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IDENTIFYING LOW-LEVEL SEQUENCE VARIANTS VIA NEXT GENERATION SEQUENCING TO AID STABLE CHO CELL LINE SCREENING

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Developing stable Chinese hamster ovary (CHO) cell lines for biotherapeutics is an irreversible process and therefore, key quality attributes, such as sequence variants, must be closely monitored during cell line development (CLD) to avoid delay in the developmental timeline, and more importantly, to assure product safety and efficacy. Sequence variants, defined as unintended amino acid substitution in recombinant protein primary structure, result from alteration at either the DNA or the protein level. Here we report the application of transcriptome sequencing (RNAseq) in an IgG1 monoclonal antibody (mAb) CLD campaign to detect, identify and eliminate cell lines containing low-level point mutations in recombinant coding sequence. Among the top eleven mAb producers chosen from transfectant, clone or subclone stages, three of the cell lines contained either missense or nonsense point mutations at a low-level of less than 2%. Subsequent LC/MS/MS characterization detected ~3% sequence variants with an amino acid change from Ser to Leu at residue 117 in the heavy chain of transfectants 11 and 27. This substitution is consistent with the RNAseq finding of a C/T mutation located at 407 base pair (TCA→TTA) in the heavy chain coding sequence. Here, for the first time, we demonstrate that RNAseq is a rapid and highly sensitive method to identify low-level genetic mutation de novo corresponding to the amino acid substitution that elicits sequence variant(s). Its implementation in CLD constitutes an early and effective step in identifying desired CHO expression cell lines. As a continued effort to expedite the turnaround time and lower the developmental cost, we are further exploring other NGS approaches including cDNA (GOI) deep sequencing via Miseq and the preliminary data indicate a comparable sensitivity in detecting low-level point mutations.