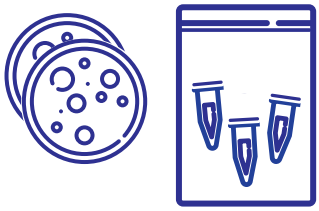
Primer concentration should be 5 uM or 5 pmol/µl. (40 ng/µl)

- For each premixed reaction please submit your sample, primer, and dH_2O in one tube.
- Use the table to see how much sample & dH_2O to provide. Then add 2 ul of primer for a total volume of 12 µl.
- Primer concentration should be 5 uM or 5 pmol/µl. *(40 ng/µl)*

NOTE: Premixed reactions are not eligible for free repeats.

Colony & Culture Samples - Direct Sequencing (DSC)

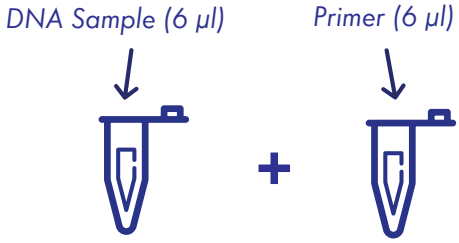
Culture: Submit 100 µl of culture in single tubes or 96-well plates.
Picked Colony: Submit in 5-6 µl of dH_2O in single tubes or 96-well plates.
Agar Plate: Label colonies of interest on the plate(s) or include picking instructions.



- If submitting culture samples please swirl before aliquoting.
- If submitting agar plates please make sure to keep replica plates for your own records.

Difficult Templates - Special Sequencing

Sample Type	**Concentration**
Plasmid < 6 kb	100-200 ng/µl
Plasmid > 6 kb	150-250 ng/µl



- **Please provide 6 µl of sample for each reaction.**
- Used for samples with difficult templates, secondary structures, or high GC content.
- Please submit samples and primers seperately (not premixed).

NOTE: Special Sequencing reactions are not eligible for free repeats.

General Recommendations

Ordering & Sample Pickup

You may include notes about your samples, pickup location or any extra info you feel like our technicians should be aware of in the special instructions box of the online order form. Daily cutoff times vary by city and location. Email **sales@etonbio.com** or your Eton sales rep for details. Sample pickup dropboxes can be set up upon request.

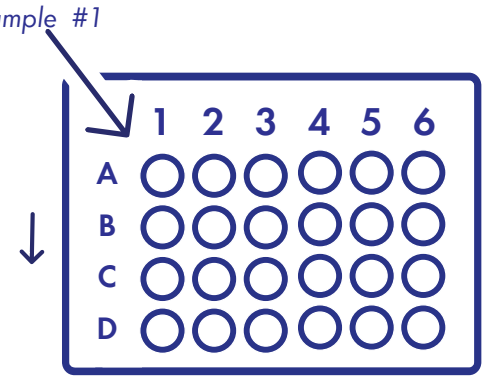
Sample Storage at Eton

Samples & primers we recieve are stored at our facilities for two weeks only in a 4°C refrigerator. After two weeks are up, we will properly discard of your samples. If you have a special situation in which you need the samples stored for longer than two weeks, please notate it on your order in the special instructions and also email our support team to let them know.

Troubleshooting and Repeats

If you were not satisfied with your results, please contact our customer service department by email at **support@etonbio.com** and we will be happy to troubleshoot your order. Upon review of the data, we can set up one-time repeats free of charge for failed reactions or reactions with unsatisfactory quality. The original reaction will still be charged but you will not be charged for the repeat reactions. To qualify for free repeats samples must meet concentration requirements and must not be premixed.

Submitting Samples in 96-well Plates



Following this format will minimize technical errors and expedite processing times.

- Load samples vertically, starting with sample #1 in well A1, sample #2 in B1, etc.
- If submitting a partial plate, please do not skip any wells.
- If providing many different primers for one order, we prefer them in tubes.
- If possible, please place samples which use the same primer close together.

Submitting DSC samples in a 96-well Plate.

- Do not use a cell culture plate and instead use a deep well plate or a PCR plate.
- Ensure that the seal over the plate is very tight to prevent leakage into other wells.

Additional Notes

- Samples should contain only DNA and dH_2O and be free of salts, EDTA, alcohol, protein, RNA, detergents, cesium and phenol.
- If you have a plasmid product, make sure all bacterial genomic DNA is removed.
- We recommend submitting only a few samples to test out your conditions before submitting a large order in the following situations*:
  - *You suspect a problem with your template.*
  - *You are testing a new template preparation method.*
  - *You are using untested primers.*
- It is important that the 260/280 ratio of your DNA sample is between 1.8 and 2.0. Otherwise your reaction may fail or have poor quality results due to contamination or improper concentration.
- We highly recommend that you run your templates on agrose gel before submission. There should be one clearly defined band on the gel representing a particular template. Please make sure to have appropriate gel percentage, duration and voltage.
- For PCR products, please remove or disable and unincorporated dinucleotides.
- Certain sequence motifs and secondary structures may prevent high-quality results. In these situations, our support team can work with you to formulate a sequencing strategy that will yield the best possible results.
- At this time we do not sequence 16s or genomic DNA samples.