NanoString: The Key to Unlocking a Treasure Trove of Data

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The challenge of obtaining enough RNA from FFPE tissue for expression analysis

Unfortunately, obtaining high quality results from FFPE tissues can be challenging. Several factors contribute to this difficulty:

1. Fixation times and preparation methods vary,
2. Age of sample and storage conditions also vary,
3. RNA and DNA are often badly degraded,
4. RNA and DNA contain modifications that inhibit enzymes, and
5. The sample often has low yields of nucleic acids.

Both microarray and RNA-Seq require high quality RNA from fresh samples to achieve reliable results, but obtaining fresh samples can be challenging especially in the case for rare human diseases. This forces researchers to either wait months or even years to collect enough patient samples. FFPE RNA extraction kits have been developed to allow researchers to utilize cached formalin fixed tissues that have been collected over the years as a viable RNA resource for input material. The caveat is that the fixation and embedding process used to create FFPE (as mentioned above) greatly hinders the enzymatic steps required for RNA-Seq and microarray, weakening the end results.

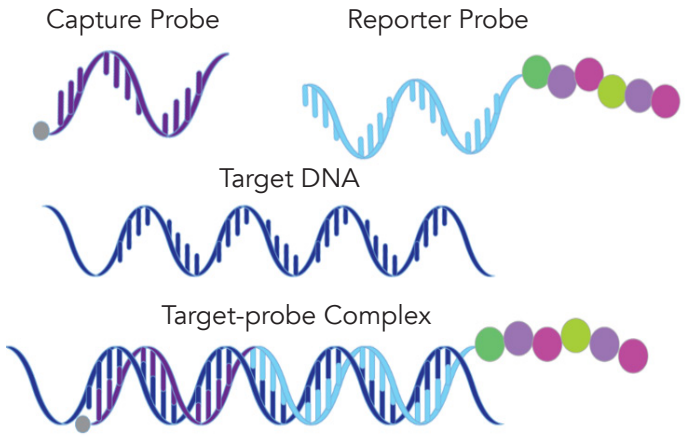

Figure 1. The capture and reporter probes hybridize juxtaposition to a 100-base target forming a tag set complex. The uniquely barcoded reporter tag is counted directly, measuring transcript abundance.

The NanoString nCounter system has circumvented the need for enzymes by utilizing a hybridization chemistry to directly count transcript abundance in a sample. The technology is an enzymatic free multiplexed assay that uses sequence-specific 50 base pair probes allowing the direct counting of nucleic acids (Figure 1). This allows the direct measurement of transcript abundance in badly degraded RNA samples. Considering that millions of archived FFPE tissue samples are stored in biobanks worldwide, and a large portion of them are tumor tissue with patient data, NanoString has now become the most appropriate key to unlocking this vast amount of data.

3 Reasons why NanoString is better suited for FFPE tissue than any other technology

1) Unlike other technologies you can reliably use crude RNA lysates from FFPE tissue

Because of the enzyme free hybridization chemistry of NanoString and that it only requires 100 bps for the probes to hybridize with the target, crude RNA lysates from FFPE tissue can be easily run on the nCounter system. The FFPE sample only requires deparaffinizing followed by a Proteinase K digestion step. At this point, the crude lysate can be used directly for the hybridization step.

To verify this, researchers at NanoString hybridized crude extracts from FFPE slices of human heart and brain in an nCounter assay with a different 96-gene CodeSet. For comparison, they also hybridized purified total heart and total brain RNA extracted from the frozen tissue and the FFPE slices (Figure 2). These results suggest that crude FFPE extracts containing degraded RNA can be used to accurately quantify gene expression levels and fold changes on the nCounter platform. The results for FFPE extracts show strong correlation with intact and purified RNA samples. This unique feature of NanoString gives it a huge advantage over other technologies.

2) Better correlation between FFPE and fresh frozen tissues than prior technologies

Several studies have shown that NanoString out performs other technologies when doing a head-to-head comparison looking at the correlation between FFPE and FF tissue (Reis et al. 2011; Vukmirovic et al. 2017; Omolo et al. 2016). Research by Reis PP et al. evaluated the results of FFPE analysis performed on the nCounter system compared to qPCR (Figure 3). Figure 3a shows nCounter data which has a correlation coefficient between FFPE and fresh-frozen of 0.90, and the

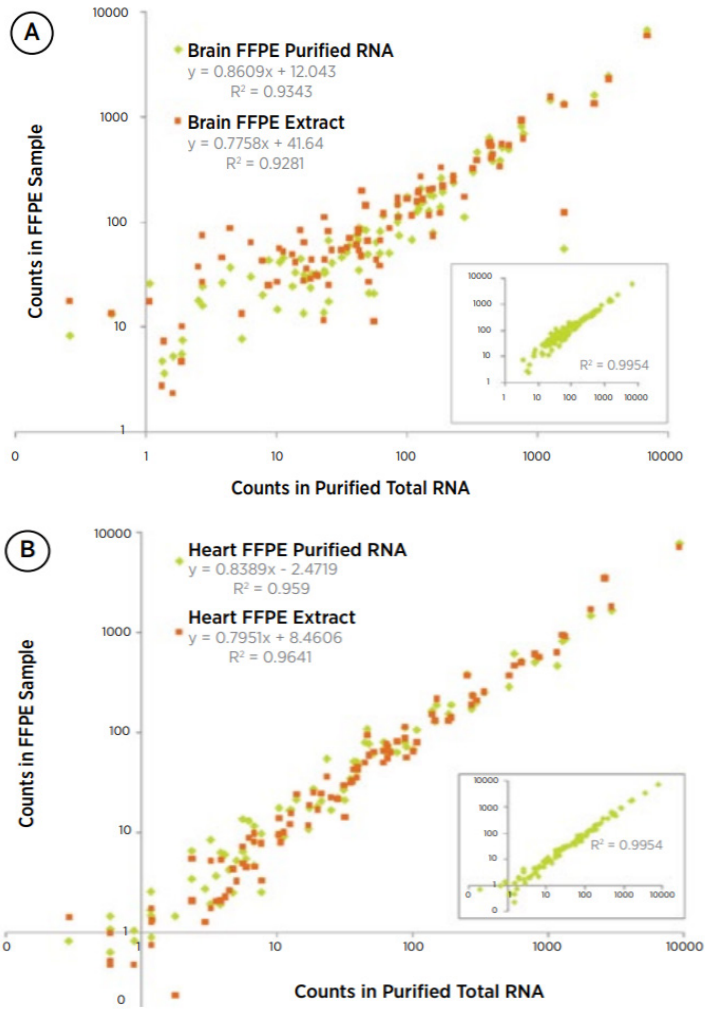

Figure 2. Correlation of counts from FFPE extracts to counts from purified RNA from FFPE slices and flash frozen tissue. A) Correlation between brain FFPE extract (orange) and brain FFPE purified RNA (green) to purified RNA from frozen brain tissue. (A inset) Correlation between brain FFPE extract and purified RNA from brain FFPE tissue. B) Correlation between heart FFPE extract (orange) and heart FFPE purified RNA (green) to purified RNA from frozen heart tissue. (B inset) Correlation between heart FFPE extract and purified RNA from heart FFPE tissue (NanoString Technologies, Inc).

figure 3b shows equivalent data for qPCR with a correlation coefficient of 0.50. This illustrates the superiority of nCounter data to that of qPCR for gene expression analysis of FFPE samples.

Omolo et al. 2016 examined adapting a gene expression signature originally created using fresh frozen tissue that's predictive of RAS pathway activation, for use with FFPE tissues.

They tested 5 different platforms; Affymetrix GeneChip (Affy), NanoString nCounter™ (NanoS), Illumina whole genome RNA-Seq (RNA-Acc), Illumina targeted RNA-Seq (t-RNA), and Illumina stranded Total RNA-rRNA-depletion (rRNA). Their results showed that of the five technology platforms tested, NanoString technology was more adaptive to the translation of the RAS pathway signature from FF tissues to widely available FFPE tissues than were the Affymetrix GeneChip and RNA-Seq technologies. They also noted that NanoString was the most forgiving FFPE technology in reproducing the "gold" standard analysis on matched FF tissues. They also state that: "NanoString technology appears to rescue samples with poor RNA quality, permitting more samples to be scored."

3) NanoString delivers reproducible data, even with old samples

NanoString produces rapid, reliable and reproducible results for several reasons. 1) It doesn't rely on enzymatic steps such as PCR amplification or cDNA conversion which take extra time and can create sample bias. 2) It only requires 100 base pair targets for accurate quantification. This is important in badly degraded samples, such as FFPE, in which most transcripts are fragmented. 3) It requires minimal hands on time which greatly reduces discrepancy in preparation contributing to its reproducibility. 4) There isn't any heavy bioinformatics involved reducing variability in data interpretation.

For example, in a study conducted by Northcott et al. 2012, a NanoString signature panel was created for subgrouping medulloblastoma into 4 subgroups using FFPE tissue. In a large series of 84 FFPE medulloblastomas from three independent pathology labs, the NanoString medulloblastoma subgroup assignment assay could assign subgroup with high confidence in 87.5% of cases in tissue that was 8 years old, illustrating the reliability of the system.

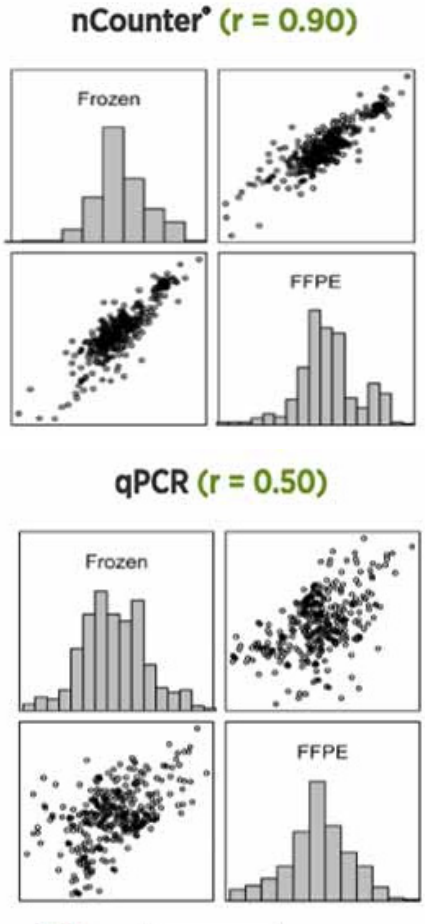

Figure 3. Comparison between qPCR and NanoString showing the correlation between FFPE and flash frozen tissue. A) NanoString had a correlation between FFPE and Frozen tissue of r = 0.90 and B) qPCR only showed a correlation of r = 0.50. Reis et al. 2011.

Conclusion

NanoString is currently the best technology available for FFPE tissue RNA analysis due to its unique non-enzymatic chemistry. As a result, FFPE tissue that has been stored for years in research labs, hospitals and pathology labs can now be used to analyze gene expression easily and reliably. This avoids the bottleneck of having to wait months to collect enough RNA from fresh tissue required for MicroArray and RNA-Seq.

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