Best Practices

RNA Handling

- Extract samples using one of the following supported extraction methods:
  - Agencourt® FormaPure® Total Nucleic Acid Extraction (A33342)
  - Promega ReliaPrep™ FFPE Total RNA Miniprep System (Z1001)
  - Maxwell® RSC RNA FFPE Kit (AS1440)
- Be sure to follow the Archer-specific protocol modification for the extraction method used above. For our current recommendations, contact tech@archerdx.com.
- Elute samples in water or a buffer that does not contain EDTA.
- Use sterile techniques. Use RNase- and DNase-free plastics and clean surfaces with a DNA and RNase cleaning reagent such as RNase AWAY™ (Molecular BioProducts™, 7008).
- Follow good RNA handling practices.
- Store RNA at -80°C when possible.
- Do not vortex RNA.
- Avoid multiple freeze-thaws. If you will be using a sample multiple times, aliquot the RNA sample prior to freezing.

Input Material Quantification

- Always use an RNA-targeting, fluorescent dye-based assay with a standard curve like the Qubit™ HS RNA Assay Kit (Life Technologies™, Q32852) for calculating the RNA concentration of your sample. You can use 20-250ng of RNA quantified by Qubit or a similar method as input for FusionPlex Assays.
- Record the raw reading from Qubit and double-check the concentration calculated based on your dilution factor if using the Qubit instruments to auto-calculate the original concentration.
- A Bioanalyzer should not be used to calculate the concentration of RNA. The RIN number is often N/A or very low for RNA from FFPE sources and should not be used to measure quality.
- A Nanodrop™ should not be used to calculate the amount of input RNA.

RNA Quality Assessment

- The Archer® PreSeq™ RNA QC assay should be used to determine if the RNA is of sufficient quality to use as input.
- Make sure the Cq Threshold is set appropriately on the qPCR instrument.
- Verify that there is a single peak centered between 80°C-83°C for the melt curve.
- Cut-offs need to be established independently at each customer site.

Reagent Storage

- Gene Specific Primers (GSPs) contain only primers. They should be stored at -20°C long term, but are stable for up to 60 weeks at 4°C. Refer to IDT's stability guide for additional information on gene-specific primer stability.
- FusionPlex lyophilized reagent pouches are stable at 4°C for long-term storage. Temporary exposure to high or low temperatures (-20°C-55°C) will not adversely affect reagent performance.
- All reagents and materials should be used prior to their expiration dates.
- Archer reagents have a shelf life of 24 months; reagents should not be used beyond their expiration date.

Pre-Treatment of Input Material

- Do not pre-treat the RNA. Pre-treatments that should be avoided include:
  - Amplification
  - Shredding or shearing of RNA
  - RT PCR
  - DNase treatment
  - Staining of FFPE slides
  - Decalcification

Agencourt® AMPure® XP Bead (A63880) Handling

- Allow the beads to equilibrate to room temperature for 30 minutes before use.
- Vortex the beads directly prior to use. The color should appear homogenous. AMPure beads can be aliquoted for future use at this point to avoid contamination.
- Avoid bead loss during washes.
- Take care to avoid bead carry over. Ligation step 1 is not affected by residual AMPure bead carryover. However all other steps in the process are affected by residual beads.
- Do not store 70% ethanol for more than one week. Always make sure the cap is tightly closed.
- Do not over-dry the beads during the drying steps, as this will reduce yield and can cause decreased performance of primers amplifying GC rich regions.

Library Preparation Protocol

- Follow the protocol without any deviations.
- Avoid using cold blocks stored in the freezer. These often introduce extra freeze-thaws during library preparation.

- Keep the tubes on ice during transfers and mixing. Avoid removing the tubes from the ice to visualize the mixing steps.
- Check volumes during transfers. Buffer conditions and enzyme concentrations have been carefully optimized. If your transfer volume is less than the required volume by 10% or more, adjust with water to the appropriate volume for the reaction step.
- When incubating at temperatures below 25°C, keep your samples on ice and allow the block to reach the desired incubation temperature before transferring the samples to the PCR block.

Avoiding Cross-Contamination

- Archer Libraries are very concentrated. Make sure to clean work surfaces and pipettes before and after procedures with a DNA cleanup reagent like RNase AWAY.
- Pre-PCR and post-PCR areas should be physically separated.
- Always clean the Illumina® platforms with fresh Wash Buffer.
- Run all samples on a single analysis job. Archer Analysis will look for and flag any intra-run contamination.

Troubleshooting:

Problem	Possible Cause	Action
Over-clustering or under-clustering on the MiSeq or NextSeq	1) KAPA quantification error	1) Repeat KAPA quantification on the individual samples and final pool. Ensure that: a) The efficiency is between 90-110% b) The thresholds are set appropriately and R^2 is greater than 0.99 c) The Ct difference between standards is between 3.1 and 3.6 d) The correct percent of denatured PhiX was added to the final pool
Libraries failed PreSeq	1) Make sure Cq thresholds were set appropriately	1) Reanalyze with adjusted thresholds if necessary. Include a sample with known Cq on each run.
	2) Verify that you are using fresh material extracted with Archer recommended protocols.	2) Re-extract with Archer-supported methods and recommendations.

Problem	Possible Cause	Action
	3) Ensure that Archer-approved SYBR® qPCR mixes are being used	3) Consult the FusionPlex protocol for a list of approved SYBR qPCR mixes
Library yield under 4nM	1) Protocol not followed exactly	2) Ensure that: a) The GSP1 was used with the First PCR pouch, and the GSP2 was used with Second PCR pouch. b) Both MBC and adapter ligation lyospheres were combined with samples prior to adapter ligation incubation.
High percentage of samples filtered out during adapter trimming steps	1) Quantification, or dilution error	1) Repeat serial dilution and double check that calculations and dilutions are correct.
	2) Input amount too low	2) Ensure the concentration of input material was appropriately measured and calculated.
	3) Bad sequencing run	3) Look at the below two metrics to make sure the sequencing run performance was optimal: a) Over 80% of your reads should pass filter on the MiSeq and over 70% should pass filter on the NextSeq. If you have higher error rates than above, this can cause errors in the common region. b) Over-clustering on the MiSeq can also lead to compromised data. For Archer libraries the cluster density should be between 800-1000 k/mm^2

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