Method for Assessing Specificity/Sensitivity with Replicates

Replicates can be used to optimize variant call quality score thresholds, minimize false positive variant calls, maximize true positives and quantify false negatives. The procedure described here is illustrated in Figure 2, and code to implement this is available at the github repository: https://github.com/krobasky/mkSProC.git. The table resulting from step 5) in this method can be used to create a ROC-like plot, which is illustrated in Figure 3. This is a plot of the proportion of false positive (discordant) variants seen at or below a particular threshold relative to the number of all false positives (discordant) variants detected for all quality scores. By traversing the curve from left to right, the proportions of concordant and discordant variants increase as the rank-ordered quality score is lessening in stringency, thus including variants with lower confidence scores.

1) Sequence and Call Variants:
   a. Starting with three or more technical and/or biological replicates, identify a sample to be used as the target for which the quality score is to be calibrated.
   b. Sequence the replicates using a blocked design and call the variants using an error model that is consistent across all loci of interest. For example, if using GATK, use the EMIT_ALL option to obtain scores for all loci, including variants and reference.

2) Classify Variants: Bifurcate the variant loci quality scores by loci in which all three sets agree and loci in which replicates agree but the target does not. Let these two quality score datasets be called concordant and discordant, respectively.

3) Repeat: If all replicates are similar in character and quality, the analysis can be repeated, choosing each of the replicates as the target, in turn. For this analysis, fibroblast was used as the target because it was closer to the subject’s germ-line genotype than either of the reprogrammed iPS cell line or the immortalized lymphocyte line. This is because the fibroblasts have undergone less experimental manipulation.

4) Rank-Order: For each of the two datasets (concordant and discordant), normalize the quality scores and rank order the normalized values.

5) Assess Specificity/Sensitivity and Select Threshold: Create cumulative distributions of the concordant and discordant frequencies for each quality score and combine these into a look-up table to quantify specificity and sensitivity of the various possible quality score thresholds.
REFERENCES
