

Incorporating Barcodes into Amplicon Fusion Primer Design

The following are guidelines for designing amplicon fusion primers with Ion Barcodes. The sequences provided are from the Ion Xpress Barcode Adapter 1-16 Kit (PN 4471250). By incorporating these barcodes (plus barcode adapter sequences), customers can simply use the Ion Xpress Barcode selection on the drop down menu in the barcode field of the PGM software without altering the analysis parameters (i.e. Barcode sequences or barcode adapter sequences).

NOTE: The inclusion of the GAT adapter sequence is not required. This is merely the simplest way to incorporate the existing Ion Xpress Barcode Adapters without altering any analysis files.

To design custom barcodes or omit the adapter sequence:

- Keep in mind that after the 10bp barcode sequence (all barcodes below have a C as the 10th base), the next base must be different as not to cause a two-mer incorporation (either A, T or G in this case). This can be done by adding a 1 base buffer or designing the target sequence accordingly.
- *If customers would like to use custom barcodes or to omit the barcode adapter sequence (GAT), then it will require alteration of the barcode analysis parameters (barcode and barcode adapter sequences).* This can be done by uploading the sequences onto Torrent Browser (References Tab → Barcodes → Add).

Please use the following information in conjunction with the guidelines provided in the [Ion Amplicon Library Preparation \(Fusion Method\) User Guide](#) (PN 4468326 Rev B).

Ion Xpress Barcode	Sequence	Barcode Adapter
1	CTAAGGTAAC	GAT
2	TAAGGAGAAC	GAT
3	AAGAGGATTC	GAT
4	TACCAAGATC	GAT
5	CAGAAGGAAC	GAT
6	CTGCAAGTTC	GAT
7	TTCGTGATTC	GAT
8	TTCCGATAAC	GAT
9	TGAGCGGAAC	GAT
10	CTGACCGAAC	GAT
11	TCCTCGAATC	GAT
12	TAGGTGGTTC	GAT
13	TCTAACGGAC	GAT
14	TTGGAGTGTC	GAT
15	TCTAGAGGTC	GAT
16	TCTGGATGAC	GAT

Barcoded Fusion PCR primers for bidirectional sequencing

A Target Forward

5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG|Barcode X| Barcode Adapter |NNNNNNNNNNNNNNNNNNNN-3'

A Target Reverse

5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG|Barcode X| Barcode Adapter |NNNNNNNNNNNNNNNNNNNN-3'

trP1 Target Forward

5'-CCTCTCTATGGGCAGTCGGTGAT|NNNNNNNNNNNNNNNNNNNN-3'

trP1 Target Reverse

5'-CCTCTCTATGGGCAGTCGGTGAT|NNNNNNNNNNNNNNNNNNNN-3'