	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	4th November, 2020
Study Objective	neoTCRs were received from imPACT and were cloned into the HR template cloning plasmid
Report generated by	Pankaj Tiwari / 24th 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:

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

Experiment List:

Date	Experiment	Description	Initials
4th November, 2020	20002077	[PD-008] neoTCR PCR amplification	JC
5th November, 2020	20002078	[PD-008] Gibson Assembly	JC
8th November, 2020	20002079	[PD-008] Colony PCR	JC
10th November, 2020	20002080	[PD-008] Miniprep, Gel Analysis, Full Plasmid Sequencing	JC
9th November, 2020	20002081	[PD-008] Innoculate liquid Culture/bacterial lawn plate	JC
11th 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
PACT424C_TCR800	PACT424C_TCR800	p952-PACT424C_TCR800	None	N/A	1/1

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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.