

How To Design Your Custom NGS Panel using the Nonacus Panel Design Tool

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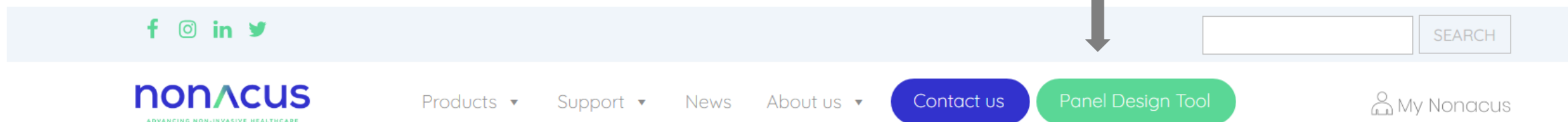



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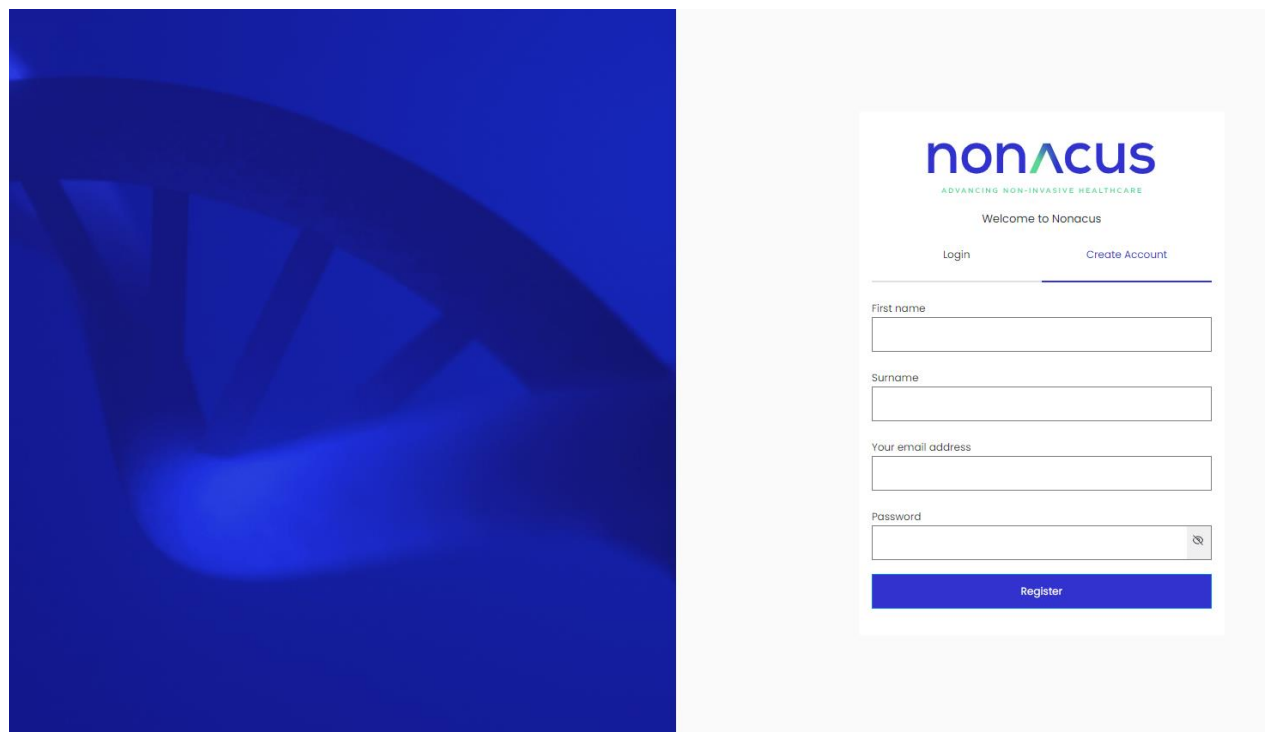
How to design your Custom NGS Panel

1. Create a Nonacus Account

- ^ Go to <https://www.nonacus.com/>
- ^ On the opening page click *Panel Design Tool* on the upper right



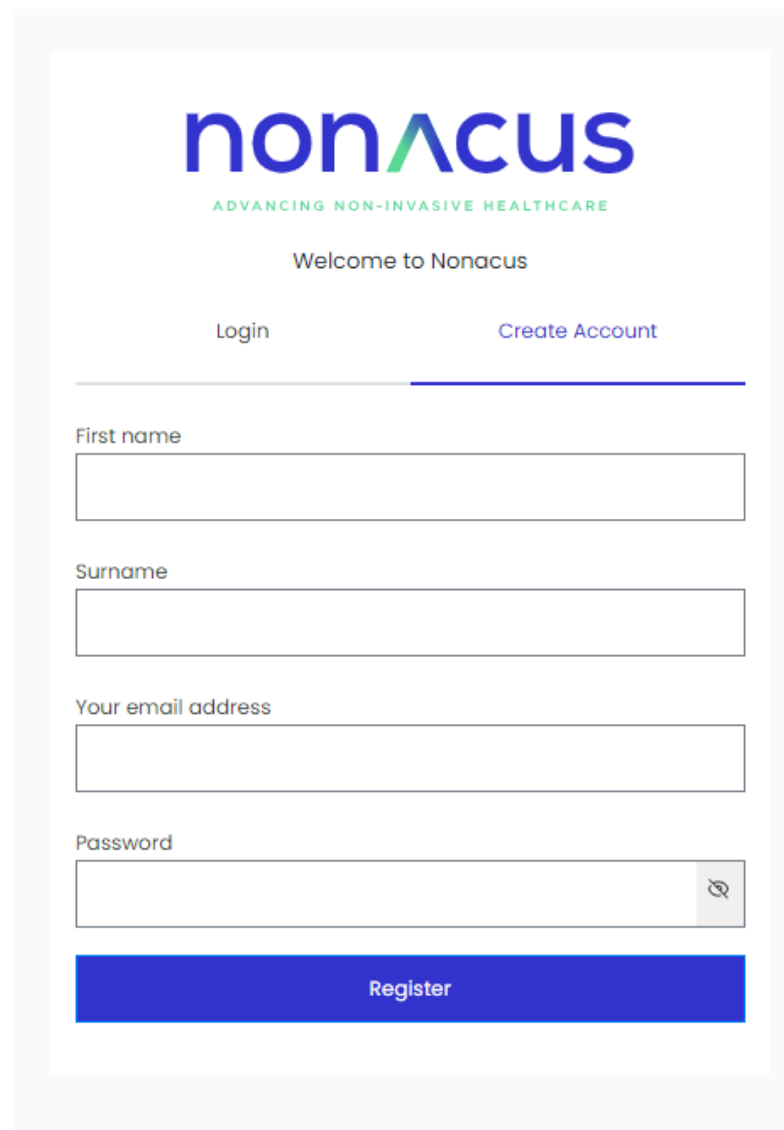
- ^ Or alternatively, go direct to <https://mynonacus.nonacus.com/>
- ^ Click 'Create Account'  My Nonacus



How to design your Custom NGS Panel

1. Create a Nonacus Account

- ▲ Fill in First Name, Surname, professional email account, setup your own password and click Register



The screenshot shows the Nonacus registration page. At the top is the Nonacus logo with the tagline "ADVANCING NON-INVASIVE HEALTHCARE". Below the logo is a "Welcome to Nonacus" message. There are two links: "Login" and "Create Account". A horizontal line separates the header from the registration form. The form consists of four input fields: "First name", "Surname", "Your email address", and "Password". The "Password" field has a toggle icon on the right. At the bottom of the form is a blue "Register" button.

nonACUS
ADVANCING NON-INVASIVE HEALTHCARE

Welcome to Nonacus

[Login](#) [Create Account](#)

First name

Surname

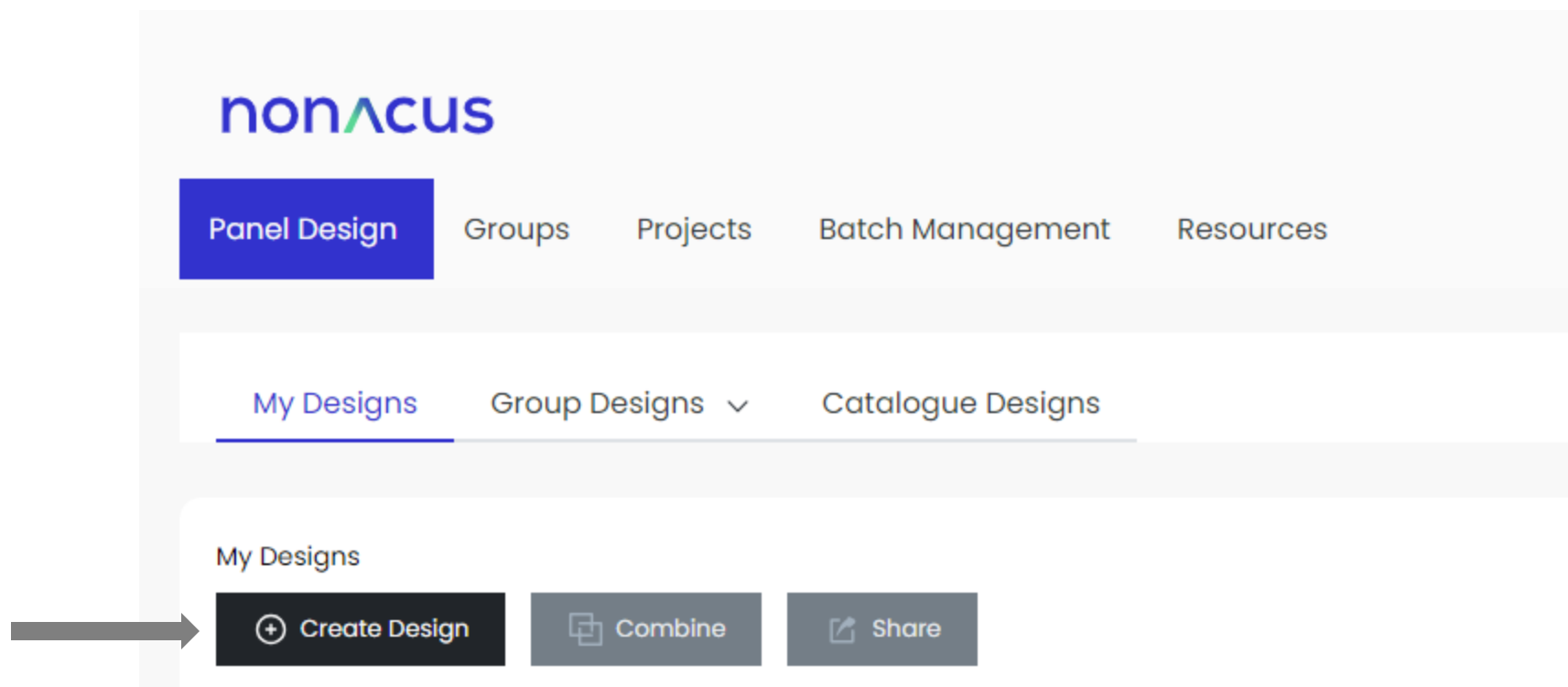
Your email address

Password

How to design your Custom NGS Panel

2. Create your own Custom NGS Panel

- ^ After registration, click *Login*, enter your email address, password and click *Continue*
- ^ On the Nonacus Probe Design Tool page, click *Create Design*



How to design your Custom NGS Panel

2. Overview of the 'Create Panel Design' page

Create Panel Design [Close]

1 Advanced Sets

2 Name*

3 Select Genome*

4 Tiling*

5 Input Type*

6 Include
 Exome

7 Gap Fill ⓘ

8 Repeat Masking

9 Input Bed File*

Click [here](#) to download an example

1. **'Advanced'** setting provides more than 2X tiling if required (cf Appendix A : Tiling)
2. Name your panel
3. Select the Genome Option (**GRCh37** or **GRCh38**)
4. Select tiling requirement based on how many times you want each base within your target region to be covered
5. Select Input Type: **BED list**, **Gene List** or **Template** ('Template' allows a mix of genes and regions in a design)
6. Selecting Exome will choose validated probes from **the Nonacus exome** (Nonacus Cell3 Target: Whole Exome NGS panel), that are appropriate for the Genes/Regions specified
7. Selecting Gap Fill will utilize validated probes from the **Nonacus exome** (Nonacus Cell3 Target: Whole Exome NGS panel) in drop out/masked regions
8. **Repeat Masking** will prevent difficult target regions from being included in your design
9. Upload your input file
10. Click the **create** button to submit your design

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3. Create a Panel Design using a BED file

Create Panel Design ✕

Advanced Sets

Name*

Select Genome*

GRCh37 **GRCh38**

Tiling*
2x

Input Type*

Bed List Gene List Template

Include

Exome

Gap Fill ⓘ

Repeat Masking

Input Bed File*

No file added

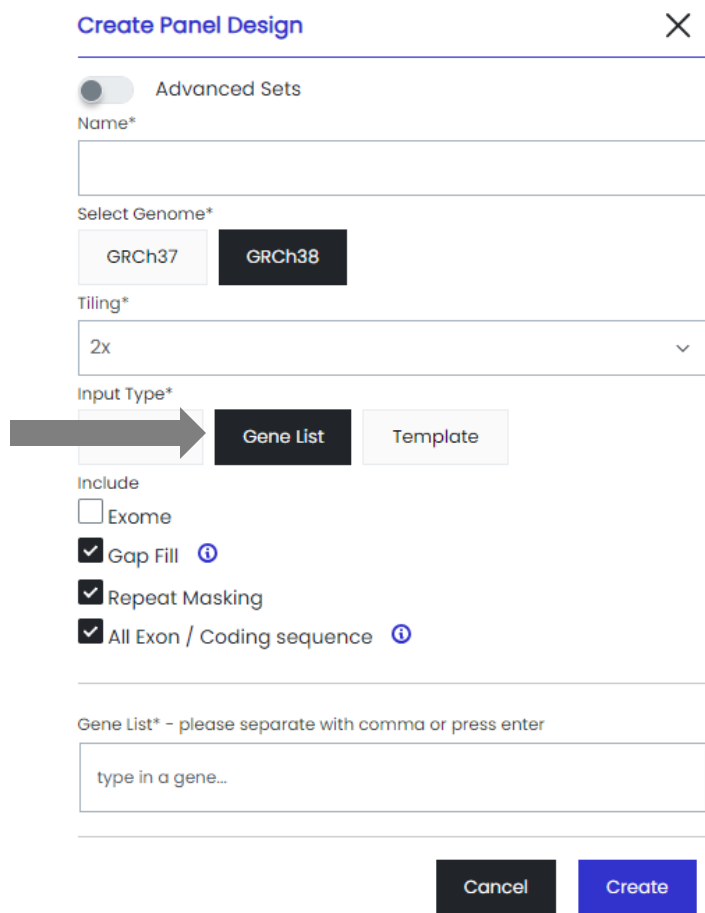
Click [here](#) to download an example

- ^ Name your panel
- ^ Select the Genome Option (**GRCh37** or **GRCh38**)
- ^ Select the level of tiling
- ^ Select Input Type: BED list
- ^ Upload the bed file and click **Create**
- ^ Note: Click here to download an example of a BED file

When the design is validated an email will be sent
You can then download the results files on your portal (CF Appendix C and D)

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4. Create a Panel Design using a Gene List



Create Panel Design ✕

Advanced Sets

Name*

Select Genome*

GRCh37 GRCh38

Tiling*

2x

Input Type*

Gene List Template

Include

Exome

Gap Fill ⓘ

Repeat Masking

All Exon / Coding sequence ⓘ

Gene List* - please separate with comma or press enter

type in a gene...

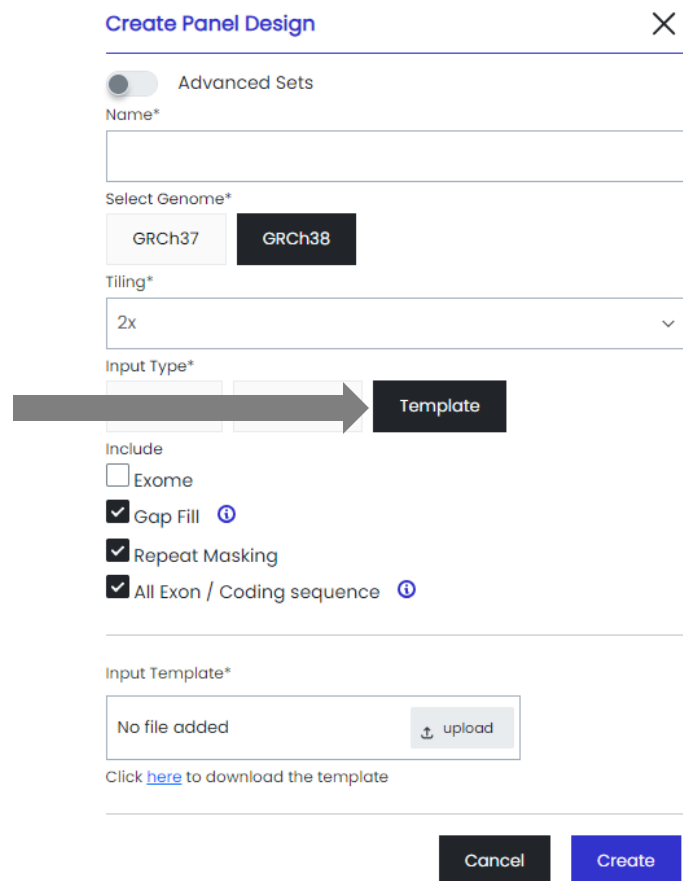
Cancel Create

- ^ Name your panel
- ^ Select the Genome Option (**GRCh37** or **GRCh38**)
- ^ Select the level of tiling
- ^ Select Input Type: Gene List
- ^ All Exon/Coding sequence: Tick for coding sequence only (exons), Untick for all exons/introns and to include 3' and 5' untranslated regions
- ^ Enter your list of genes in the Gene list box, one per line
- ^ Click **Create**

When the design is validated an email will be sent
You can then download the results files on your portal (CF Appendix C and D)

How to design your Custom NGS Panel

5. Create a Panel Design using a Template File



The screenshot shows the 'Create Panel Design' interface. At the top right is a close button (X). Below it is a toggle for 'Advanced Sets'. The 'Name*' field is empty. Under 'Select Genome*', 'GRCh37' is selected and 'GRCh38' is highlighted. The 'Tiling*' dropdown is set to '2x'. In the 'Input Type*' section, 'Template' is selected, indicated by a grey arrow pointing to it from the left. Below this are 'Include' options: 'Exome' (unchecked), 'Gap Fill' (checked), 'Repeat Masking' (checked), and 'All Exon / Coding sequence' (checked). The 'Input Template*' section shows 'No file added' and an 'upload' button. At the bottom, there is a link 'Click [here](#) to download the template' and 'Cancel' and 'Create' buttons.

A template file lets you input a mixture of gene and regions in one design. You can also use the "Combine" feature (cf Appendix B)

- ^ Name your panel
- ^ Select the Genome Option (**GRCh37** or **GRCh38**)
- ^ Select the level of tiling
- ^ Select Input Type: Template
- ^ All Exon/Coding sequence: Tick for coding sequence only (exons), Untick for all exons/introns and to include 3' and 5' untranslated regions
- ^ Browse the file to upload it and click *Add*

Note: Click *here* to download an example of a Template file

When the design is validated an email will be sent

You can then download the results files on your portal (CF Appendix C and D)

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6. Downloading your Panel Design stats

Each panel design generates 3 BED files; covered regions, non-covered and target regions and a 'design stats' PDF which shows target coverage and probe information.

The screenshot shows the 'My Designs' page in the nonACUS application. The table lists several panel designs with columns for ID, Name, Input Type, Genome, Tiling, Exome, Gap Fill, Created, and Action. The 'Action' column for the first row is highlighted with a red circle '2', and a dropdown menu is open showing options: Request Help, Download, Request Quote, Share, and Delete. A red circle '1' highlights the 'Select' checkbox in the first row.

Select	ID	Name	Input Type	Genome	Tiling	Exome	Gap Fill	Created	Action
<input type="checkbox"/>	1939	AF2 - RefSeq	Bed List	GRCh38	2x	×	✓	24/04/2023 13:11:21	⋮
<input type="checkbox"/>	1938	AF1 - coding 16-17	Bed List	GRCh38	2x	×	✓	24/04/2023 13:10:12	⋮
<input type="checkbox"/>	1931	HRD_HRR_combined_v2	Bed List	GRCh37	2x	×	✓	21/04/2023 10:43:41	⋮
<input type="checkbox"/>	1550	Panel for NKI	Gene List	GRCh38	2x	×	✓	09/11/2022 14:26:22	⋮
<input type="checkbox"/>	1546	Panel for Jitendra Badhai (NKI)	Gene List	GRCh38	2x	×	✓	08/11/2022 14:20:16	⋮

DESIGN REPORT

DESIGN INFORMATION

Design ID: XXXX

Design Name: XXXXXXXX

Genome Build: GRCh38

TARGET INFORMATION

Total Target Size (bp): XXXX

Number of Merged targets: XX

PROBE INFORMATION

Total Number of Probes: XXX

Total Covered Region (bp):

XXXXXX

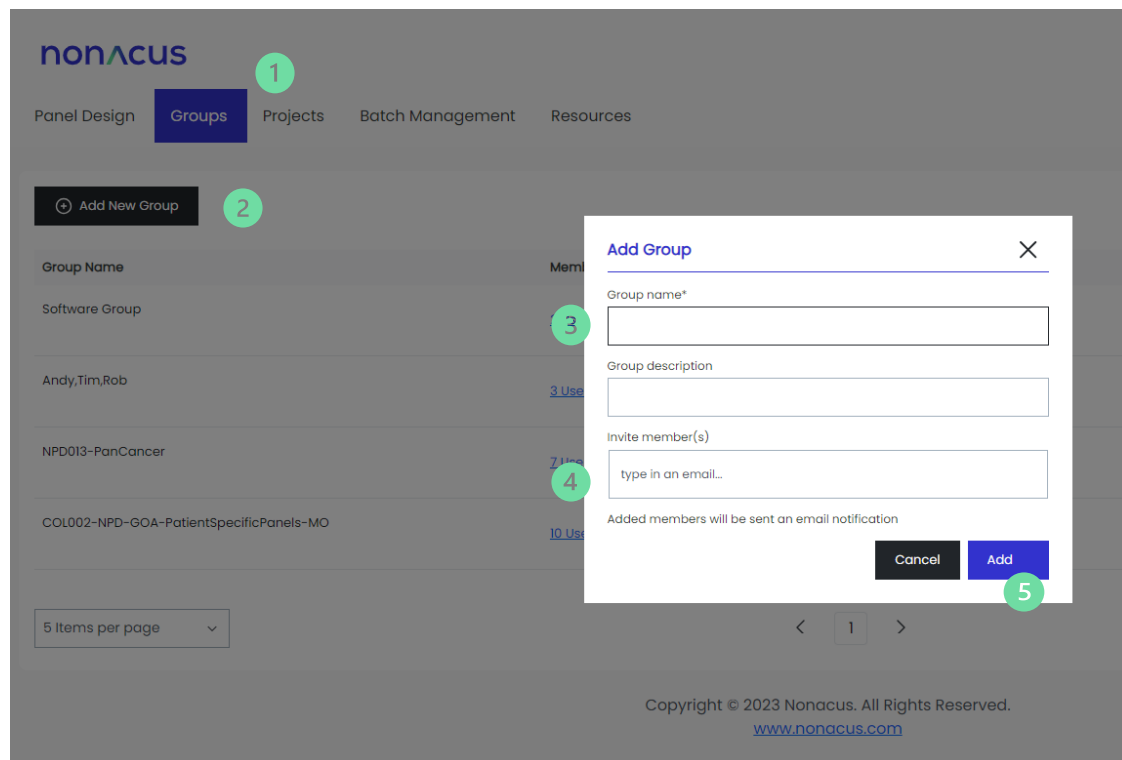
Total Covered (%): 100.0

Total Not Covered (%): 0.0

1. Find the panel you wish to view the design stats for
2. Click the Actions ellipsis '...' and choose Download. Files will be downloaded to your 'Downloads' folder

How to design your Custom NGS Panel

7. How to create a group to share your panel designs



The Group feature allows you to create a common repository where you can share your designs with colleagues and collaborators (with a mynonacus account)

1. Select the Groups tab
2. Click on '**Add new group**'
3. Give your group a name
4. Add each member's email address
5. Click '**Add**'

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7. How to share your panel designs

The screenshot shows the nonACUS interface with the 'Share with' dialog box open. The dialog box is titled 'Share with' and has a close button (X) in the top right corner. It contains a search bar, a list of groups, and a 'Share' button. The groups listed are 'Andy, Tim, Rob', 'COL002-NPD-GOA-PatientSpecificPanels-MO', 'NPD013-PanCancer', and 'Software Group'. There is also a 'Create New Group' button. The 'Share' button is highlighted with a green circle 4. The 'My Designs' table in the background has a 'Share' button highlighted with a green circle 2. The first row of the table has a checkmark in the 'Select' column, highlighted with a green circle 1.

Select	ID	Name	Gene List	GRCh38	2x	Gap Fill	Created	Action
<input checked="" type="checkbox"/>	1939	AF2 - RefSeq				✓	24/04/2023 13:11:21	⋮
<input type="checkbox"/>	1938	AF1 - coding 16-17				✓	24/04/2023 13:10:12	⋮
<input type="checkbox"/>	1931	HRD_HRR_combined_v2				✓	21/04/2023 10:43:41	⋮
<input type="checkbox"/>	1550	Panel for NKI				✓	09/11/2022 14:26:22	⋮
<input type="checkbox"/>	1546	Panel for Jitendra Badhai (NKI)				✓	08/11/2022 14:20:16	⋮

1. Select the panel(s) you wish to share
2. Click the **Share** button
3. Select the group(s) you wish to share with
4. Click 'Save'

An email will be sent to all the members of the group notifying them that a panel has been shared with them.

Q: Why has my design failed?

A: You may have entered an incorrect gene name (please check you are using NCI naming convention), if you entered a gene list, make sure it's in the right format – ie a list not in a line. If none of these work, please contact tech support: support@nonacus.com

Q: How do I find out how well the design covers my regions?

A: A pdf file called 'design stats' which contains information about your panel including percentage coverage can be downloaded by clicking the Actions ellipsis associated with your panel.

Q: My coverage is less than 100% how do I find out which regions are missing?

A: Download the BED file labelled 'covered' for your panel design. The BED file can be found by clicking the Actions ellipsis associated with your panel.

Q: I have some really important regions missing from my design – how can I get these covered?

A: Our design algorithm automatically masks highly repetitive regions of the genome to improve panel success and prevent excess capture and sequencing cost associated with these regions. If you have a region important to your work that is being masked and preventing probe design across that region, please contact technical support (support@nonacus.com) who can help you improve coverage.

Q: How much will my panel cost?

A: You can request a quote by clicking the Actions ellipsis associated with your panel. We price panels based on the number of probes in the panel:

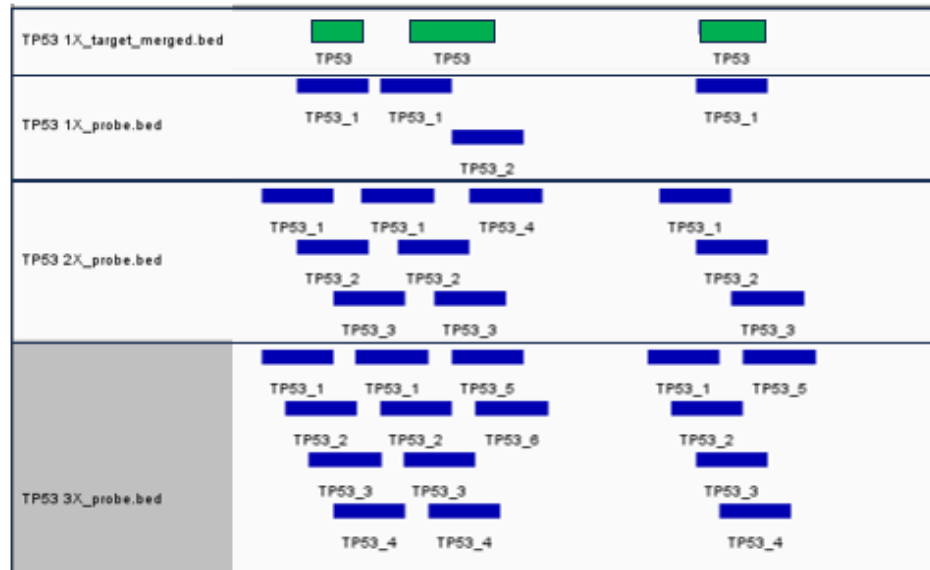
Catalogue number	Product Description	Number of probes
C3448CU	Cell3™ Target: Custom Panel, Tier 1 (48 samples)	10,000
C3496CU	Cell3™ Target: Custom Panel, Tier 1 (96 samples)	10,000
C3548CU	Cell3™ Target: Custom Panel, Tier 2 (48 samples)	20,000
C3596CU	Cell3™ Target: Custom Panel, Tier 2 (96 samples)	20,000
C3648CU	Cell3™ Target: Custom Panel, Tier 3 (48 samples)	50,000
C3696CU	Cell3™ Target: Custom Panel, Tier 3 (96 samples)	50,000
C3748CU	Cell3™ Target: Custom Panel, Tier 4 (48 samples)	100,000
C3796CU	Cell3™ Target: Custom Panel, Tier 4 (96 samples)	100,000

Region targeted

Tiling 1X

Tiling 2X

Tiling 3X



1. Tiling refers to the strategic arrangement of probes covering your region of interest. The goal is to minimise gaps and overlaps ensuring every part of the regions of interest is effectively captured.
2. The choice of tiling density depends on the complexity of the region
3. Configuring the tiling density in a design (1X, 2X, etc.) will apply the chosen density uniformly across all regions, regardless of their complexity
4. For optimal region performance, we recommend using the "exome" option, which enables the utilization of tiling and validated probes from the Nonacus exome
5. You can also manually "boost" a region by creating another design with the selected region and merge this design to the original one

My Designs

<input type="checkbox"/> Select	ID	Name	Input Type	Genome	Tiling
<input checked="" type="checkbox"/>	2219	Cancer panel amend	Gene List	GRCh38	2x
<input checked="" type="checkbox"/>	2218	Cancer panel	Gene List	GRCh38	2x

1. The combined feature allows you to merge two or more panel designs
2. If the same region appears multiple times, the tiling for this region will be combined
3. It can be used to combine any number of panel designs

1. The design zip is composed of 4 files:
2. Design stats : pdf file containing all the informations about the design
3. Covered : Bed file containing the region covered by the probes
4. Non_covered : Bed file containing the region NOT covered by the probes
5. Target_merged : Bed file containing the region targeted by the initial design

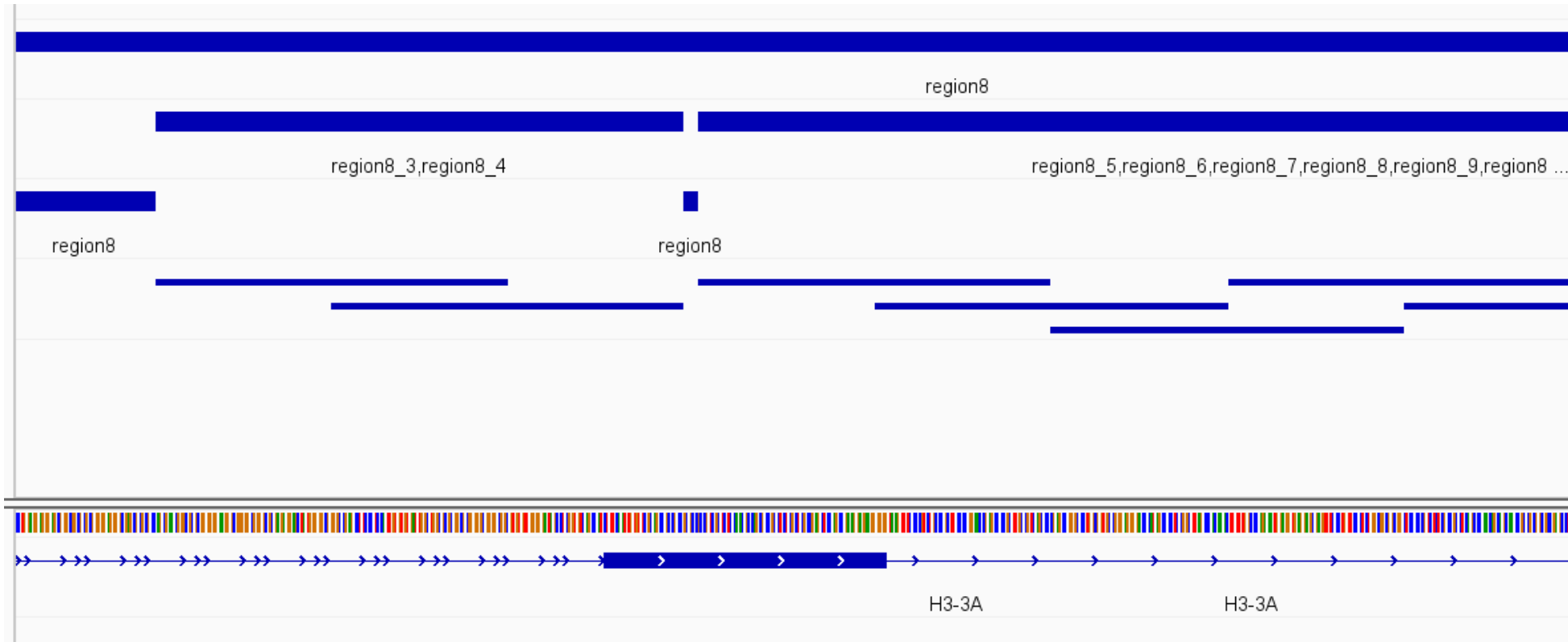


DESIGN REPORT
DESIGN INFORMATION
Design ID: XXXX
Design Name: XXXXXXXX
Genome Build: GRCh38

TARGET INFORMATION
Total Target Size (bp): XXXX
Number of Merged targets: XX

PROBE INFORMATION
Total Number of Probes: XXX
Total Covered Region (bp): XXXXXX
Total Covered (%): 100.0
Total Not Covered (%): 0.0

Appendix D : bed files



Region targeted

Region covered

Region non covered

Detail of the probes on the covered region (non provided)

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