

	<b>Study-20-568 PACT424C neoTCR cloning</b>	<b>Version 1.3</b>
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<b>Study #</b>	20-568
<b>Study ID</b>	PACT424C NeoTCR cloning
<b>Responsible Scientist</b>	Jessica Carlson
<b>Date</b>	4th November, 2020
<b>Study Objective</b>	neoTCRs were received from imPACT and were cloned into the HR template cloning plasmid
<b>Report generated by</b>	Pankaj Tiwari / 24th May, 2021
<b>Initial Study Reviewed By</b>	Review Pending
<b>Final Study Reviewed By</b>	Review Pending
<b>Treatment</b>	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
<b>Readouts</b>	Amplicon sequencing Full plasmid sequencing Agarose gel electrophoresis

1.0 Material and Methods

Reagent IDs:

<b>Reagent:</b>
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

PACT424C_TCR801	PACT424C_TCR801A01	N/A	Insertions/deletions due to strand slippage	PCR generated mutation	0/2
	PACT424C_TCR801A02	N/A	Insertions/deletions due to strand slippage	PCR generated mutation	
PACT424C_TCR802	PACT424C_TCR802	p953-PACT424C_TCR802	None	N/A	1/1
PACT424C_TCR804	PACT424C_TCR804B01	N/A	96 bp deletion	PCR generated mutation	0/2
	PACT424C_TCR804B03	N/A	96 bp deletion	PCR generated mutation	
PACT424C_TCR807	PACT424C_TCR807	p956-PACT424C_TCR807	None	N/A	1/1
PACT424C_TCR808	PACT424C_TCR808A06	p955-PACT424C_TCR808A06	Patient derived mismatch mutations	N/A	1/4
	PACT424C_TCR808A01	N/A	N/A	Unable to amplify	
	PACT424C_TCR808A02	N/A	N/A	Unable to amplify	
	PACT424C_TCR808A07	N/A	Various point mutations	Other	

Conclusions:

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