# Ion Torrent by life COLOR.jpg

# Assay Design Report

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| Request | WG\_IAD154041, THCAF\_v3 |
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# Goals and Considerations:

* Amplicon size: 125-150bp
* Number of pools: 1 pool design
* Genome: hg19
* Keep the designs from previous design (IAD154041) which contains 82 fusion targets, 36 RNA genes but:
  + remove 6 genes: AFM, APOB, CFHR5, ITGB7, MTTP, MYC
  + add 3 genes: SUGCT (C7orf10), TRIM61 and NKX2-1
* Copy the following amplicons from WG\_IAD105559 to this design:
  + ETV6-NTRK3.E4N14.COSF1535 (AMPL0000000983)
  + STRN-ALK.S3A20.COSF1430 (AMPL0000000435)
* Add 27 new fusion targets
* Add expression imbalance assay and remove RNA expression assay for 12 genes: RET, NTRK1, NTRK3, ALK, THADA, FGFR2, MET, BRAF, LTK, ERBB4, ROS1, RAF1
* The following fusions share the same reverse primer:
  + EML4-ALK.E6A19.COSF1296 AMPL0000000025\_F AMPL0000001171\_R
  + KIF5B-ALK.K15A19.COSF1061 AMPL0000001171\_F AMPL0000001171\_R
* Delivery format: No special instructions (standard AmpliSeq shipping)

# Assay Design:

* To increase coverage rate and minimize risk, multiple rounds of tiling/pooling are carried out.
* No SNP under primer for bulk of the design and it is relaxed in later rounds (one SNP is allowed at 5’ half of primers) to increase coverage.
* Stringent primer specificity filters are applied for bulk of the design and the specificity parameters are relaxed in later design rounds to increase coverage.

# Results Summary

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| **Number of targets** | WG\_IAD105559 – 2 fusions  IAD154041 – 18 genes, 82 fusions  New RNA target – 3 genes  New fusion target – 27 fusions  New imbalance assay – 12 genes |
| **Number of total amplicons** | 156 |
| **Number of primers** | 138 unique forward primers  80 unique revers primers |

**Notes on coverage**

Some amplicons are longer than 150bp in order to fit into single pool design and also improve coverage.

Customer agree to use AMPL0000001171\_R to be the shared primer, although EML4-ALK.E6A19.COSF1296 will become 191bp:

EML4-ALK.E6A19.COSF1296 AMPL0000000025\_F AMPL0000001171\_R

KIF5B-ALK.K15A19.COSF1061 AMPL0000001171\_F AMPL0000001171\_R

*Disclaimer: Deviating from the Ampliseq Designer standard result always bears the risk of lower performance.  We are currently investigating in the lab these types of more lenient designs with higher coverage and some of the results look promising, but caution is advised.*