

IMPROVEMENTS IN NEXT GENERATION MASSIVELY-PARALLEL HID-STR MARKER SEQUENCING AND IDENTIFICATION USING AMPLISEQ HD

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Reliable sequencing of Short Tandem Repeats (STRs) is paramount for the identification and discrimination of DNA profiles. Due to their polymorphic nature, HID-STRs are a valuable tool used in forensics and paternity testing.

The presence of significant regions of secondary structure in STR markers can often lead to stutter or polymerase slippage. This can inhibit any next generation sequencing (NGS) workflow from achieving high quality results similar to the gold standard of sequencing - capillary electrophoresis - making it challenging to separate major from minor DNA contributors. In the past, through optimization of templating conditions on the Ion Chef and sequencing conditions on the Ion S5, we developed an NGS approach (Precision ID NGS System) allowing us to sequence a complex STR panel which includes the core set of STR loci (CODIS) used to distinguish individuals, as well as additional region-specific markers.

While the current Precision ID chemistry works well, it does produce a significant amount of stutter products, which limits the ability to identify minor contributors.

To reduce STR stutter, we have applied the Ampliseq HD technology, which incorporates unique molecular tags (UMTs) to each amplicon. Using this new chemistry and bioinformatics pipeline, we are able to eliminate stutter in many HID-STR markers.