BNX File Format Specification Sheet

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BNX v1.3 File Format Specification Sheet

This file format specification sheet details the file format specifications for BNX (*.bnx) file version 1.3.

Introduction

The Bionano Genomics® BNX file is a raw data view of molecule and label information and quality scores per channel identified during a run or runs if data from multiple runs are merged. BNX v1.3 supports one or two label channels (colors).

The BNX file presents the general molecule information [data] in two sections: the BNX information header, which describes the specific format of the data; and the molecule information block, which contains the data values. This file format specification sheet provides descriptions, with examples, of the BNX header and molecule information block format of the file.

For easy readability, BNX files can be opened in Excel or in any tab-delimited, text-based editor. However, raw BNX files can be gigabytes in size and may not be viewable on a computer with limited memory. BNX v1.3 is not supported in IrysView®. BNX v1.3 files can only be generated by Saphyr Instruments, and not Irys Instruments. Previously generated BNX v1.2 files may be imported into Bionano Access®. Please contact Bionano Support for version compatibility issues.

When molecules in a BNX file are aligned to a reference (typically in a CMAP file), the alignment tool, RefAligner, checks that the BNX label motif header line in the BNX is consistent with that in the CMAP. This is to ensure that the input data are compatible, and that the resulting alignment data can be interpreted.

Understanding Run Headers

Run headers in the BNX file are used to identify the origin of the molecule data collected. This includes the software versions, label channels, enzyme recognition sites, information about the origin of molecules, and information about the molecule data that will follow.

For data generated on the Saphyr instrument, each cohort has a separate Run Data line. A cohort is defined as a subset of a flowcell for a given scan. For example, since ICS v4.8, each flowcell is divided into eight cohorts. So the number of Run Data lines will be eight times the number of scans for that flowcell. Each of the Run Data lines is given a unique sequential RunID. Each molecule listed in the BNX file has a RunID associated with it, so each molecule can be traced back to its original cohort. The scan number of all molecules from a Saphyr Chip is always 1. Downstream Bionano software can continue to assume that all molecules sharing the same RunID and scan number share the same scaling factor. For this to work, it is not necessary to identify precisely which cohort is associated with a specific RunID. However, to support customer applications and to trace back individual

molecules to the image data, the specific cohort location on each chip can be identified from the SourceFolder field on the Run Data line. In general, the SourceFolder field identifies the folder of images from which the molecules associated with this Run Data line are derived (NOTE: these image folders are deleted by default immediately after extracting the molecule information, unless the user requests that they be preserved and set it up before the run starts). For Saphyr Chips, this field ends in a 4 or 5 digit number. The lowest order digit indicates the cohort within the bank, the 2nd lowest digit indicates the bank and the high order 2 or 3 digits indicate the scan number. In the Run Data example below, the data came from scan 17, bank 3 and cohort 2.

Format

The BNX file contains the following sections:

- BNX header
  - # BNX File Version:
  - # Label Channels:
  - # Nickase Recognition Site 1 and color:
  - # Nickase Recognition Site 2 and color: (optional)
  - # Software Version: (optional)
  - # Bases per pixel (optional)
  - # Number of molecules (optional)
  - # rh
  - # Run Data
  - # 0h
  - # 0f
  - # 1h
  - # 1f
  - # 2h (optional)
  - # 2f (optional)
  - # Qh
  - # Qf
- Molecule information block
  - Molecule header (as defined in #0h)
  - Label position for Channel 1 (as defined in #1h)
  - Quality Score 1 (label SNR) for Channel 1
  - Quality Score 2 (label average intensity) for Channel 1
  - Label position for Channel 2 (as defined in #2h)
  - Quality Score 1 (label SNR) for Channel 2
  - Quality Score 2 (label average intensity) for Channel 2

Note: The data are broken down into sections. Each section is a group of data rows associated with a single molecule, and is then repeated for all data.

Example Single Color BNX file

BNX File Version: 1.3

Software Version: 4.8.19085.2, merco 1.3.8041.8044 Tue Oct 30 14:23:20 PDT 2018

Label Channels: 1

Nickase Recognition Site 1: CTTAAG;BNGFLGR001

Bases per Pixel: 375

#rh SourceFolder InstrumentSerial Time NanoChannelPixelsPerScan StretchFactor BasesPerPixel NumberofScans ChipId Flowcell LabelSNRFilterType MinMoleculeLength MinLabelSNR1 RunId

Run Data /home/bionano/access/local/SAPHYR_F09/2017-06/SN_CDLERX6NPNRX7NWU,Run_6c1a79ef-141a-4096-9d82-314634ab0357/FC1/Cohort1732 SAPHYR_F09 2019-05-07 01:24:35 PM 68819821 0.83 375 1 chips,SN_CDLERX6NPNRX7NWU,Run_6c1a79ef-141a-4096-9d82-314634ab0357,0 1 dynamic 15 3 1

…

Number of Molecules: 12978111

#0h LabelChannel MoleculeId Length AvgIntensity SNR NumberofLabels OriginalMoleculeId ScanNumber ScanDirection ChipId Flowcell RunId Column StartFOV StartX StartY EndFOV EndX EndY

#0f int int int float float int int int int string int int int int int int int int int

#1h LabelChannel LabelPosition[N]

#1f int int

#Qh QualityScoreID QualityScores[N]

#Qf string float

Quality Score QX11: SNR for channel 1

Quality Score QX12: Intensity for channel 1

…

Example Two Color BNX file

BNX File Version: 1.3

Software Version: 4.9.19225.1, merco 1.3.8041.8044 Tue Oct 30 14:23:20 PDT 2018

Label Channels: 2

Nickase Recognition Site 1: GCTCTTC;BNGFLRD001

Nickase Recognition Site 2: CTTAAG;BNGFLGR001

Bases per Pixel: 375

#rh SourceFolder InstrumentSerial Time NanoChannelPixelsPerScan StretchFactor BasesPerPixel NumberofScans ChipId Flowcell LabelSNRFilterType MinMoleculeLength MinLabelSNR1 RunId

Run Data SAPHYR_D-BETA2 2019-08-14 06:25:57 PM 68819821 0.83 375 1 chips,SN_4MSLRTONPOAXZNWU,Run_2ca21a30-2c55-4cda-92f9-a6b6cd169ea0,0 2 dynamic 15 3 1

…

Number of Molecules: 7198645

#0h LabelChannel MoleculeId Length AvgIntensity SNR NumberofLabels OriginalMoleculeId ScanNumber ScanDirection ChipId Flowcell RunId Column StartFOV StartX StartY EndFOV EndX EndY

#0f int int int float float int int int int string int int int int int int int int int

#1h LabelChannel LabelPosition[N]

#1f int int

#2h LabelChannel LabelPosition[N]

#2f int int

#Qh QualityScoreID QualityScores[N]

#Qf string float

Quality Score QX11: SNR for channel 1

Quality Score QX12: Intensity for channel 1

Quality Score QX21: SNR for channel 2

Quality Score QX22: Intensity for channel 2

…

Header Specifications

Header rows are prefixed by the pound sign (#).

Header Line Tag	Header Line Description
# BNX File Version:	**Indicates the version of the BNX file.**
# Label Channels:	**Defines the number of label channels.**
# Nickase Recognition Site 1:	**Comma separated list of enzyme recognition sequences for channel 1 followed by semicolon and channel 1 color (or fluorescent dye part number or name). There can be no spaces in this string. Color or dye is optional. This can also refer to the label recognition sequence for a non-nicking enzyme (i.e. DLE-1).**
# Nickase Recognition Site 2:	**Comma separated list of enzyme recognition sequences for channel 2 followed by semicolon and channel 2 color or dye. There can be no spaces in this string. Color is optional. This can also refer to the label recognition sequence for a non-nicking enzyme (i.e. DLE-1).**
# Software Version:	**Indicates software tool and version that generated the BNX file. In merged BNXs, this field is optional. Tools that handle BNX may not fill this in.**
# bases per pixel	**This field is optional. Tools that handle BNX may not fill this in.**
# Number of Molecules	**This field is optional. Tools that handle BNX may not fill this in.**
# rh	**Defines required tab-separated columns for headers in the # Run Data section.**
# Run Data	**Defines data that conforms to tab-separated headers specified in # rh.**
#0h	**Defines required columns for backbone data rows (rows labeled "0").**
#0f	**Defines format for columns in backbone data rows (rows labeled "0").**
#1h	**Description of fields in label channel 1.**

#1f	**Defines format for data in label channel 1.**
#2h	**Description of fields in label channel 2.**
#2f	**Defines format for data in label channel 2.**
#Qh	**Description of a quality score fields (ID and scores).**
#Qf	**Description of the quality score line format.**

Header Specification Details

The following tables provide the BNX header's descriptions (including any specific formatting, limitations and requirements) and examples.

# BNX File Version	
Header	**# BNX File Version:**
Description	**Indicates the version of the BNX file.**
Example	**# BNX File Version:<TAB>1.3**

# Label Channels	
Header	**# Label Channels:**
Description	**Defines the number of label channels. Available values: [1,2].**
Example	**# Label Channels:<TAB>2**

# Nickase Recognition Site 1	
Header	**# Nickase Recognition Site 1:**
Description	**Sequence of enzyme for channel 1, optionally followed by the laser color or dye name or dye part number for the channel and separated by a semicolon. This field is case insensitive.**
Example	**# Nickase Recognition Site 1:<TAB>CCTCAGC;BNGFLGR001**

# Nickase Recognition Site 2	
Header	**# Nickase Recognition Site 2:**
Description	**Sequence of enzyme for channel 2, optionally followed by the laser color or dye name or dye part number for the channel and separated by a semicolon.This field is case insensistive.**
Example	**# Nickase Recognition Site 2:<TAB> CCTCAGC;BNGFLGR001**

Note: If no color arguments are given, Access assumes site 1 is green and site 2 is red. A color argument may be the string “unknown”.

# Software Version	
Header	**# Software Version:**
Description	**Indicates the detection software and version that generated the BNX. For merged runs, this header may be dropped or replaced by a comment with the command used to generate the merged BNX.**
Example	**# Software Version:<TAB> 4.8.19085.2, merco 1.3.8041.8044 Tue Oct 30 14:23:20 PDT 2018**

# rh		
Header	**# rh**	
Description	**Description of the required tab-separated columns for headers specified in # Run Data rows. See Understanding BNX Headers above:**	
	SourceFolder	**The original images folder for the run**
	InstrumentSerial	**Instrument name or identifier (or UNKNOWN)**
	Time	**Beginning run time (or 1999, if unknown)**
	NanoChannelPixelsPerScan	**Effective pixels per nanochannel**
	StretchFactor	**Chip stretch factor**
	BasesPerPixel	**Estimated or calculated from stretch factor**
	NumberofScans	**Number of scans performed in the run**
	ChipId	**This field is used to identify the chip that was used. It may contain comma separated values with no spaces. It must contain ‘,’ followed by an integer on the end that uniquely identifies the chip run (UID).**
	Flowcell	**Flowcell number**
	LabelSNRFilterType	**Type of filter applied to label SNR for individual run (static or dynamic)**
	MinMoleculeLength	**Minimum molecule length filter for individual run (in kilobases)**
	MinLabelSNR	**Value of minimum label SNR for individual run**
	RunId	**Unique run Id (optional if there is only one Run Data line, with a value of 1 implied)**
Example	**#rh<TAB>SourceFolder<TAB>InstrumentSerial<TAB>Time<TAB> NanoChannelPixelsPerScan<TAB>StretchFactor<TAB>BasesPerPixel <TAB>NumberofScans<TAB>ChipId<TAB>Flowcell<TAB> LabelSNRFilterType<TAB>MinMoleculeLength<TAB>MinLabelSNR <TAB>RunId**	

Note: # rh entries must be in the order listed. Versions 1.0 and 1.1 only have the first 9 entries up to Flowcell.

# Run Data	
Header	**# Run Data**
Description	**Defines data that conforms to the tab-separated headers specified in #rh. Multiple Run Data lines will exist when BNX combines data from multiple irys runs or multiple Saphyr cohorts. ChipId entry must be a comma-delimited list of values, with at least one comma, and the last value must be an integer. Some values may be the string "UNKNOWN".**
Example	**# Run Data <TAB>D:\Data\EColi\2012-08\PL-Ecoli\Detect_Molecules** **<TAB>SAPHYR_F09<TAB> 2019-05-07 01:24:35 PM<TAB>** **68819821<TAB>0.83<TAB>375<TAB>1<TAB> chips,SN_CDLERX6NPNRX7NWU,Run_6c1a79ef-141a-4096-9d82-314634ab0357,0<TAB>2<TAB>dynamic<TAB>15<TAB>3<TAB>1**

#0h		
Header	**#0h**	
Description	**Description of the required tab-separated columns for molecule backbone data rows (rows labeled "0"):**	
	LabelChannel	**Channel 0 corresponds to the molecule backbone channel**
	MoleculeId	**Molecule ID**
	Length	**Molecule length in bases**
	AvgIntensity	**Average backbone molecule intensity**
	SNR	**Average backbone molecule SNR**
	NumberofLabels	**Total number of labels detected for this molecule on all channels**
	OriginalMoleculeId	**When multiple runs are merged, molecule IDs will be re-numbered and reported in the #0h MoleculeId column for each run. This field reports the molecule ID from the original BNX file. For a single run, this field reports the value reported in the #0h MoleculeId header line tag.**
	ScanNumber	**Scan number from the run. For Saphyr Chips, this value is always '1'.**
	ScanDirection:	**Description:**
	-1	**Unknown**
	0	**Forward**
	1	**Backward**
	ChipId	**Serial number of the chip of the run**
	Flowcell	**Flow cell number**
	RunId	**A positive integer that identifies the # Run Data header line: A value N corresponds to the Nth header line.**

	Column	**The imaging column in which the molecule was detected.**
	StartFOV	**The field of view within the column in which the molecule begins.**
	StartX	**The X coordinate in which the molecule begins.**
	StartY	**The Y coordinate in which the molecule begins.**
	EndFOV	**The field of view within the column in which the molecule ends.**
	EndX	**The X coordinate in which the molecule ends.**
	EndY	**The Y coordinate in which the molecule ends.**
	GlobalScanNumber	**Unique global ID computed from RunId and ScanNumber. (Not present in all BNX files)**
Example	**#0h<TAB>LabelChannel<TAB>MoleculeId<TAB>Length<TAB>SNR <TAB> AvgIntensity<TAB>NumberofLabels<TAB>OriginalMoleculeIdA <TAB>ScanNumber<TAB>ScanDirection<TAB>ChipId <TAB>Flowcell<TAB>RunId<TAB>Column<TAB>StartFOV<TAB>StartX<TAB> StartY<TAB>EndFOV<TAB>EndX<TAB>EndY<TAB>GlobsalScanNumber**	

Note: BNX 1.0 has only a single "# Run Data" header and does not include the RunId or any subsequent field.

Note: The 7 fields from Column to EndY may be absent from the header, in which case all molecule headers will be missing this information. This may be the case for early versions of BNX 1.3 data.

#0f	
Header	**#0f**
Description	**Defines the format for columns in backbone data rows (rows labeled "0").**
Example	**#0f<TAB>int<TAB>int<TAB>int<TAB>float<TAB>float<TAB>int <TAB>int<TAB>int<TAB>int<TAB>string<TAB>int<TAB>int<TAB>int<TAB>int<TAB>int<TAB>int<TAB>int<TAB>int<TAB>int**

# 1h	
Header	**#1h**
Description	**Description of the fields in label channel 1. For LabelPosition[N], [N] is variable [1...N].**
Example	**#1h<TAB>LabelChannel<TAB>LabelPosition[N]**

#1f	
Header	**#1f**
Description	**Defines the format for data in label channel 1.**
Example	**#1f<TAB>int<TAB>int**

# 2h	
Header	**#2h**
Description	**Description of the fields in label channel 2. For LabelPosition[N], [N] is variable [1...N].**
Example	**#2h<TAB>LabelChannel<TAB>LabelPosition[N]**

#2f	
Header	**#2f**
Description	**Defines the format for data in label channel 2.**
Example	**#2f<TAB>int<TAB>int**

#Qh	
Header	**#Qh**
Description	**Description of the fields in QX<m><n>; where QualityScoreID is QX[*m*=channel][*n*=score sequence]; and QualityScores[N], where [N] is variable [1...N].**
Example	**#Qh<TAB>QualityScoreID<TAB>QualityScores[N]**

#Qf	
Header	**#Qf**
Description	**Defines the format for data in QX<m><n>.**
Example	**#Qf<TAB>string<TAB>float**

Molecule Information Block Specification

Molecule information block rows are prefixed by the backbone (0) and channel (1 or 2) designations and the quality designation for labels (e.g., QX11, QX12, QX21, QX22). Each molecule information block adheres to the following convention:

- Molecule information block
  - Backbone data row (values for header #0h)
  - Channel 1 data row (values for header #1h)
  - Channel 1 quality score field ID and values for SNR (values for header # Quality Score QX11:)
  - Channel 1 quality score field ID and values for average intensity (values for header # Quality Score QX12:)
  - Channel 2 data row (values for header #2h)
  - Channel 2 quality score field ID and values for SNR (values for header # Quality Score QX21:)
  - Channel 2 quality score field ID and values for average intensity (values for header # Quality Score QX22:)

Note: A molecule information block has the data rows for a single molecule. Molecule information blocks are repeated for each molecule's data.

Molecule Information Block Specification Details

The following tables provide the BNX molecule information block descriptions (including any specific formatting, limitations and requirements) and examples.

0	
Header	**Backbone data row (values for header #0h)**
Description	**Required**
Example	**0<TAB>1<TAB>898875<TAB>15795.7<TAB>438.111<TAB>224<TAB>** **55226<TAB>1<TAB>-1<TAB>chips,SN_CDLERX6NPNRX7NWU,Run_6c1a79ef-141a-4096-9d82-314634ab0357,0<TAB>1<TAB>1<TAB>32<TAB>1<TAB>103<TAB>217<TAB>2<TAB>100<TAB>760**

1	
Header	**Channel 1 data row (values for header #1h)**
Description	**Required**
Example	**1<TAB>15416<TAB>20446<TAB>22515<TAB>32389** **<TAB>36148 38079<TAB>47018 <TAB>54062<TAB> 76852<TAB>80806<TAB>94432<TAB>96995<TAB>99773** **<TAB>122543<TAB>138487<TAB>150504<TAB>153590** **<TAB>154790<TAB>159545….**

QX11	
Header	**QX11**
Description	**Channel 1 quality score field ID and values (label SNR)**
Example	**QX11<TAB>39.3287<TAB>6.2613<TAB>6.9517<TAB>15.9162<TAB>17.8695**

QX12	
Header	**QX12**
Description	**Channel 1 quality score field ID and values (label average intensity)**
Example	**QX12<TAB>0.1233<TAB>0.0431<TAB>0.0471<TAB>0.0641<TAB>0.0767<TAB>0.0489**

2	
Header	**Channel 2 data row (values for header #2h)**
Description	**Required**
Example	**2<TAB>15416<TAB>20446<TAB>22515<TAB>32389** **<TAB>36148 38079<TAB>47018 <TAB>54062<TAB> 76852<TAB>80806<TAB>94432<TAB>96995<TAB>99773** **<TAB>122543<TAB>138487<TAB>150504<TAB>153590** **<TAB>154790<TAB>159545**

QX21	
Header	**QX21**
Description	**Channel 2 quality score field ID and values (label SNR)**
Example	**QX21<TAB>39.3287<TAB>6.2613<TAB>6.9517<TAB>15.9162<TAB>17.8695**

QX22	
Header	**QX22**
Description	**Channel 2 quality score field ID and values (average label intensity)**
Example	**QX22<TAB>0.1233<TAB>0.0431<TAB>0.0471<TAB>0.0641<TAB>0.0767<TAB>0.0489**

Molecule Information Block 2 Color Example

0 183 241500 4321.49 80.67 66 12967 1 -1 chips,SN_CDLERX6NPNRX7NW
U,Run_6c1a79ef-141a-4096-9d82-314634ab0357,0 1 1 4 4 405 813
4 399 1456
1 8431 15317 18719 20337 22378 23461 26511 38148 43239 44940 46152
48618 50418 55040 58304 62986 65026 67284 73302 75041 79238 83161 86625
90744 93541 98059 100275 103807 106615 108569 113238 115342 119415 123874 127147
128836 132386 136947 139303 143796 146698 148737 153243 156795 158939 163425 166261
169608 171133 173298 180030 182590 187506 189308 191170 194120 199178 205613 212731
215843 218206 221351 224684 229039 233971 238808 241500

2 372 30454 33754 53263 68284 73545 75769 80890 85650 87569 121950
132445 134124 161983 164214 183781 205368 208468 213711 227576 233488 239277 244203
252851 256800 264283 273708 277056 279563 285553 290158 297320 310045 311687 326948
336634 339543 344722 348611 352278 357012 358578 362169 364573 366917 368454 372675
376614 385439 387369 389689 395302 399662 405791 409123 416338 418047 423318 425846
429461 438289 442342 444530 464606 467240 472875

QX11 10.08348 6.33244 6.25445 6.27364 4.90548 6.82648 3.94645 20.34334 8.32105
7.80591 9.32729 11.34582 9.53998 7.06546 11.75305 11.87323 5.79447 6.69021

8.47028 12.94812 14.33600 8.09798 21.59392 5.61965 21.69473 21.09607
19.16816 9.50938 34.36756 11.66572 30.09792 41.02215
73.67329 17.74068 29.42975 39.03440 28.29641 28.13300
21.77220 30.53836 12.88604 5.35750 41.67753 17.60822 16.60717
7.78603 13.97649 11.65518 21.52665 15.09029 17.83440
16.97878 14.53676 12.57979 15.61269 15.79564 7.96846 28.43458
16.56446 4.34261 8.42204 14.80289 15.17917 12.42019 4.78720 6.89824

QX21 7.64803 9.44464 14.81698 8.44297 12.80191 12.53211 9.51094 6.65061
10.88102 9.18963 11.08927 10.47844 18.68086 5.27135 8.22451 9.80301
7.41648 15.81695 14.93037 13.41137 12.47227 9.26461 32.88548
19.00489 13.21570 10.60119 10.19845 14.87359 14.36357
9.82766 14.30509 20.68182 8.14954 17.95187 10.97401 16.51873
10.16308 33.78429 17.33000 5.89374 21.92048 8.46575 22.60254
18.22422 18.64907 11.70299 23.17835 9.13149 8.74133 14.90674
8.69611 9.19728 9.79332 15.49938 15.17399 12.41004 13.67709 17.42826
22.22139 9.82052 11.87555 22.77458 12.69888 8.88651 12.80701

QX12 583.12 366.20 361.69 362.80 283.68 394.77 228.22 1176.44 481.20 451.41 539.39
656.12 551.69 408.59 679.67 686.62 335.09 386.89 489.83 748.78 829.04 468.30 1248.76
324.98 1254.59 1219.97 1108.48 549.92 1987.45 674.62 1740.54 2372.28 4260.47 1025.93 1701.90
2257.33 1636.36 1626.91 1259.07 1766.01 745.19 309.82 2410.18 1018.27 960.38 450.26 808.25
674.01 1244.87 872.66 1031.35 981.87 840.65 727.48 902.87 913.45 460.81 1644.35 957.91
251.13 487.04 856.04 877.80 718.25 276.84 398.92

QX22 449.87 555.55 871.56 496.63 753.03 737.16 559.45 391.20 640.04 540.55 652.29
616.36 1098.84 310.07 483.78 576.63 436.25 930.38 878.23 788.88 733.64 544.96 1934.38
1117.90 777.37 623.58 599.89 874.89 844.89 578.08 841.45 1216.54 479.37 1055.96 645.51
971.66 597.81 1987.25 1019.38 346.68 1289.40 497.97 1329.52 1071.98 1096.97 688.39 1363.39
537.13 514.18 876.84 511.52 541.00 576.06 911.70 892.56 729.98 804.51 1025.16 1307.10
577.66 698.54 1339.64 746.97 522.72 753.33

Appendix

For data generated on the Irys instrument, each chip run of a single flow cell is given a single Run Data line with only one RunID in the header. It describes data across all scans of a single flowcell; individual molecules identify the scan number they originated from. If multiple flowcells, multiple chip runs (reuse of the same chip), or multiple chips are run, and the BNX files are merged, there will be more than one Run Data line, each with a unique sequential RunID. Individual molecules now identify both the RunID and scan number they originated from. Downstream Bionano software assumes that all BNX molecules sharing the same RunID and scan number share the same scaling factor.

Technical Assistance

For technical assistance, contact Bionano Genomics Technical Support.

You can retrieve documentation on Bionano products, SDS's, certificates of analysis, frequently asked questions, and other related documents from the Support website or by request through e-mail and telephone.

Type	Contact
Email	**support@bionanogenomics.com**
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