

Protocol for generating a reference file for feature barcoded reagents

Materials:

- List of dCODE® and TotalSeqC™ reagents, aligned with the attached barcode sequences
- CSV file / Excell file (CSV format)

Structure of the reference file:

id	name	read	pattern	sequence	feature_type
Reagent annotation	Reagent annotation	R2 <i>The barcode is read from this direction</i> Do not change!	Oligonucleotide structure including 15 nt barcode sequence (BC) Do not change!	Sequence of the 15nt barcode	Type of library Do not change!

Protocol:

Example 1: using simple text editor program as notepad.txt

- Open a notepad.txt simple text editor document or download a reference file template at our website: <https://immudex.com/resources/protocols/>
- Write in the header dCODE® (10x) and TotalSeqC™ reagent information separated with a "," (comma), exactly as shown in the example below:
Important: no special characters are allowed (as "*" or "/")

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	id,name,read,pattern,sequence,feature_type												
2	CD8,CD8_TotalSeqC,R2,5PNNNNNNNNNN(BC)NNNNNNNNNN,GCGCAACTTGATGAT,Antibody Capture												
3	A_0201_Neg_Control,A_0201_Neg_Control,R2,5PNNNNNNNNNN(BC)NNNNNNNNNN,TGACGAAGTACCTAT,Antibody Capture												
4	B_0801_Neg_Control,B_0801_Neg_Control,R2,5PNNNNNNNNNN(BC)NNNNNNNNNN,ATGCGGTAGCGAGAC,Antibody Capture												
5													

- Save the document as a .csv file:
name it: ex. "feature_ref-file.txt" no special characters allowed, and the file name must not contain a "space".

Example 2: csv file format using Microsoft Excel

- Open new document in Excel
- Fill in columns as shown below, with dCODE® (10x) and TotalSeqC™ and reagent information exactly as shown in the example below
Important: no special characters are allowed (as "*" or "/")

	A	B	C	D	E	F
1	id	name	read	pattern	sequence	feature_type
2	CD8	CD8_TotalSeqC	R2	5PNNNNNNNNNN(BC)NNNNNNNNN	GCGCAACTTGATGAT	Antibody Capture
3	A_0201_Neg_Control	A_0201_Neg_Control	R2	5PNNNNNNNNNN(BC)NNNNNNNNN	TGACGAAGTACCTAT	Antibody Capture
4	B_0801_Neg_Control	B_0801_Neg_Control	R2	5PNNNNNNNNNN(BC)NNNNNNNNN	ATGCGGTAGCGAGAC	Antibody Capture
5	"Fill_In"	Fill_In	R3	5PNNNNNNNNNN(BC)NNNNNNNNN	ATGCGGTAGCGAGAC	Antibody Capture
6	"Fill_In"	Fill_In	R4	5PNNNNNNNNNN(BC)NNNNNNNNN	ATGCGGTAGCGAGAC	Antibody Capture

Save the file as CSV (Comma delimited) (*.csv)

- name it: example: "feature_ref-file" no special characters allowed, and the file name must not contain a "space".

Critical:

Immudex® DNA barcodes are not unique to a given dCODE Dextramer® specificity but are assigned randomly during production. It is crucial, during planning of the experiment and ordering of the products, to ascertain that their barcode sequences are different if used together with another order of dCODE® reagents.

An excel file with the dCODE® (10) reagents and cognate DNA barcodes can be requested from customer@immudex.com

- Follow the 10x ranger analysis protocol, for upload of the reference file.
- 10x Ranger cloud analysis portal: <https://cloud.10xgenomics.com/cloud-analysis>
- 10x software support: <https://www.10xgenomics.com/support/software/cloud-analysis/latest/tutorials/CA-upload-via-cli-for-linux>