**Smart-Plexer: a breakthrough workflow for hybrid development of multiplex PCR assays**

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**Supplementary Table 1 | Fitting model performances**. Data source: <https://github.com/am5113/pyACA>

|  |  |  |  |
| --- | --- | --- | --- |
| Model name | Equation | data shape (N) | MSE |
| Four-parameter Sigmoid |  | 16,188 | 0.017085 |
| Five-parameter Sigmoid |  | 16,188 | 0.003603 |
| Six-parameter Sigmoid |  | 16,188 | 0.011825 |

**Supplementary Table 2 | Combination table for 3-plex**

**Table

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**Supplementary Table 3 | ADS and MDS scores for the three curve representations in 3-plex**

Table

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**Supplementary Fig. 1 | Correlation of “c” ADS and MDS for 3-plex.**

**Supplementary Table 4 | “c” parameter stats for 3-plex**

Table

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**Supplementary Table 5| Combination table for 7-plex**

**Application, table, Excel

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**Supplementary Table 6 | “c” parameter stats 7-plex**

Table

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**Supplementary Fig. 2 | Standard curves for all targets in the BEST selected 7-plex.**

**Supplementary Table 7 | Primer table for 3-plex**

**Table

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**Supplementary Table 8 | Assay table for 3-plex**

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**Supplementary Table 9 | Primer table for 7-plex**

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**Supplementary Table 10 | Assay table for 7-plex**





**Supplementary Fig. 3 | Overall development of Smart-Plexer. Stream 1.** Pipeline for combo selection based on simulated multiplex assays. Before combination selection, operations including 3-fold data manipulation (Background removal, Late amplification filter and Noisy curve removal), data processing (Sigmoid fitting and Curve FFI normalization) and simulated score computation (The types of data are raw curve, normalized curve, fitted parameters and “c” parameter) are conducted. The principle of selection is then based on MDS-ADS ranking system. Combinations from 5 groups (BEST, TOP-ADS, TOP-MDS, MID, BOT) are chosen for validation progress. **Stream 2**. Pipeline for result validation based on empirical multiplex assays. With empirical experiment, same pre-operations (3-fold data manipulation and data processing) are taken. Then, the empirical scores are computed, and the distributions of classification accuracy are evaluated versus scores. The last validation step is based on clinical samples with best assay combination developed so far.