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|  | Study-20-568 PACT424C neoTCR cloning | Version 1.3 |
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| Study # | 20-568 |
| Study ID | PACT424C NeoTCR cloning |
| Responsible Scientist | Jessica Carlson |
| Date | 5th November, 2020 |
| Study Objective | neoTCRs were received from imPACT and were cloned into the HR template cloning plasmid |
| Report generated by | Pankaj Tiwari / 25th May, 2021 |
| Initial Study Reviewed By | Review Pending |
| Final Study Reviewed By | Review Pending |
| Treatment | Day 1: Amplification and purification of TCR alpha and beta genes Day 2: Gibson Assembly, bacterial transformation Day 3: Colony PCR, amplicon sequencing, single colony streaking Day 4: Colony PCR amplicon sequencing analysis, liquid culture inoculation, bacterial lawn plate streaking Day 5: Mini-prep, plasmid gel analysis, full plasmid sequencing Day 6: Full plasmid sequencing analysis, bacterial plate sent for maxi plasmid production |
| Readouts | Amplicon sequencing Full plasmid sequencing Agarose gel electrophoresis |

1.0 Material and Methods

Reagent IDs:

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| Reagent: |
| RT/PCR-3 (3rd round) amplification cDNA plate |
| Plate ID: |
| PACT424C_M01_EXP20002042_Plate_out |

Experiment List:

| Date | Experiment | Description | Initials |
|---------------------|------------|--|----------|
| 5th November, 2020 | 20002077 | [PD-008] neoTCR PCR amplification | JC |
| 6th November, 2020 | 20002078 | [PD-008] Gibson Assembly | JC |
| 9th November, 2020 | 20002079 | [PD-008] Colony PCR | JC |
| 11th November, 2020 | 20002080 | [PD-008] Miniprep, Gel Analysis, Full Plasmid Sequencing | JC |
| 10th November, 2020 | 20002081 | [PD-008] Inoculate liquid Culture/bacterial lawn plate | JC |
| 12th November, 2020 | 20002082 | [PD-008] Full Plasmid Sequencing Analysis | JC |

Raw data location: "W:\PACT-0101\0030_PACT424C\06_GE \1_Raw_Data"


2.0 RESULTS

Summary

- 10 TCRs delivered to Gene Editing
- Initiated cloning for 15 TCR plasmids including TCR variants
- 8 TCR plasmids delivered to downstream processes
 - 7 plasmids were dropped
 - PACT424C_TCR801A01
 - PACT424C_TCR801A02
 - PACT424C_TCR804B01
 - PACT424C_TCR804B03
 - PACT424C_TCR808A01
 - PACT424C_TCR808A02
 - PACT424C_TCR808A07

Summary Table

| TCRs sent to GE | TCR cloning initiated | TCR plasmids delivered | Mutations present | Reason for dropped TCR | TCR variants cloned |
|-----------------|-----------------------|------------------------|-------------------|------------------------|---------------------|
| PACT424C_TCR796 | PACT424C_TCR796 | p948-PACT424C_TCR796 | None | N/A | 1/1 |
| PACT424C_TCR797 | PACT424C_TCR797 | p949-PACT424C_TCR797 | None | N/A | 1/1 |
| PACT424C_TCR798 | PACT424C_TCR798 | p950-PACT424C_TCR798 | None | N/A | 1/1 |
| PACT424C_TCR799 | PACT424C_TCR799 | p951-PACT424C_TCR799 | None | N/A | 1/1 |

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| PACT424C_TCR800 | PACT424C_TCR800 | p952-PACT424C_TCR800 | None | N/A | 1/1 |
| PACT424C_TCR801 | PACT424C_TCR801A01 | N/A | None | PCR generated mutation | 0/2 |
| | PACT424C_TCR801A02 | N/A | None | PCR generated mutation | |
| PACT424C_TCR802 | PACT424C_TCR802 | p953-PACT424C_TCR802 | None | N/A | 1/1 |
| PACT424C_TCR804 | PACT424C_TCR804B01 | N/A | None | PCR generated mutation | 0/2 |
| | PACT424C_TCR804B03 | N/A | None | PCR generated mutation | |
| PACT424C_TCR807 | PACT424C_TCR807 | p956-PACT424C_TCR807 | None | N/A | 1/1 |
| PACT424C_TCR808 | PACT424C_TCR808A06 | p955-PACT424C_TCR808A06 | None | N/A | 1/4 |
| | PACT424C_TCR808A01 | N/A | None | Unable to amplify | |
| | PACT424C_TCR808A02 | N/A | None | Unable to amplify | |
| | PACT424C_TCR808A07 | N/A | None | Other | |

Conclusions:

8 TCR plasmids (8 TCRs) were delivered to downstream processing.
7 TCR plasmids (2 TCRs) were dropped.