Notices

Limitations of use

For research use only. Not for use in diagnostic procedures. Unless otherwise agreed to in writing, IDT does not intend these products to be used in clinical applications and does not warrant their fitness or suitability for any clinical diagnostic use. Purchaser is solely responsible for all decisions regarding the use of these products and any associated regulatory or legal obligations.

Safety data sheets pertaining to this product are available upon request.

Safety Notices

Reminder symbols call attention to minor details that may be easily overlooked and compromise the procedure resulting in decreased assay performance.

Caution symbols denote critical steps in the procedure where risk of protocol failure or damage to the product itself could occur if not carefully observed.

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Additional resources

View videos and additional resources for Archer products at http://archerdx.com/videos.

Technical support

Visit https://support.archerdx.com for a list of helpful answers to frequently asked questions or contact us directly at archer-tech@idtdna.com.

Overview

Intended use

The Archer PreSeq DNA QC protocol is intended for research applications in conjunction with Archer reagent kits and corresponding target-enrichment panels to produce high-complexity libraries for use with Illumina next- generation sequencing (NGS) platforms.

Test principle

The Archer PreSeq DNA QC Assay utilizes quantitative PCR (qPCR) to identify DNA samples of sufficient quality to produce adequate sequencing library using Archer VariantPlex assays. The Archer DNA QC Assay is used to evaluate the quantity of amplifiable DNA in a sample in relation to a known Assay Standard. This SYBR® Green assay amplifies a 100bp genomic DNA sequence in the sample and the Assay Standard in two separate reactions. A comparison of the two resultant quantification cycle (Cq) values results in a ΔCq, or DNA QC Score. The DNA QC Score holds predictive value for library yield and can be used to gauge the amount of input material required for successful Archer VariantPlex library preparation.

Using amplifiable genomes to determine recommended starting input amounts:

1. Calculate the DNA QC Score for each sample:

$$\textbf{DNA QC Score} = \textbf{Sample } Cq_{AVG} - \textbf{Standard } Cq_{AVG}$$

2. Visit archerdx.com/genome-calculator to calculate amplifiable genome equivalents per nanogram or use the following equation:

$$\textbf{Amplifiable genomes per ng} = 2^{-(\text{DNA QC Score})} \times 64{,}626$$

3. Review application-specific minimum input requirements. Thresholds and pass criteria should be set experimentally. The values below are recommended minimums.

Application	Required Quantity of Amplifiable Genomes
4nM library yield	1,100
Archer Analysis Variations QC Pass	3,750
≥2% allelic frequency mutation detection	6,500
CNV calling (≥3-fold change)	1,900

4. Adjust input quantity as needed.

Using DNA QC score for input recommendations

The DNA QC Score obtained is a direct measurement of functional DNA templates, which provides an indicator of library yield and library quality metrics when using Archer VariantPlex assays.

Samples with DNA QC Scores greater than 11 have a lower probability of yielding sufficient library to sequence; the addition of more input will significantly increase this probability. Samples with DNA QC Scores above 15 are typically too low in quality to produce sufficient library yield or quality sequencing metrics, regardless of input amount. Samples with DNA QC Scores above 15 are not recommended for library preparation.

PreSeq DNA QC Assay Reagents (AK0067-16)

Store at -30°C to -10°C -

Description	Part Number (AK0067-16)	Quantity
PreSeq DNA QC Assay Standard	*SA0597*	*32 µL*
PreSeq DNA QC Assay 10X Primer Mix	*SA0598*	*120 µL*

Materials required, but not supplied have been optimized for proper application of the Archer Library preparation. Use of other materials have not been tested by IDT.

Materials Required, but not Supplied		
Description	**Supplier**	**Part Number**
Ultrapure water (molecular biology grade)	*Various*	-
RNase AWAY™	*Thermo Fisher Scientific*	*7003*
Real-Time PCR thermal cycler	*Various*	-
qPCR tubes	*Various*	-
Plate centrifuge	*Various*	-
Pipettes (P10, P20, P200 and P1000)	*Pipetman or equivalent*	-
Sterile, nuclease-free aerosol barrier pipette tips	*Various*	-
Vortex mixer	*Various*	-

PCR tube cooling block	*Various*	-
Gloves	*Various*	-
Qubit® Fluorometer (3.0 or higher)	*Thermo Fisher Scientific*	*Q33216*
Qubit® dsDNA HS Assay Kit	*Thermo Fisher Scientific*	*Q32851*
(optional) AMPure® XP beads	*Beckman Coulter*	*A63880*
(optional) DynaMag™-96 Side Magnet	*Thermo Fisher Scientific*	*12331D*

Before getting started

Important precautions

- **Read through the entire protocol before starting your library preparation.**
- Use good laboratory practices to prevent contamination of samples by PCR products.
- Use nuclease-free PCR tubes, microcentrifuge tubes, and aerosol-barrier pipette tips.
- Wipe down workstation and pipettes with nuclease and nucleic acid cleaning products (e.g., RNase AWAY, Thermo Fisher Scientific).
- Verify that the thermal cycler used for library preparation is in good working order and currently calibrated according to manufacturer specifications.
- Reaction cleanup with AMPure XP beads (Beckman Coulter) is performed at room temperature (20°C to 25°C). Ensure that AMPure XP beads are equilibrated to room temperature and fully resuspended by vortexing until homogenous in both color and appearance prior to drawing out material for **each** use.

Input nucleic acid

- Input nucleic acid (TNA or DNA) in EDTA-free buffer (pH 7-8) or ultrapure water is the optimal starting template for AMP library preparation. Do NOT use EDTA-containing buffers.
- Use the maximum allowable input mass (ng) whenever possible. Higher input quantities enable more sensitive variant detection:
    - 10 - 250 ng of DNA for VariantPlex (somatic mutation detection)
- Contact technical support (archer-tech@idtdna.com) for commercially available extraction kit recommendations.

Reagents to prepare before starting

- Make **10 mL** fresh 10 mM Tris-HCl, pH 8.0 from 1M Tris-HCl, pH 8.0 and ultrapure water.
    - Mix **100 µL** 1M Tris-HCl, pH 8.0 with **9,900 µL** ultrapure water.
    - 10 mM Tris-HCl, pH 8.0 can be used for up to one week after mixing.
- Make **50 mL** fresh 70% ethanol from 100% ethanol and ultrapure water.
    - Add **35 mL** 100% ethanol to **15 mL** ultrapure water.
    - Tightly close the cap to minimize evaporation when not in use.
    - 70% ethanol is appropriate for use for up to one week after mixing.

Protocol

Before beginning, review "Before Getting Started" section for additional information regarding best practices.

Input DNA Cleanup (Optional)

If your input DNA or total nucleic acid (TNA) is already free of divalent cations and EDTA, proceed directly to the PreSeq DNA QC Assay portion of the protocol.

Purify DNA or TNA with AMPure XP Beads. Note that some TNA or DNA will be lost during purification; provide 2x the amount needed for your intended sequencing application.

1. Adjust purified DNA samples to a final volume of **50 µL** with ultrapure water. If the volume is greater than **50 µL**, scale the corresponding volume of AMPure XP beads proportionately.
2. Completely resuspend AMPure XP beads by vortexing.
3. Add **2.5X** volume (**125 µL**) of AMPure XP beads to each input sample.
4. Vortex well or pipette 10 times to mix and visually inspect the color of the sample to ensure a homogenous mixture.
5. Incubate for **5 minutes** at room temperature (20°C to 25°C).
6. Briefly spin down tubes.
7. Place tubes on the magnet for **4 minutes or until beads are fully pelleted** against the tube wall.
8. Without disturbing the bead pellet, use a pipette to remove and discard the supernatant. If the pellet becomes dislodged from the magnet and a portion is drawn into the pipette tip, return contents to the tube and repeat magnet incubation step.
9. Wash beads **two times** with 70% ethanol while still on the magnet. For each wash:
    a. Add **200 µL** 70% ethanol.
    b. Incubate for **30 seconds** at room temperature (20°C to 25°C).
    c. Carefully remove ethanol and discard.
10. After the final wash, use a pipette (≤20 µL capacity) to completely remove visible supernatant residue and allow tubes to dry for **3-5 minutes** at room temperature with open lids. **Take care not to over-dry beads** as this will significantly decrease overall recovery (yield) of nucleic acid.
11. Elute DNA by resuspending beads in **55 µL** 10mM Tris-HCl, pH 8.0.

12. Place tubes back on the magnet for **2 minutes**.

13. Carefully transfer **53 µL** of the DNA solution to a new PCR tube. Be sure to avoid transferring beads to the new tube.

PreSeq DNA QC Assay

This protocol is for preparation of a single qPCR reaction. If duplicates or triplicates are preferred, adjust the volumes accordingly.

1. Quantify DNA samples using a DNA-specific method. The Qubit dsDNA HS Assay kit is recommended for use with Archer kits. End-users should not rely on quantification from spectrophotometric-based methods.

2. Dilute an aliquot of each input sample to **1 ng/µL**. 6 µL is the minimum required amount for this assay.

3. Using the supplied **10X Primer Mix**, prepare a sufficient volume of qPCR master mix for each input sample, the PreSeq Assay Standard and negative control reaction (NTC), including the desired number of replicate reactions. **20 µL** qPCR reaction volumes are recommended for 96-well qPCR instruments.

Component	Part number	Reaction volume
2X SYBR® Green qPCR Reagent Mix	Not supplied	10µL
10X Primer Mix	SA0598	2µL
Ultrapure water	Not supplied	3µL
Total		15µL

4. Transfer **15 µL** of master mix to each well in the qPCR plate.

5. Transfer and combine **5 µL** of input samples, PreSeq DNA QC Assay Standard (SA0597), or ultra-pure water in the corresponding assigned wells.

6. Mix reaction well by slowly pipetting without introducing air bubbles. Cap tubes or seal the plate. Collect the reaction mixture at the bottom of the wells using centrifugation.

7. Incubate in a real-time thermal cycler using the thermal profile in the table below.

   a. Using a heated lid (≥100°C)

Step	Temperature (ºC)	Time	Cycles
Activation	95	3 min	1
Denaturation	95	15 sec	35
Annealing/Extension	64	1 min	

8. Determine DNA QC Score

a. Apply the same cycle threshold setting across all samples in the qPCR plate and determine the average Cq values for the PreSeq Assay Standard and DNA sample replicate reactions.

b. Calculate PreSeq DNA QC Scores:

(Average Sample Cq) – (Average Standard Cq) = DNA QC Score

c. Visit our website at archerdx.com/genome-calculator to determine the corresponding amplifiable genome (AG) equivalents per nanogram of DNA input or use the following equation:

AG/ng = 2 – (DNA QC Score) x 64,626

9. Determine assay requirements (see table at archerdx.com/genome-calculator) and requisite corresponding amount of starting material.

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