



BioXp[®] mRNA synthesis kits with CleanCap[®] Reagent AG User Guide

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Introduction to mRNA synthesis on the BioXp system

The BioXp system allows you to synthesize purified capped and tailed mRNA. The input to the workflow is a digital sequence with a length between 0.4kb and 1.8kb, and the output material will be newly synthesized mRNA as well as the DNA template used as the input for the in vitro transcription step of the process. The BioXp mRNA synthesis kit contains all the necessary reagents to synthesize synthetic mRNA. The instrument run begins with *de novo* synthesis of error-corrected gene fragments generated by using the BioXp system. The DNA template is purified and used for in vitro transcription, followed by co-transcriptional capping, enzymatic addition of a poly (A) tail, and mRNA purification.

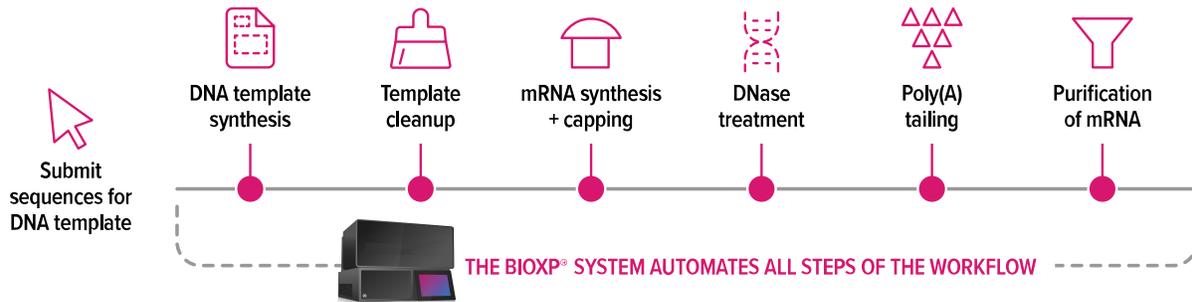


Figure 1. Graphical representation of the end-to-end steps to generate mRNA from a digital sequence on the BioXp system.

Overview of the BioXp mRNA synthesis workflow

The workflow below describes the steps done to produce *de novo* mRNA using the BioXp system from a digital sequence.

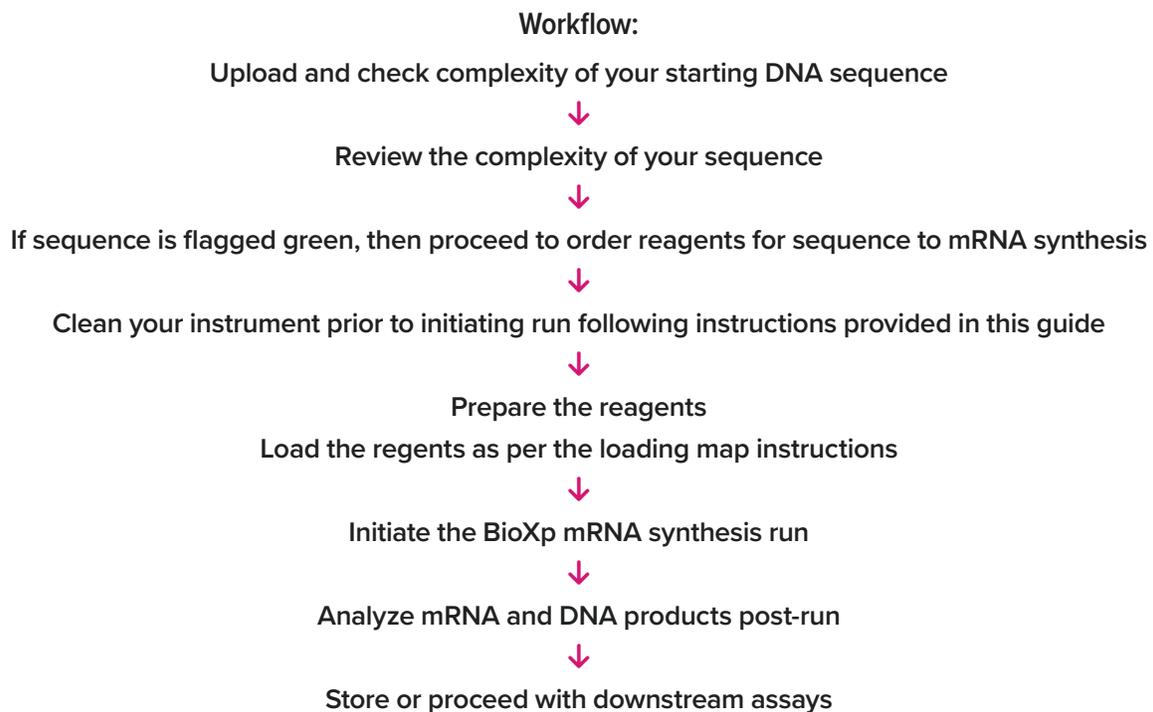


Figure 2. End to end workflow to generate mRNA on the BioXp 3250 : The user can upload sequences using the myBIOXP and once they submit the sequence, checks are performed to ensure that the sequence is qualified to be built into mRNA. Once the user submits an order, they will receive the reagent kit within 7 business days of order. They can then proceed with generating the DNA template and the mRNA and within 18 hours they will have mRNA as well as DNA template to recover and perform quality checks and use in their downstream applications.

Input requirements and guidelines for mRNA sequences

1. Acceptable length of sequence : 300-2000b; 300-400b and 1800-2000b are outside of the current complexity requirements but will still be allowed to proceed for ordering (see below).
2. Sequence features to include: 5' UTR , Kozak sequence, ORF and 3' UTR sequences.
3. Sequence features to exclude: T7 Promoter sequence, and poly-A sequence. The T7 promoter sequence will be added automatically by the system. Inclusion of the sequence will flag an error.
4. For the CleanCap (AG) kit, the following promoter sequence will be added: TAATACGACTCACTATAAGG.
5. For the BioXp workflow, since the poly-A sequence feature will be added enzymatically after transcription, do not add the poly-A sequence while submitting the DNA template sequence through the portal.

Initiating your mRNA project and ordering your mRNA through myBioXperience

Visit the Telesis Bio ordering platform, **myBioXperience** to initiate a new project. If you're new to myBioXperience, you will need to register and provide your work email, shipping, and billing information. Once registered, you will be able to access myBioXperience and initiate a new project, and order your sequences. You can submit one or multiple sequences for use with the BioXp 3250 systems. For this workflow, up to 16 sequences may be submitted and built in one run of the BioXp 3250 systems. The supported number of reactions is from 1 to 8 using an 8-reaction kit, or 9 to 16 using the 16-reaction kit size.

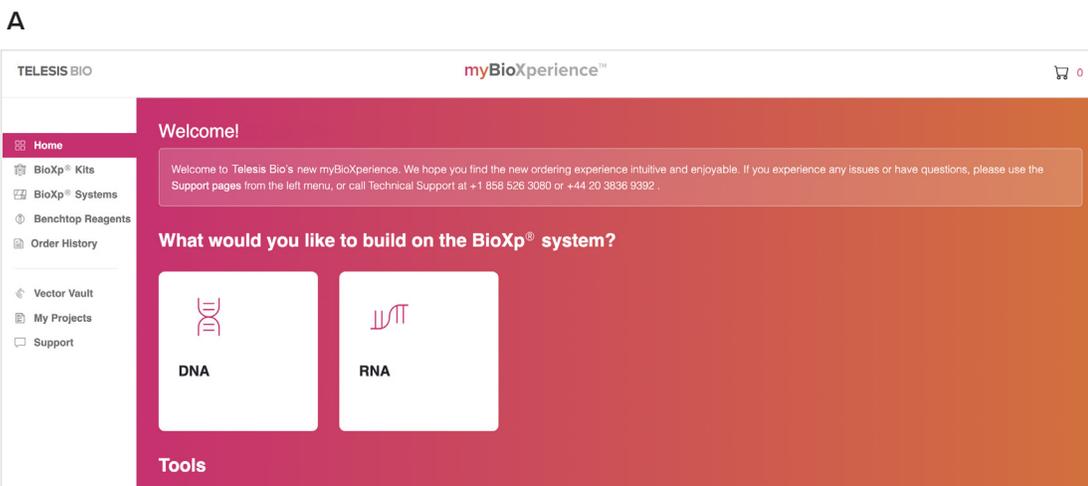


Figure 3. Screenshots of MyBioXperience showing A) selection of the mRNA kit options once you have logged into myBioXperience (continued on next page).

B

1. Upload 2. Design 3. Review ← BACK

Project name

BioXp[®] mRNA Synthesis Kits, Modified, CleanCap[®] AG (3'OMe)

Contains all the Gibson Assembly[®] reagents necessary to synthesize up to ten micrograms of biologically active capped and tailed synthetic mRNA using de novo synthesized, error-corrected gene fragments (DNA template) of up to 1.8 kb in length. Final output includes both the single-stranded mRNA and the linear double-stranded DNA fragments. Note: This kit includes N1-methyl-pseudouridine and the CleanCap[®] AG (3'OMe) cap analog

[LEARN MORE](#)

Drag and drop files.
Accepted file formats: CSV, Genbank, FASTA/text

[UPLOAD FILE](#)

OR

You can use below options

[Create sequences](#) | [Download file templates](#)

NEXT: DESIGN

C

TELESIS BIO myBioXperience™

1. Upload 2. Design 3. Review ← BACK

Test2

[NEXT: REVIEW](#)

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<input type="checkbox"/>	Sequence name	bp count	Flanking sequence added	Complexity	Action
<input type="checkbox"/>	1 1600_N_AG_Correct	1601	--	✓	⋮

D

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1. Upload 2. Design 3. Review ← BACK

Test3

[NEXT: REVIEW](#)

Your sequence is not qualified for purchase, please return to the first step and reupload.

[DOWNLOAD SEQUENCES](#)

<input type="checkbox"/>	Sequence name	bp count	Flanking sequence added	Complexity	Action
<input type="checkbox"/>	1 1600_N_AG_Incorrect	1620	--	✗	Edit ⋮

Figure 3 (continued). B) Selection of the specific mRNA kit and upload of the sequences of interest. C) Shows a screenshot when the sequence submitted by the user has passed the complexity checker and has been successfully added into the cart. D) Shows a screenshot when the sequence submitted by the user has not met the requirements for it to be built successfully.

Sequence submission and complexity check:

Select the mRNA application option to get started. Upload your sequence(s) by using the upload tool or pasting your sequence (Figure 3B). The supported sequence formats are: CSV, Genbank and FASTA/text. Once uploaded, your sequence will be displayed as illustrated in Figure 3. Proceed with the complexity check by selecting the button on the right side of the uploaded sequence. After the complexity check is done, if the sequence(s) are “green” or “yellow”, you may proceed to order. For sequences flagged “yellow”, you may still proceed accepting the risk that the synthesis is not fully guaranteed as the sequences flagged “yellow” are considered outside of our complexity requirements. If the sequence is flagged “black”, you will not be able to proceed forward.

If you have an incorrect sequence, then you will get a message that says your sequence is not qualified.

When you click next it will show you a summary of your order and at this point you can add the order to the cart.

Complexity requirements

Length – The current maximum recommended length for the uploaded sequence is 1.8 kb and the lower limit is 0.4 kb. However, the portal allows upload of sequences between 0.3-2.0kb, but sequences with length between 0.3-0.4kb and 1.8kb-2.0kb will be flagged “yellow” as they are outside the recommended length requirements for this kit. Sequences shorter than 0.3kb and longer than 2kb will not be uploaded and these will be flagged “black”. Sequences flagged “black” may not be ordered under any circumstance. For further questions regarding the length limit, please contact our support team at help@telesisbio.com.

GC content – sequences with GC content in the range of 20% - 70% are supported for sequences of length 0.3-1kb. For sequences of length between 1 – 2kb, GC content of 40%-60% is supported.

In addition to the length and the GC content, our Complexity checks also include analysis of the GC extent, presence of homopolymers and repeats. A sequence may be flagged accordingly if any of these parameters exceed the criteria for synthesis. Please contact our technical support team at help@telesisbio.com for additional questions about your sequences and requirements.

Preparing the BioXp system prior to run

It is imperative that the system is free from contaminants that could interfere with the synthesis of the mRNA. Spray an RNase decontamination solution, such as RNase AWAY™ (Thermo Fisher cat. No. 700TS1) onto a lint-free wipe and decontaminate all the exposed BioXp system surfaces, including the four pipettors of the pipette head. Do not spray the solution directly on the BioXp as this may leave residual liquid which could interfere with proper functioning of the instrument. To further decontaminate the system, spray 70% ethanol (RNase-free) or 70% isopropanol (RNasefree) onto a lint-free wipe and clean the same surfaces. Again, do not spray directly into the BioXp system, as this could also damage the circuitry in the system. Once the surfaces are dry, proceed towards loading reagents.

Thawing and preparation of reagents

Thaw the mRNA synthesis plate (stored at -80°C) at room temperature (+25°C) for 30 minutes prior to placement on the BioXp system deck. Freshly prepare 12ml of 70% ethanol using nuclease-free water and add it to the designated reservoir before the run.

Load ethanol reservoir in the rightmost reservoir retainer position of the instrument deck (component #3 in Fig. 4). Do not discard the ethanol reservoir after the run as this is a reusable unit and will be needed for future runs.

Understanding the BioXp system

In preparation for initiating the run, it is helpful to understand the various components of the deck in order to correctly place all the reagents and consumables into the system. Familiarize yourself with the BioXp 3250 system deck:

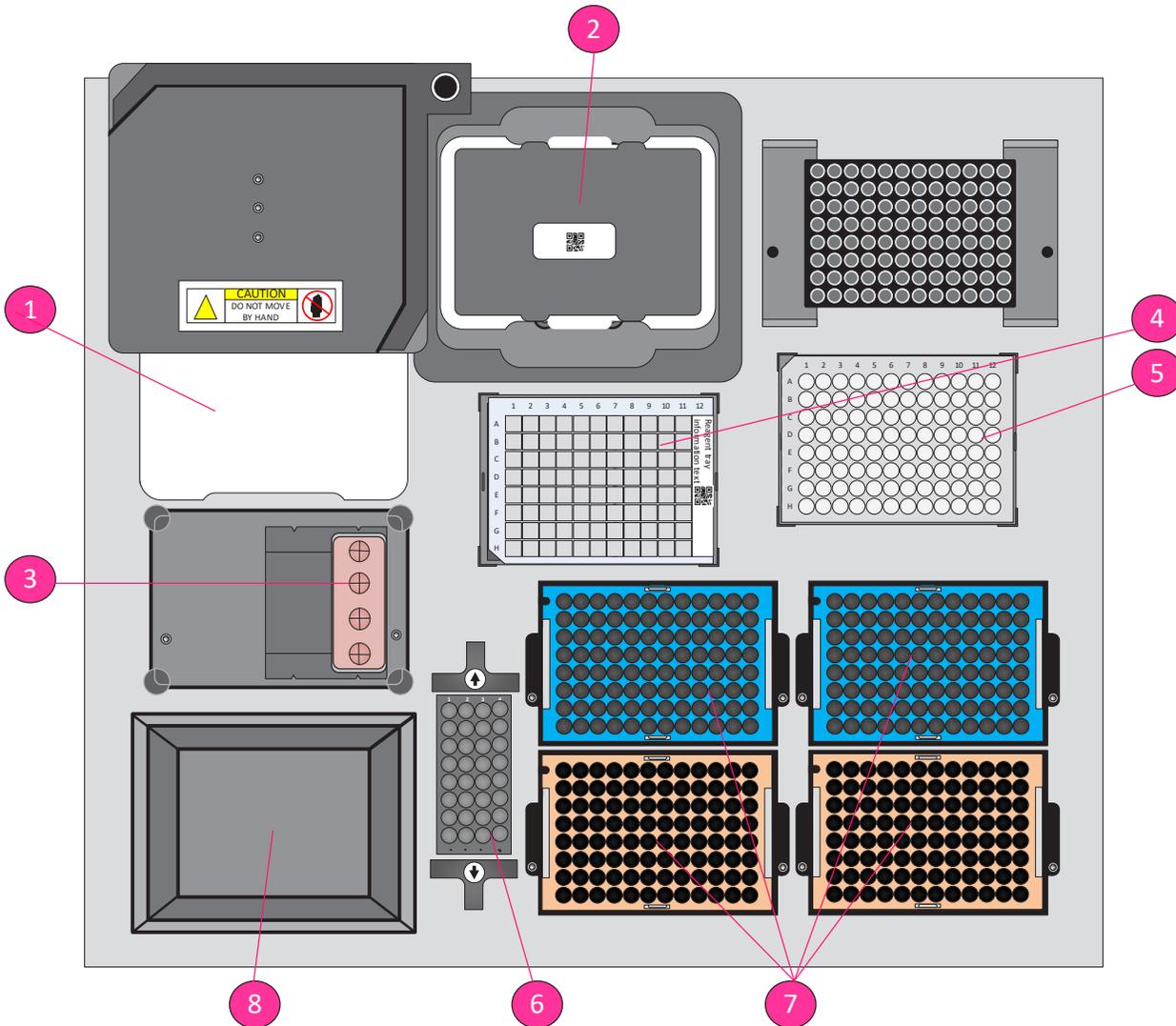


Figure 4. Image of the instrument deck with the components listed below labeled.

The key components are:

1. Thermal cover
2. Oligo vault location
3. Ethanol reservoir holder
4. Synthesis plate start point
5. Recovery plate start point
6. Reagent strip location
7. Location of tip trays

8. Waste bin location

Loading the BioXp system with the BioXp mRNA synthesis kit

Loading of reagents and consumables:

1. Load the recovery plate onto the recovery chiller with the notch in the upper left corner. Ensure a snug fit of the recovery plate. If it does not fit right, the orientation of the plate may not be correct.
2. Load the Oligo Vault™ plate so that the notch is positioned in the upper left corner of the thermocycler. Similar to the recovery plate, the Oligo Vault™ has directionality. If it does not fit right, the orientation of the plate is likely to be incorrect.
3. Follow these steps to load the appropriate purification strips depending on the reaction size of your kit (8 or 16):
 - a. For an 8-reaction run, load the (black) BX2300-16 BioXp DNA purification strip into position 1. Then, load the (white) BX3300-16 BioXp RNA purification strip into position 3.
 - b. For a 16-reaction run, load the (black) BX2300-32 BioXp DNA purification strip into position 1. Then, load the (white) BX3300-16 BioXp RNA purification strip into position 3 and the (white) BX3300-32 BioXp RNA purification strip into position 4.

Note: Do not forget to secure the spring-loaded arms while holding the strips securely in place.

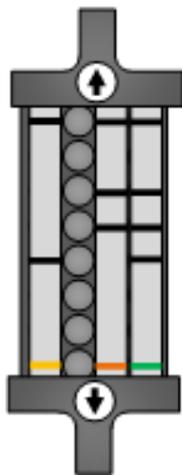


Figure 6. Shown here is an image of the strip holder with correctly loaded DNA and mRNA purification strips for a 16-reaction CleanCap mRNA synthesis kit.

4. Spin the thawed BioXp mRNA synthesis plate for one minute at 500 rcf. Visually inspect the wells to ensure they are completely thawed before loading the plate. Load the BioXp mRNA synthesis plate onto the reagent chiller, with notch in the lower left corner and barcode on the right. Note: Please ensure that the plate is properly seated within the chiller.
5. Load freshly prepared 70% ethanol using the ethanol reservoir at the rightmost reservoir retainer position of the instrument deck.
6. Load fresh (previously unopened) tips by aligning the tip tray notch with the upper left corner of each tip tray retainer. Load 1 × 50 µL tips (tray 1) for an 8-reaction job, or load 2 × 50 µL tips (trays 1 and 2) for a 16-reaction run. Load 2 × 200 µL tips (trays 3 and 4) for both 8 and 16 reaction kits. Please note that similar to the reagent plates, the tip trays have directionality. If the

tip tray does not snap into place, it is not placed correctly. Rotate the tray 180° and try to place the tips again. The tip tray should have a snug fit and not wiggle.

Initiating the BioXp mRNA synthesis run

Prior to initiating the run, refer to the Loading Map document to ensure that all reagents and consumables have been correctly placed onto the BioXp system deck. Once confirmed, close the system door and press Start to begin the run. The typical run time will be about ~18 hrs. It is recommended that freshly synthesized mRNA, maintained at 4°C after the run completion on the BioXp, be removed immediately and stored at -80C or used for downstream assays.

Retrieving your mRNA

Once the run is complete, the User Interface will indicate that the run is done, see figure 7 for reference.

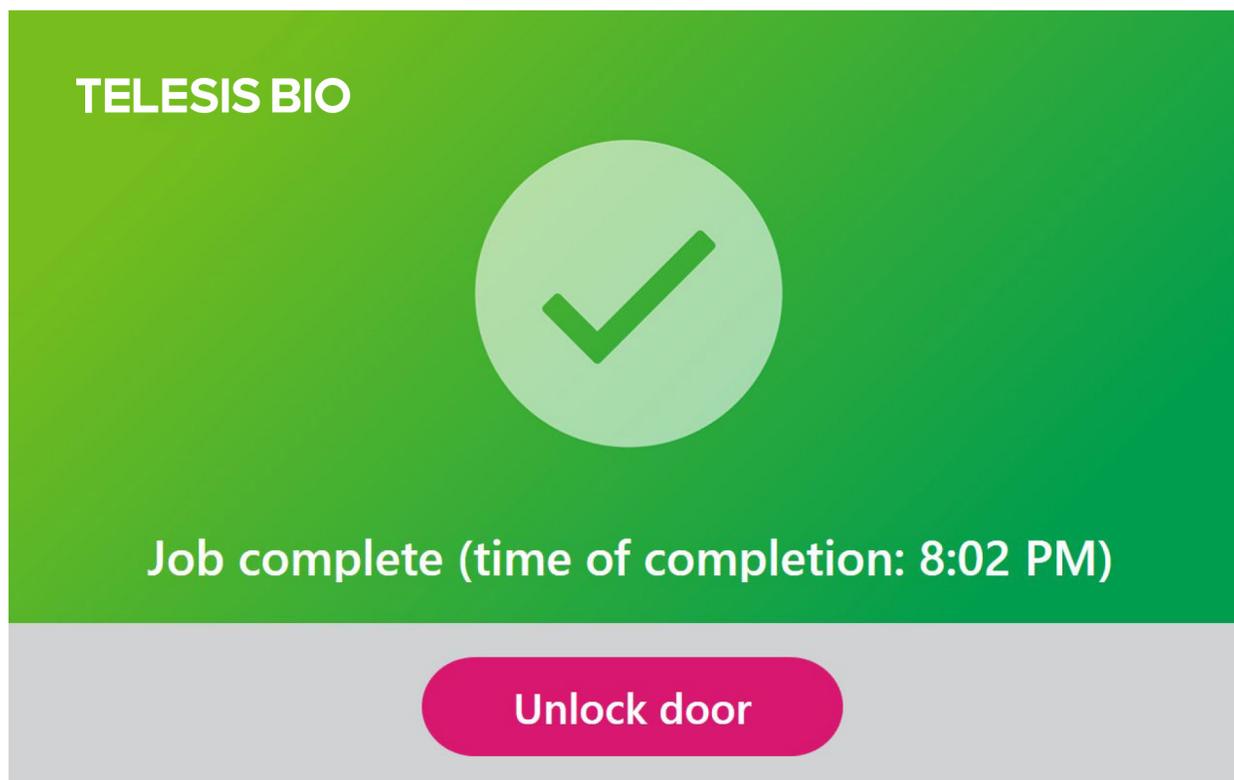


Figure 7. User screen on the BioXp 3250 when the run is completed.

The newly synthesized mRNA is located in column 1 for an 8-reaction run, or columns 1-2 for a 16-reaction run of the recovery plate.

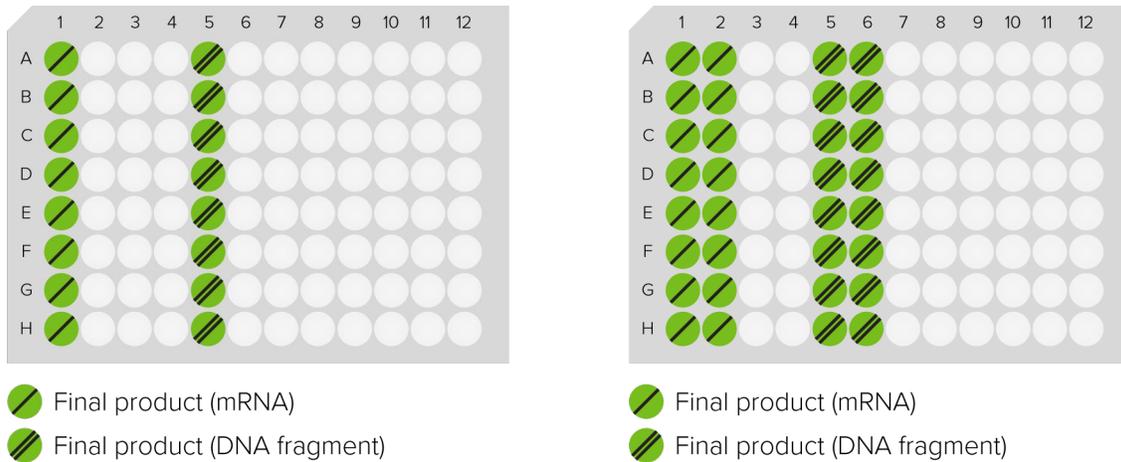


Figure 8. Included in the recovery plate you will also find the newly synthesized DNA template for your sequences. These will be available on column 5 for an 8-reaction run, and columns 5 and 6 for a 16-reaction run.

mRNA storage recommendations

The newly synthesized mRNA is suspended in 1mM Sodium Citrate buffer (pH 6.4) and is ready for use in QC and downstream assay. We recommend aliquoting the mRNA into appropriate volume and store at -80°C . The mRNA in the product plate has been tested to withstand up to three freeze-thaw cycles. While it is best practice to store mRNA at -80°C to ensure long-term stability, the product plate can also be stored at -80°C for up to a month.

Quality check of newly synthesized mRNA

Prior to use of the mRNA in downstream assays, it is highly recommended that you evaluate the success of the synthesis of both the DNA and the mRNA. We recommend measuring concentration of the mRNA using fluorescence-based assays (Qubit or Ribogreen or QuantiFluor) and checking the quality of the mRNA synthesized using a Bioanalyzer or a TapeStation.

Qubit Assay to determine mRNA concentration

The Qubit Assay is a fluorescence-based method that uses target-selective dyes when specifically bound to RNA, to emit a fluorescence signal for RNA concentration determination. It is more sensitive and specific to RNA compared to the UV absorbance-based measurement. We recommend the Qubit RNA BR (Broad-Range) Assay Kit to measure mRNA concentration accurately and specifically.

Materials required for Qubit assay

Nuclease Free Water, RNase-free PCR tubes or plates, RNase-free 5 ml clean Eppendorf snap cap tube (Eppendorf, Cat. No. 0030108310), Qubit RNA BR assay kit – Broad Range (ThermoFisher Scientific, Cat. No. Q10211), Qubit Assay Tubes (ThermoFisher Scientific, Cat. No. Q32856), and Qubit® 3.0 Fluorometer.

Recommended Protocol

- To perform Qubit Assay, the mRNA outputs (column 1 or columns 1 and 2 in the output plate) generated by the BioXp system need to be diluted 5 to 10-fold. Mix 18 μl of RNase-free water with 2 μl mRNA of each sample for the dilution in a chilled 96-well plate. Maintain the diluted sample on ice. Remember to keep the output plate on ice throughout the assay.

- To prepare the Qubit BR buffer Master Mix, calculate the required amount of Qubit BR buffer (Component B) and Qubit RNA BR Reagent (Component A) for each sample. In general, one reaction volume requires 200 µl of Qubit BR buffer (Component B) and 1 µl Qubit RNA BR Reagent (Component A) per sample/Standard Control. Include three additional reaction volumes in the final master mix for the Standards (2) and potential pipetting volume loss. For example, to quantify 8 mRNA samples, use 11 reaction volumes (2.2 ml of Qubit BR buffer (Component B) with 11 µl Qubit RNA BR Reagent (Component A)) for the Master Mix.
- Aliquot 198 µl (for the mRNA sample) /190 µl (for the Standard Controls, Component C and D) of the Master Mix to the clean Qubit Assay Tubes (ThermoFisher Scientific, Cat. No. Q32856). Mark each tube before adding the samples and Standard Controls.
- Add 2 µl of each diluted mRNA sample or 10 µl of The Standard Controls (Component C and D) to the designated tubes. Mix each tube by vortexing 3 to 5 seconds, spin down briefly in a centrifuge and incubate at room temperature for 3-5 minutes.
- For the concentration quantification, select the Qubit RNA BR Assay and calibrate the instrument every time before use by using the Standards 1 and 2 in that order by selecting 'read standard' at the bottom of the screen. The Fluorometer will show the measurement range on its screen once two Standard Controls are measured.
- Place each sample tube for the measurement. Set the product volume to 2 µl as the input and select the 'read tube' on the bottom of the screen. Once done, record the concentration of each sample.
- To make sure the measurement is correct, re-measure the Standard Control 2 again at the end, the concentration of Standard Control 2 should be around 500 ng/ µl + 5%.
- The output volume for the BioXp run would be ~70 µl of mRNA. The concentration measured using Qubit would be processed as follows to get the yield:
 - $[\text{Qubit concentration} \times 10 \text{ (dilution factor)} \times 70 \text{ (volume)}] / 1000 = X \text{ µg of mRNA yield per well.}$
- Once the concentrations are determined, proceed to the mRNA TapeStation analysis.
- The yield specification for the small-scale mRNA synthesis kit is > 5µg/well. The mRNA yield is highly dependent on the length and the sequence of the mRNA. Yields of 100 µg/well have been observed for this kit.

mRNA Yield			mRNA Yield		
Well	Conc. (ng/µL)	Yield (µg)	Well	Conc. (ng/µL)	Yield (µg)
A1	1410	98.7	A2	868	60.67
B1	919	64.33	B2	557	38.99
C1	766	53.62	C2	543	38.01
D1	722	50.54	D2	531	37.17
E1	618	43.26	E2	548	38.36
F1	674	47.18	F2	728	50.96
G1	557	38.99	G2	501	35.07
H1	204	14.28	H2	165	11.55

Table 1. Shows the concentration of the mRNA measured by Qubit in 2 replicates.

TapeStation Electrophoresis Analysis

The mRNA TapeStation electrophoresis is an assay to examine the mRNA size as well as the purity when produced by small-scale mRNA synthesis kits.

Required materials

Nuclease free water, RNase-free PCR tubes or plates, Agilent TapeStation strip tube (and cap) (Agilent, Cat. No. 401425 & 401428), 96-well Armadillo plate, clear wells (ThermoFisher Scientific, Cat. No. AB2396), 96-well plate foil seal (Agilent, Cat. No. 5067-5154), Agilent RNA ScreenTape (Agilent, Cat. No. 5067-5576), Matrix tips (Agilent, Cat. No. 5067-5599), Agilent ScreenTape Sample Buffer (Agilent, Cat. No. 5067-5577), Agilent ScreenTape Ladder (Agilent, Cat. No. 5067-5578), Vortex Mixer, Microcentrifuge/Low speed platefuge, TapeStation System (4200) with Laptop.

Protocol

- This step should be performed after the RNA Qubit assay. Allow the ScreenTape Sample Buffer to equilibrate at room temperature for 30 minutes, vortex before use. Place and thaw the ScreenTape Ladder. Remember to keep the output plate on ice throughout the assay.
- Dilute the RNA samples to about 50 ng/μl in concentration. For example, if the mRNA concentration is 1000 ng/μl, dilute the sample 20-fold by mixing 2 μl of RNA with 38 μl of nuclease free water to reach 50 ng/μl, gently mix the diluted sample. Place and maintain the diluted samples on ice. Use nuclease-free water for dilution.
- Once dilution is completed, aliquot 5 μl of Agilent TapeStation buffer to each well in the Agilent TapeStation strip tube, briefly centrifuge the reagent to the bottom of the tube.
- Add 1 μl of the ScreenTape Ladder at the position A1 in the strip tube and add diluted RNA samples to the other wells in the strip tube.
- Place caps to the strip tubes or foil seals to the 96-well plate.
- Mix the liquid using the IKA MS3 vortexer at 2000 rpm for 1 minute, tape down the strip tube or the 96-well plate to the vortexer before vortex mixing.
- Spin down the ladder and samples using microcentrifuge/low speed platefuge for 30 seconds.
- Denature the ladder and samples at 72 deg C for 3 minutes.
- Immediately place the ladder and on ice for 2 minutes.
- Spin down the ladder and samples again for 30 seconds.
- Load the ladder and samples into the TapeStation System (4200). Remember to place the strip tube with ladder in the position A1 on the strip holder.
- Flick the RNA ScreenTape and tap it to make sure there are no bubbles in the reagents. Insert it into the ScreenTape nest in the TapeStation instrument. Make sure to fill in the tips within the tip holder.
- Carefully remove the strip tube caps. Make sure the liquid is positioned at the bottom of each well. Once completed, close the TapeStation system cover.
- Set up the TapeStation Controller software, select **Run a ladder** and how many wells are required for the assay. Click **Start** to start the run.
- The results will be automatically displayed on the TapeStation Analysis software once the electrophoresis is completed.
- At the end of the run, normalize the gel according to the tallest peak of each sample shown on the electropherogram by clicking **Scale to Sample** on the Display section.

An Example TapeStation result of 16-reaction mRNA synthesis kit. The first lane shows the bands of ScreenTape ladder range from 25 nt to 6000 nt.

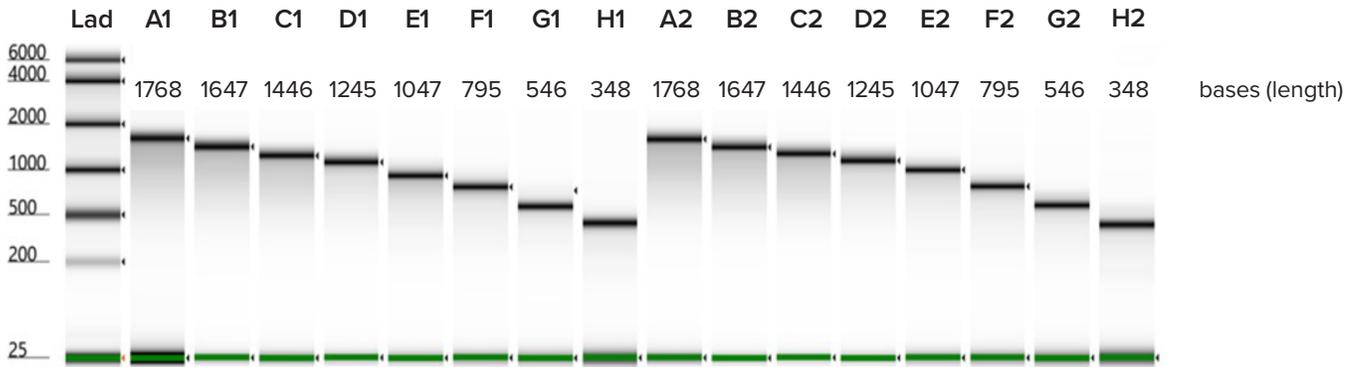


Figure 9. Shows a run image of a TapeStation run for the mRNA generated in 2 replicates. Lanes labeled A1 to H1 and again, A2 to H2 show the mRNA sample bands varying size from 1800b to 300b. Specifically, 1800b, 1600b, 1400b, 1200b, 1000b, 750b, 500b and 300b. The green band at the bottom of the gel in each lane is the 25 nt control mRNA from the ScreenTape Sample Buffer that tracks and indicates that the electrophoresis for mRNA sample in that lane was successful.

As part of the quality check, it is also recommended that the DNA is also analyzed using an agarose gel or an E-gel. Depending on the type of the gel 2-5µl of the DNA template from the output plate (Column 5 or Columns 5 and 6) will need to be loaded. An example gel demonstrating DNA template synthesis.

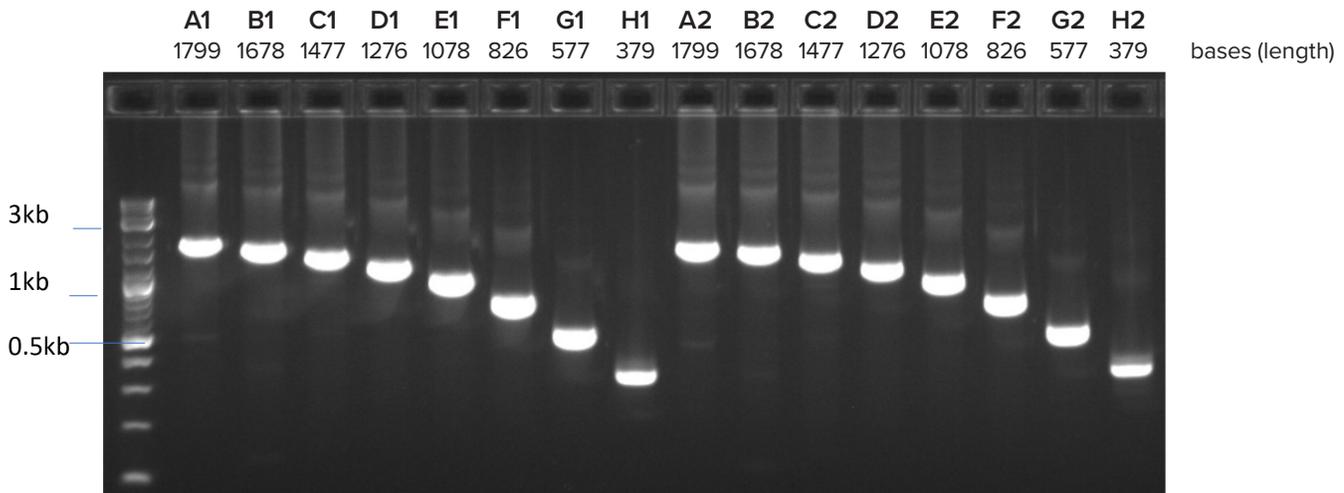


Figure 10. Shows an agarose gel image of the DNA template that was synthesized used to generate the mRNA. Lanes labeled A1 to H1 and again, A2 to H2 show the DNA template bands varying in size from 1800bp to 300 bp. Specifically, 1800bp, 1600bp, 1400bp, 1200bp, 1000bp, 750bp, 500bp and 300bp. (Ladder: 1kb Plus DNA Ladder, New England Biolabs, Catalog number: N3200S).

Troubleshooting Recommendations

Problem	Cause	Proposed Solution
mRNA is longer than DNA	Enzymatic polyA tailing adds a median of 100 bases	Expect ~100 bases added to DNA template
mRNA is truncated	DNA template is truncated	Redesign DNA template to obtain full length assembly; contact help@telesisbio.com for design assistance
mRNA is truncated but DNA template built to the expected size	Presence of T7 terminators in the sequence	Redesign DNA template to avoid T7 terminator like sequences
mRNA is expected size, but additional small molecular weight species are present	Full-length DNA template was built, but there off-target products were assembled as well	Gel-purify full-length DNA template and repeat IVT on bench if there is no tolerance for presence of smaller species; alternately, redesign DNA template
There is no mRNA product	DNA template DID NOT build	Redesign DNA template to obtain full length assembly; contact help@telesisbio.com for design assistance
There is no mRNA product	DNA template DID build	Test product for RNase contamination; decontaminate BioXp

Table 2. Shows recommendations for troubleshooting when you have a problem with the intended use of the BioXp small scale mRNA Kit.

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