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

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ADVANCING NON-INVASIVE HEALTHCARE

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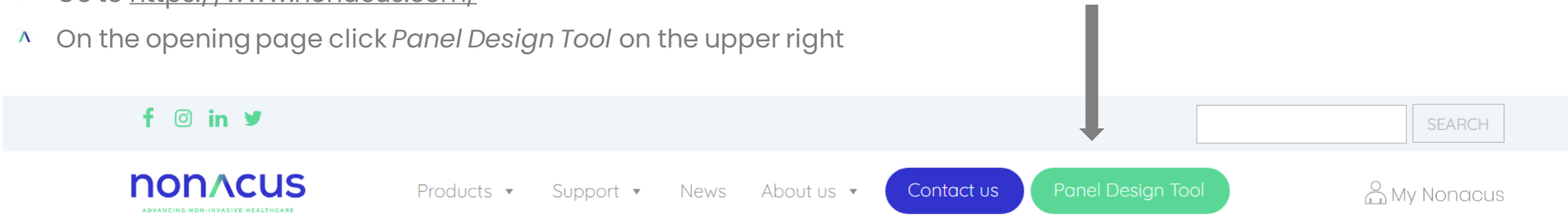
Content

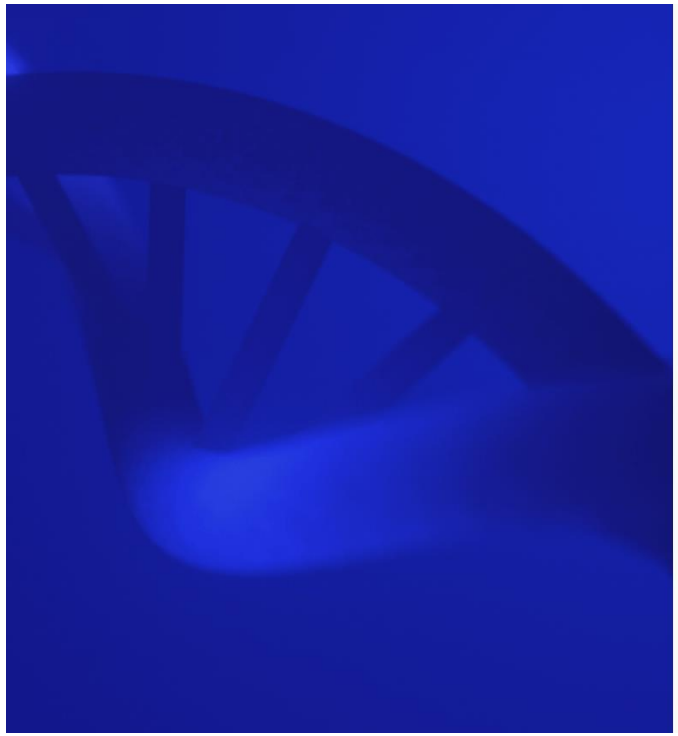
How to design your Custom NGS Panel

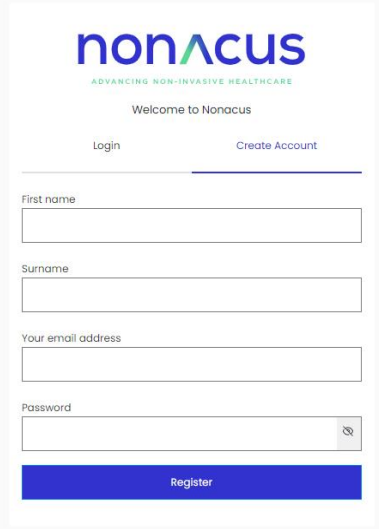
1. Create a Nonacus Account

- 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'

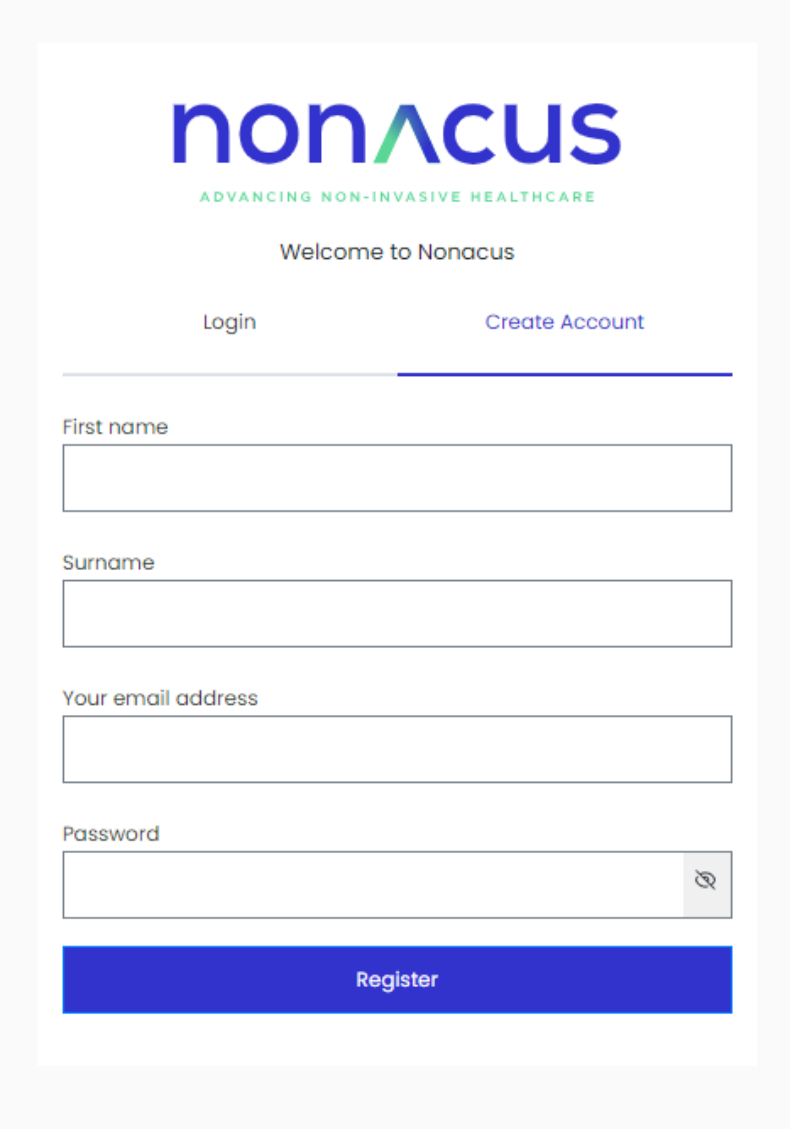




How to design your Custom NGS Panel

1. Create a Nonacus Account

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



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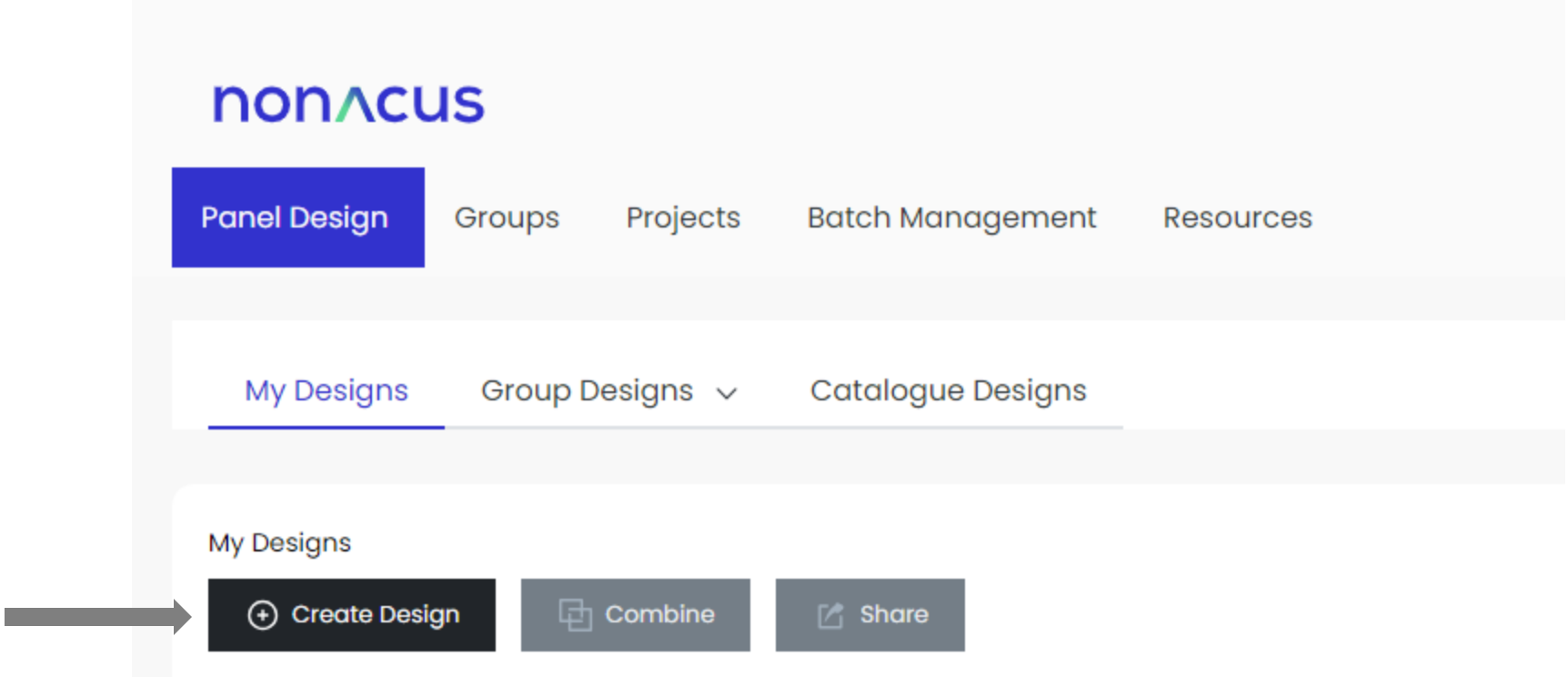
2. Create your own Custom NGS Panel

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



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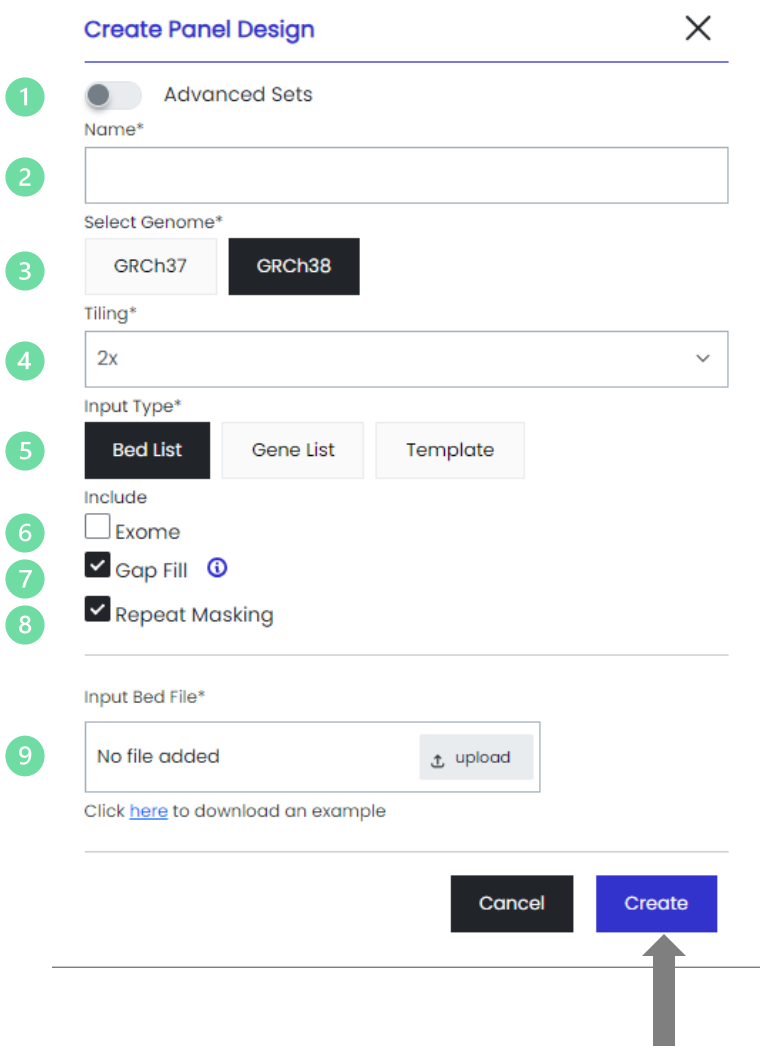
2. Overview of the 'Create Panel Design' page



1. **'Advanced'** setting provides more than 2X tilling if required (cf Appendix A : Tilling)
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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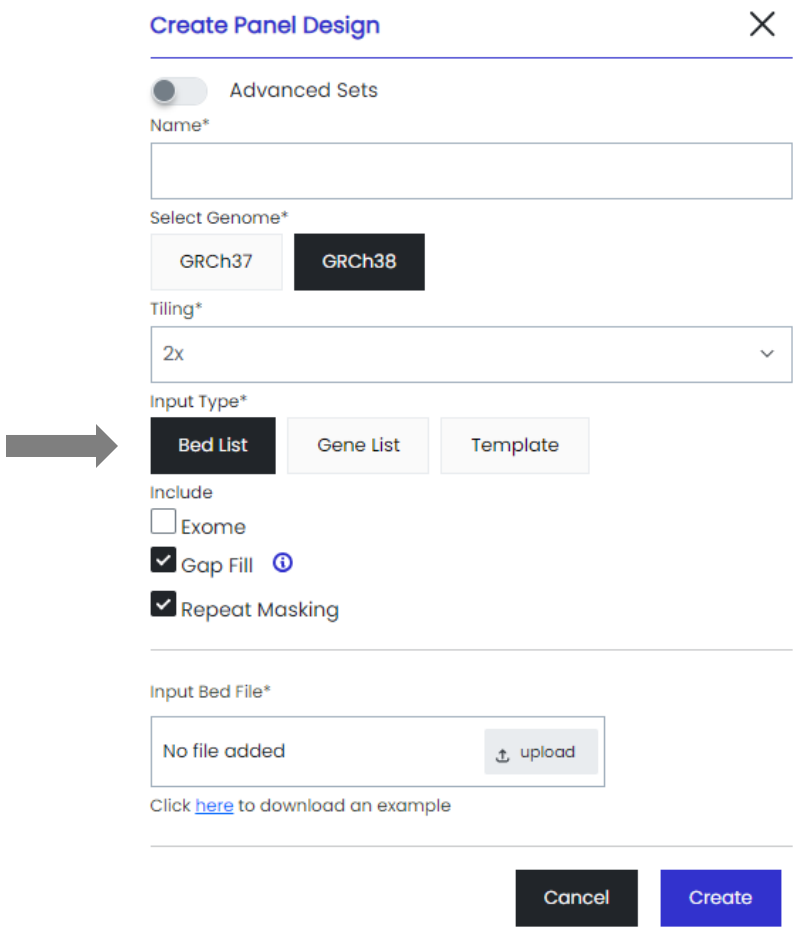
3. Create a Panel Design using a BED file



- 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

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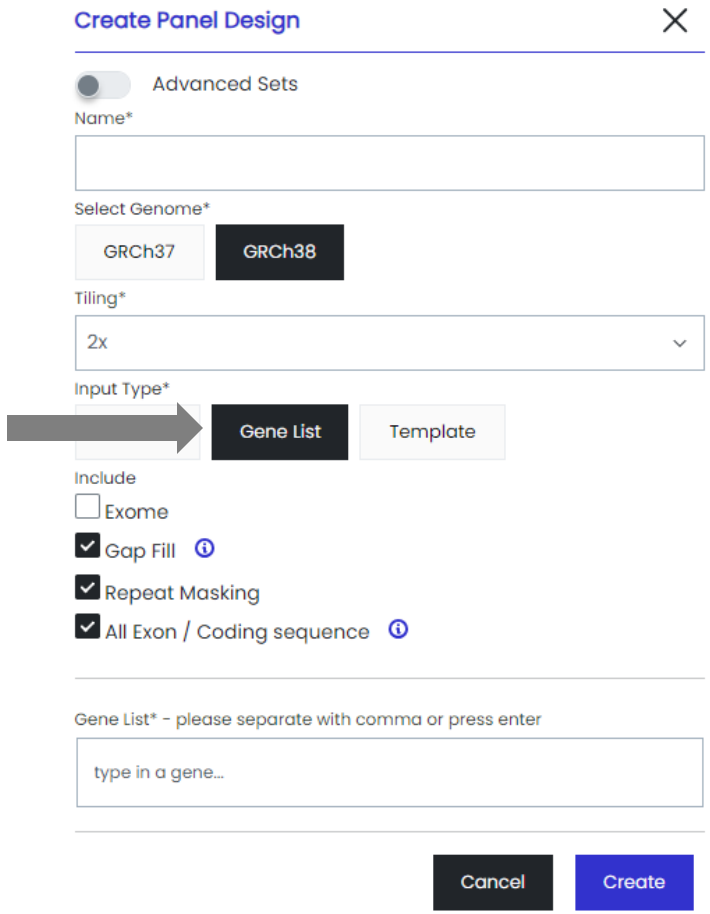

- Name your panel
- Select the Genome Option (**GRCh37** or **GRCh38**)
- Select the level of tiling
- Select Input Type: Gene List
- All Exon/Coding sequence: Tick for coding sequence only (exons), Untick for all exons/introns and to include 3' and 5' untranslated regions
- Enter your list of genes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

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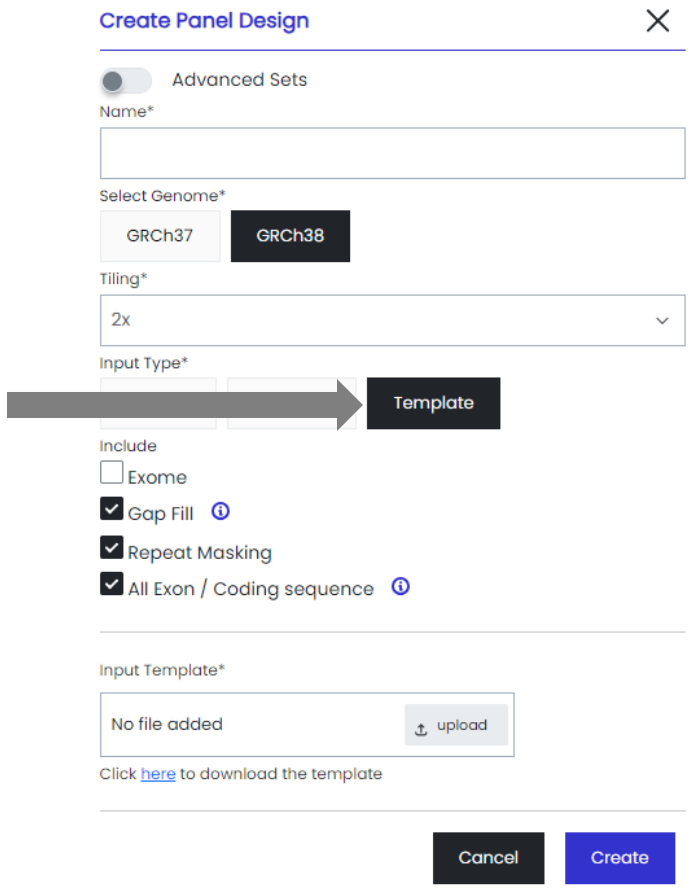
5. Create a Panel Design using a Template File



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

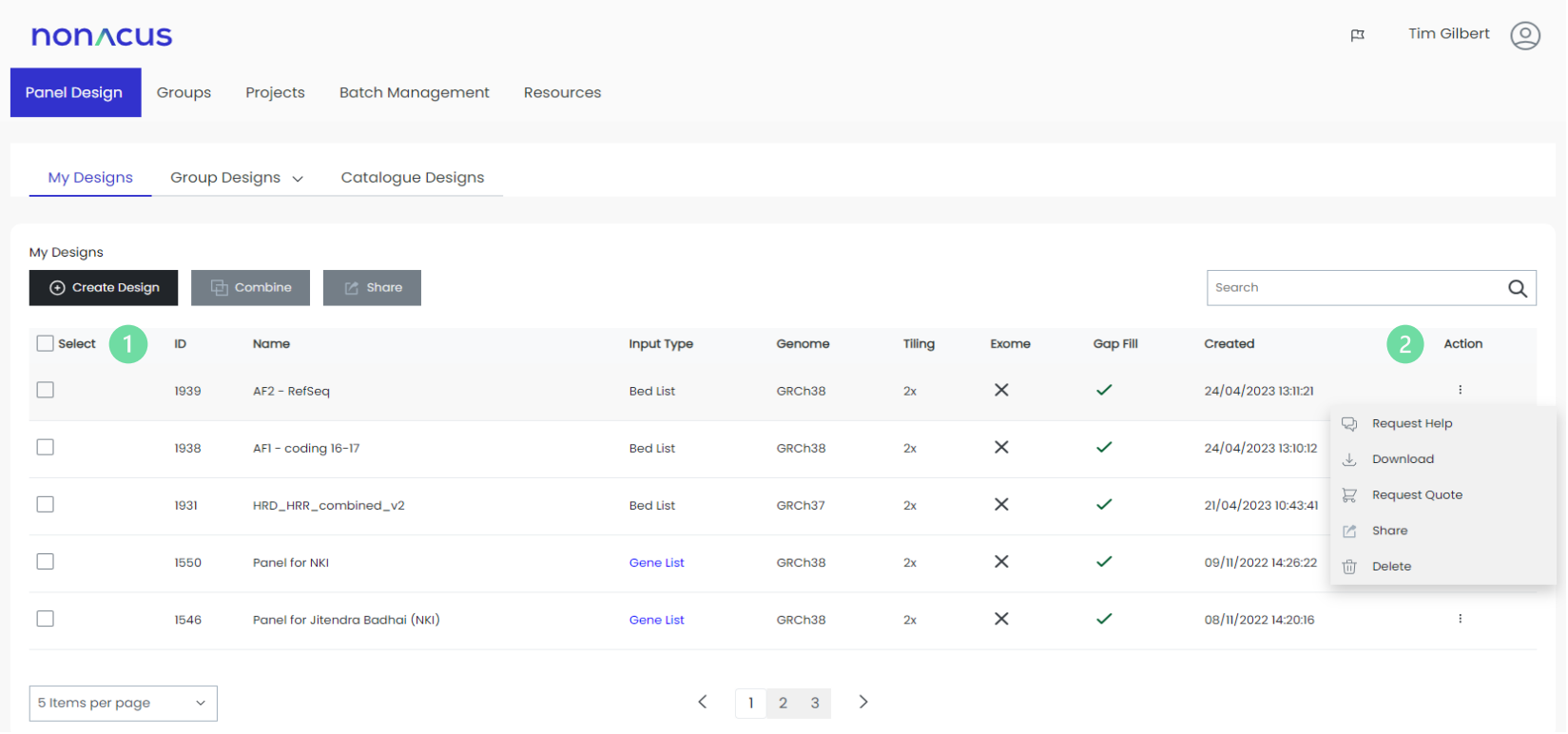
When the design is validated an email will be sent

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

How to design your Custom NGS Panel

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.



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

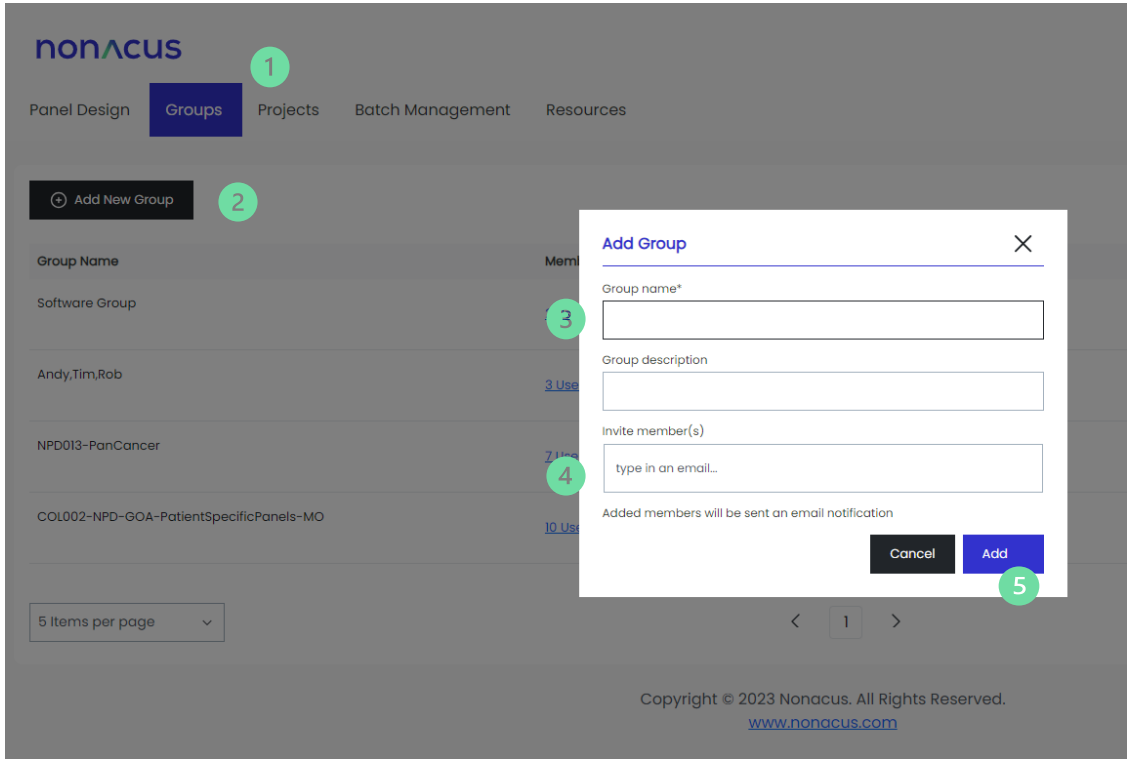
TARGET INFORMATION
Total Target Size (bp): XXXX
Number of Merged targets: XX

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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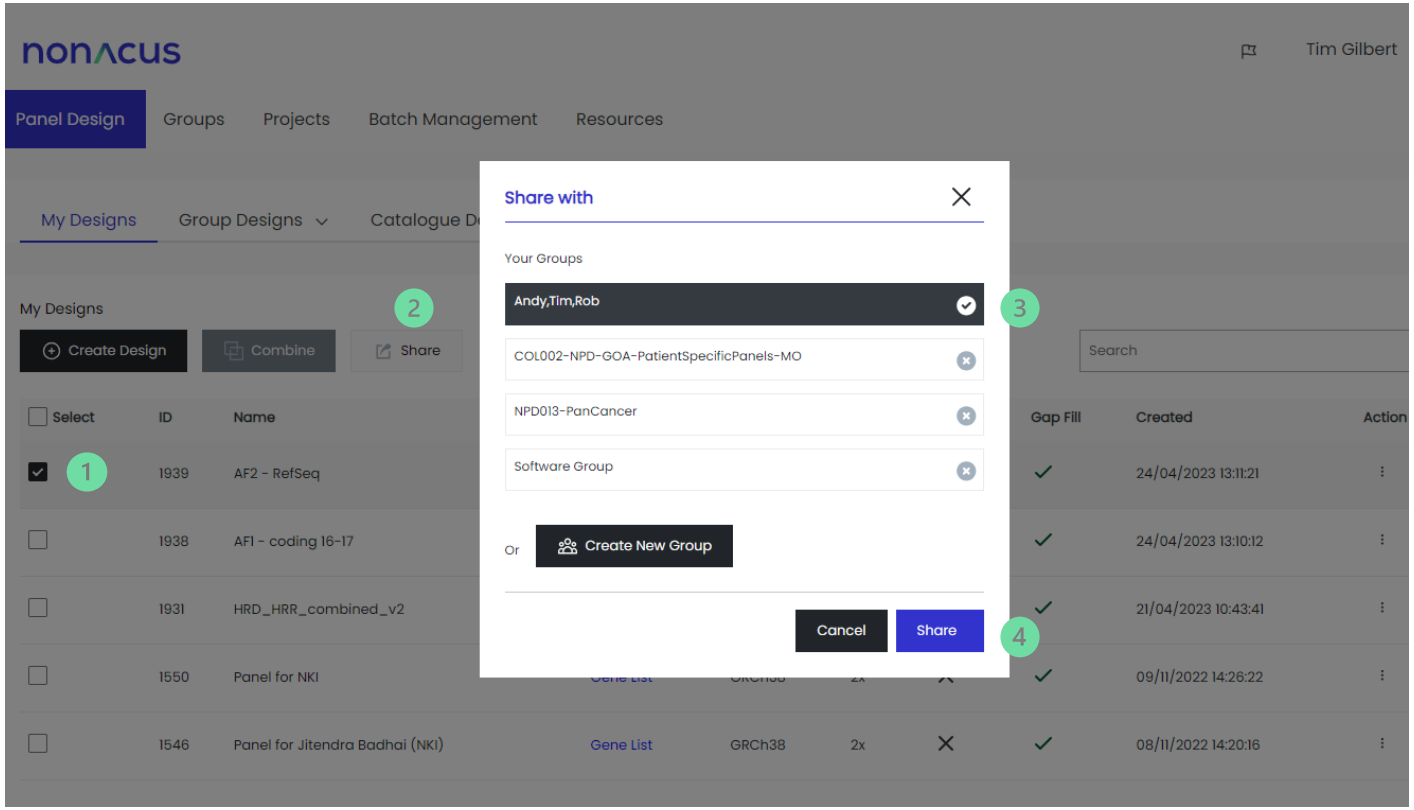
7. How to share your panel designs



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.

FAQs

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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.

FAQs

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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

Appendix A : Tilling

Region targeted

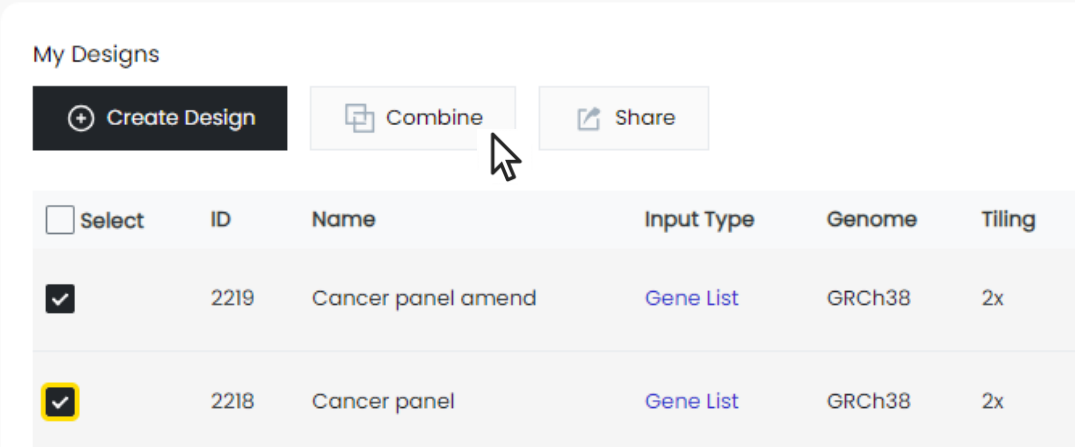
Tiling 1X

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

Appendix B : Combine



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

Appendix C : Design results

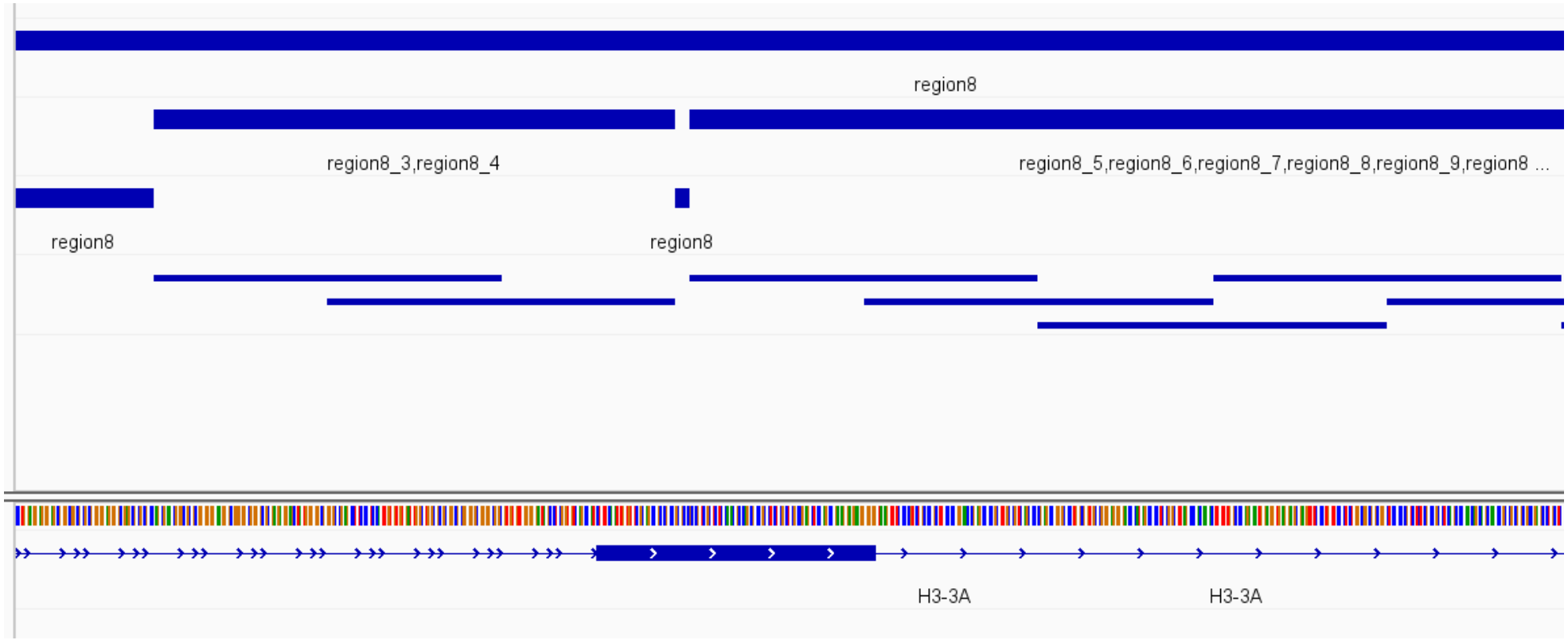
1. The design zip is composed of 4 files :
2. Design stats : pdf file containing all the informations about the design
3. Covered : Bed file containing the region covered by the probes
4. Non_covered : Bed file containing the region NOT covered by the probes
5. Target_merged : Bed file containing the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