

Australian Grains Genebank Strategic Partnership

Digital Tools Workshop

Duration: 4 hours, including breaks

Format: Hands-on with guided exercises

Prerequisites:

- An interest in using digital tools for genomic data analysis
- Your own laptop

Learning Objectives:

- Understand the capabilities of the digital tools *Genolink*, *FairyBread* and *Pretzel* and when to use them
- Search passport data effectively
- Explore the diversity of germplasm in the Australian Grains Genebank (AGG)
- Load genotype data and explore allelic diversity
- Search for AGG accessions containing a specific haplotype
- Use the digital tool ecosystem to help make more informed selections of germplasm from the AGG



The Australian Grains Genebank Strategic Partnership is a \$30M joint investment between the Victorian State Government and Grains Research and Development Corporation (GRDC) that aims to unlock the genetic potential of plant genetic resources for the benefit of the Australian grain growers. The Partnership is genotypically characterising the AGG plant genetic resource collection and building novel digital tools to improve accessibility and utility of the genebank for industry.

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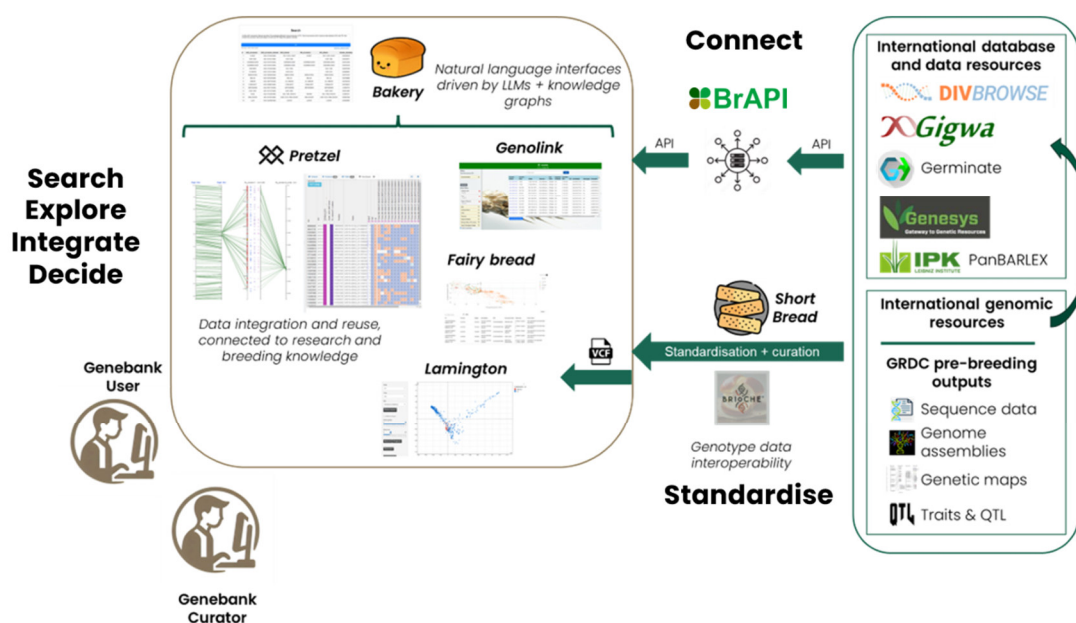
Introduction to the AGG digital tool ecosystem

Why does the digital tool ecosystem exist?

The Australian Grains Genebank (AGG) conserves a vast collection of crop genetic diversity that contains genes and traits that may be important for future crop improvement. While genotypic and passport datasets are becoming increasingly available, access to data alone does not necessarily help researchers and breeders make decisions about which accessions to use in their work.

A key challenge is that the data required to identify useful germplasm is often distributed across multiple databases, stored in different formats, and requires specialised bioinformatics expertise to analyse. As a result, there is often a gap between having access to data and being able to use that data to answer practical research and breeding questions.

The AGG Strategic Partnership digital tool ecosystem was developed to bridge this gap. The ecosystem transforms large and complex datasets into accessible, connected and actionable information, enabling users to move from searching for germplasm through to making evidence-based selection decisions.



What can the digital tool ecosystem do?

The ecosystem enables users to:

- Search and discover accessions held in the AGG.
- Access passport and genotype information through connected interfaces.
- Explore genetic diversity across thousands of accessions.
- Compare germplasm within the context of the broader genebank collection.
- Connect AGG accessions to published research, markers, genes, QTL, pangenomes and other genomic resources.
- Identify accessions carrying specific alleles, haplotypes or genomic regions of interest.
- Export information for downstream analyses and breeding decisions.
- Support both simple discovery tasks and advanced genomic investigations without requiring extensive bioinformatics expertise.

The tools are designed to support a progression from Search → Explore → Integrate → Decide, with different tools providing functionality at each stage of the workflow.

The tools in the ecosystem

Genolink

<https://genolink.plantinformatics.io>



Purpose: Search and discover germplasm.

Genolink is the entry point to the ecosystem. It connects accession passport information from global genebank resources such as Genesys-PGR (<https://www.genesys-pgr.org/>) with genotype datasets generated through the AGG Strategic Partnership.

What Genolink does:

- Searches AGG accessions using passport information.
- Identifies whether an accession has been genotyped.
- Links accession records directly to genotype datasets.
- Filters accessions using taxonomy, country of origin, donor information and other passport fields.
- Exports accession and genotype information.
- Provides an API that allows other tools in the ecosystem to access its data.

What makes *Genolink* different?

Unlike traditional genebank databases that focus primarily on passport information, *Genolink* seamlessly connects passport and genotype data. Users do not need to know where the underlying datasets are stored or manually cross-reference multiple databases. It acts as the integration layer that connects the ecosystem together.

FairyBread

<https://fairybread.plantinformatics.io>

Purpose: Explore genetic diversity.

FairyBread provides interactive visualisations of genetic diversity based on genotype data. It helps users understand how accessions are related to one another and where they sit within the diversity of the broader collection.

What *FairyBread* does:

- Visualises genetic diversity using PCA and related approaches.
- Displays accessions within the context of the complete collection.
- Integrates directly with passport information through *Genolink*.
- Allows users to identify diverse, unusual or genetically similar germplasm.
- Supports selection of accessions for research, pre-breeding and breeding activities.

What makes *FairyBread* different?

Many diversity analyses require specialised software and statistical expertise. *FairyBread* makes these analyses accessible through an interactive web interface, allowing users to visually explore thousands of accessions and immediately connect observations back to accession metadata and genotype information.

Pretzel

<https://agg.plantinformatics.io>

Purpose: Connect germplasm to genomic knowledge.



Pretzel is the ecosystem's advanced data integration platform. It links AGG genotype data to external genomic resources, enabling users to investigate genes, markers, haplotypes, QTL, pangenomes and genome assemblies within a single environment.

What *Pretzel* does:

- Visualises genebank genotype data.
- Connects AGG datasets with external genomic resources.
- Supports searches for genes, markers, genomic regions and haplotypes.
- Enables identification of accessions carrying specific genetic variants.
- Integrates genotype data generated from different technologies and time periods.
- Supports advanced research and breeding use cases, such as identifying introgressions, tracking trait-linked haplotypes and linking genomic discoveries back to AGG accessions.

What makes *Pretzel* different?

Pretzel is designed specifically to connect genebank collections with modern genomic knowledge. *Pretzel* aims to be a "Google Maps for genomic data", allowing users to navigate genomic information and connect it directly to germplasm resources. Rather than simply viewing markers, users can combine diverse datasets and knowledge sources to answer practical breeding questions.

Brioche

Purpose: Enable interoperability behind the scenes.



Brioche is a data translation tool that supports interoperability across different genome assemblies and genotyping platforms. Most end users will not interact directly with *Brioche*, but it plays a critical role in making the ecosystem function.

What *Brioche* does:

- Re-maps marker positions onto different reference genomes.
- Generates standardised genotype files.
- Supports comparisons across genome assemblies.
- Enables integration of historical and newly generated datasets.
- Allows pangenome assemblies to be translated into genotype data that can be compared directly with AGG collections.

What makes *Brioche* different?

Brioche solves one of the major barriers to data reuse: differences between genome assemblies and marker coordinates. By standardising data and making genotype datasets portable between reference genomes, it enables interoperability across projects, technologies and time.

Bakery (under development)



Purpose: Simplify access through artificial intelligence.

Bakery is the next-generation interface being developed for the ecosystem. It combines genebank data, genomic information and knowledge from literature with a large language model interface.

What *Bakery* will do:

- Allow users to ask questions in natural language.
- Connect information from multiple ecosystem tools automatically.
- Help users find accessions and answer research questions without requiring specialist knowledge of datasets or software.
- Translate complex analyses into straightforward recommendations.

What makes *Bakery* different?

Traditional genomic tools require users to understand the data structure [of their genomic data](#) and [the necessary](#) workflows [for analysis](#). *Bakery* aims to allow users to focus on their biological question rather than the mechanics of data analysis, creating a direct pathway from question to answer.

What makes the AGG ecosystem different?

The AGG Strategic Partnership Digital Tool Ecosystem is not a collection of independent applications. It is an integrated, open-source ecosystem built around interoperability, data standards and practical decision-making. The tools are designed to work together, allowing users to seamlessly move from accession discovery, to diversity exploration, to genomic investigation, and ultimately to germplasm selection decisions.

Most importantly, the ecosystem focuses on helping users answer real research and breeding questions with minimal bioinformatics expertise. Rather than simply making data available, the ecosystem aims to transform data into actionable knowledge and decisions. For more information on the Australian Grains Genebank Strategic Partnership and links to all the digital tools and datasets, visit the website:

<https://agg.plantinformatics.io/strategic-partnership/>

Workshop Overview

Today's workshop will cover:

- Comparing barley accessions in the AGG using passport data in *Genolink*
- Exploring genetic diversity of the collection in *FairyBread*
- Bringing knowledge from published manuscripts into *Pretzel* to search for a haplotype to tag the gene *Rrs2* for scald resistance
- Search for germplasm in the AGG potentially carrying *Rrs2* and investigate their overall genetic diversity using *FairyBread*
- Using information from literature, search for a haplotype to tag the *nud* gene conferring the hulless (naked caryopsis) trait in *Pretzel*
- Understanding how to use null calls in *Pretzel*
- Search for AGG accessions carrying the hulless haplotype in *Pretzel* and investigate their overall genetic diversity in *FairyBread*
- Optional exercises if time permits

Workshop tasks

Preliminary tasks

Task 1: Download files

Ensure e-mailed files are downloaded and ready for use or saved from <https://docs.plantinformatics.io/Workshops/workshops/>.

Task 2: Load each digital tool in a separate tab of your web browser

1. *Genolink*

<https://genolink.plantinformatics.io>

2. *FairyBread*

<https://fairybread.plantinformatics.io>

3. *AGG Pretzel*

<https://agg.plantinformatics.io>



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Task 3: Log in to *Pretzel* and complete Data Licence Agreement

If you haven't already requested a *Pretzel* account, click the 'Sign Up' link.

Otherwise, continue to log in with the 'Log In' link.

The screenshot shows the AGG Pretzel website. The URL <https://agg.plantinformatics.io> is circled in the browser's address bar. In the top right corner, the version 'v3.11.5 2026-07-21' is displayed next to three links: 'Documentation', 'Sign Up', and 'Log In', each enclosed in a green circle. A green callout box points to the 'Documentation' link and contains the text: 'Includes: User Stories, Use Cases, Basic Functions, Workshop manuals'. The main header features the AGG Pretzel logo and a description: 'Pretzel is an interactive web application designed to enable rapid translation of genomics outputs for any species into concrete answers to research and breeding questions'. Below this, a 'Recent Updates' section lists three updates with dates and links to DOI pages. On the right, a partial view of the application interface is shown, including a 'Dataset' tab and a 'Genotypes' section with a chromosome plot.

<https://agg.plantinformatics.io>

v3.11.5 2026-07-21

[Documentation](#) [Sign Up](#) [Log In](#)

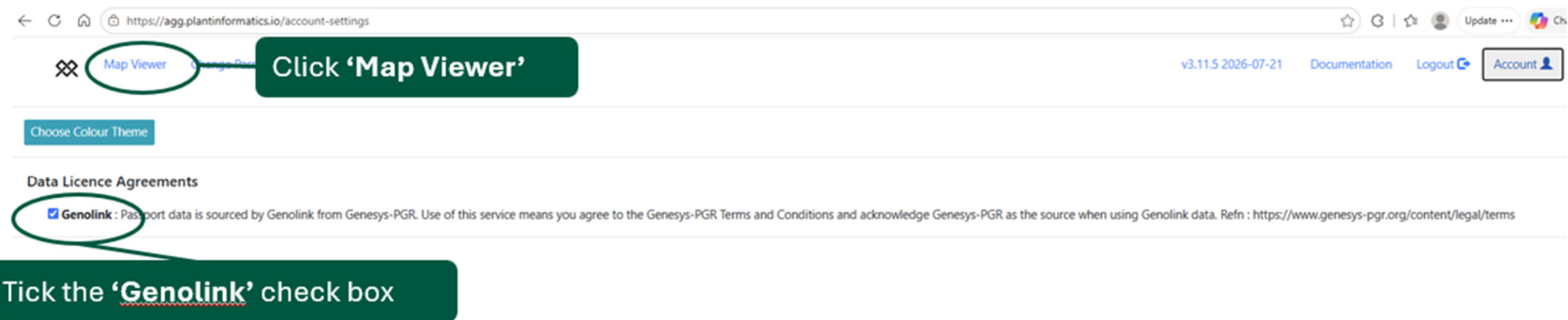
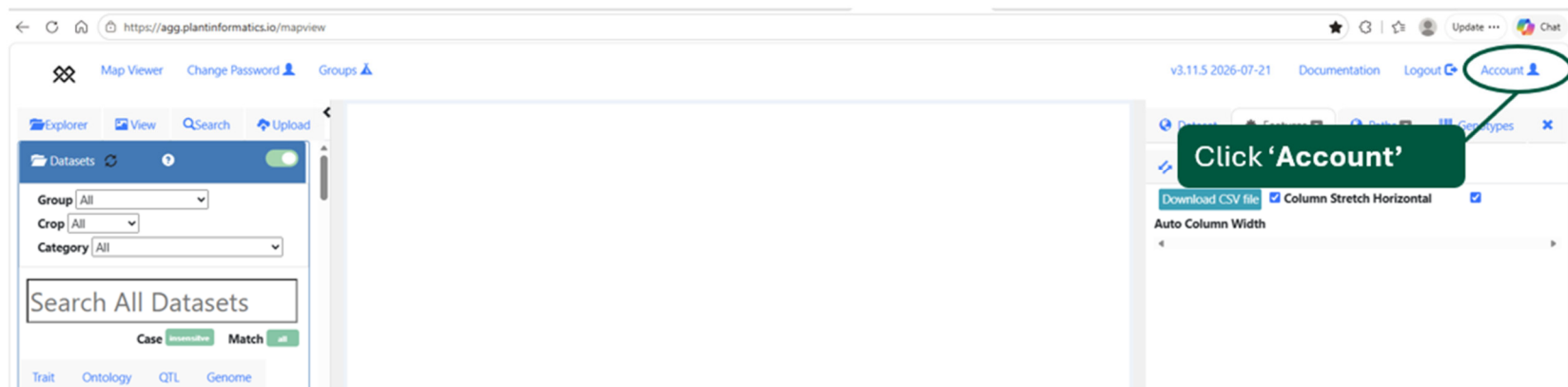
AGG Pretzel
Pretzel is an interactive web application designed to enable rapid translation of genomics outputs for any species into concrete answers to research and breeding questions

Recent Updates

- 30/07/2026 - Added genotypes for 32,656 Wheat AGG Plant genetic resources (<https://doi.org/10.7910/DVN/MOBT8>) genotyped by the AGG Strategic Partnership; updated in-silico genotype calls generated for assemblies in the 10+ Wheat Genomes project (Walkowiak et al. 2020) using Brioche v2; and updated marker positions for the Wheat Barley Infinium 40k BeadChip in IWGSC RefSeq v2.1 and IWGSC RefSeq v1.0 using Brioche v2.
- 17/06/2026 - New Pretzel version v3.11.0 released! Includes new functionality for displaying and filtering null alleles from genotype data. Additional new datasets loaded include: 27,242 barley AGG plant genetic resources (<https://doi.org/10.7910/DVN/LXU0WD>) genotyped by the AGG Strategic Partnership; in-silico genotype calls generated for assemblies in the Barley Pangeome v2 (Jayakodi et al. 2024) using Brioche v2; and updated marker positions for the Wheat Barley Infinium 40k BeadChip in Morex v3 using Brioche v2.
- 08/09/2025 - Added genotypes for 5,635 Field pea AGG Plant genetic resources (<https://doi.org/10.7910/DVN/A6WGYS>) genotyped as part of the AGG Strategic

Includes:
User Stories
Use Cases
Basic Functions
Workshop manuals

Agree to terms of using *Pretzel* - Click on 'Account' then tick the 'Genolink' box to agree to the Genesys-PGR Terms and Conditions.



Genolink tasks

Task 4: Navigating the Genolink interface

Go to Genolink (<https://genolink.plantinformatics.io/>). The Australian Grains Genebank (Institute Code AUS165) is pre-filtered by default.

The screenshot shows the Genolink web interface. The browser address bar displays <https://genolink.plantinformatics.io>. The interface has a green header with the Genolink logo and the text "Powered by Genesys-PGR". Below the header, there is a navigation bar with "Passport Data" and "Genotype Data" tabs. The "Passport Data" tab is active. On the left side, there is a "Filters" panel. It shows "Total Accessions: 178265" and "Filter Mode: Passport Filter". Under "Active Filters", "Institute Code: AUS165" is listed. Below this, there are several filter categories with dropdown menus: Date, Holding Institute, Crops, Taxonomy: Genus, Taxonomy: Genus Species, Taxonomy: Species, Origin Of Material, Donor Institute, Biological Status Of Accession, Type Of Germplasm Storage, Available for distribution, Curation type, FIGS set, and Subsets. The "Check for genotype" checkbox is also present. The main area displays a table of passport data. The table has columns: #, Institute Code, Holding Institute, Accession Number, Accession Name, Aliases, Remarks, Taxonomy, Crop Name, and Ge. The first 20 rows are visible, all with Institute Code AUS165. At the bottom of the table, there are buttons for "More Results (156)" and "Export All Passport Data".

#	Institute Code	Holding Institute	Accession Number	Accession Name	Aliases	Remarks	Taxonomy	Crop Name	Ge
1	AUS165	Australian Grains Ge...	AGG 400270 BARL	ANABEE	PI 320209, selection f...	N/A	Hordeum vulgare L.	Barley	Ho
2	AUS165	Australian Grains Ge...	AGG 400280 BARL	APIZACO	PI 467741, VDB 818.5	N/A	Hordeum vulgare L.	Barley	Ho
3	AUS165	Australian Grains Ge...	AGG 400268 BARL	AMAKEI NO.13	VDB 2130.1	N/A	Hordeum vulgare L.	Barley	Ho
4	AUS165	Australian Grains Ge...	AGG 400293 BARL	ARIANA	PI 55525, Cih 2524, ...	N/A	Hordeum vulgare L.	Barley	Ho
5	AUS165	Australian Grains Ge...	AGG 400265 BARL	ALL WRONG	Cih 6178, VDB 1248.1	N/A	Hordeum vulgare L.	Barley	Ho
6	AUS165	Australian Grains Ge...	AGG 400292 BARL	ARGENTINE 48/2127	VDB 17.1	N/A	Hordeum vulgare L.	Barley	Ho
7	AUS165	Australian Grains Ge...	AGG 400295 BARL	ARIMAR	selection from landra...	N/A	Hordeum vulgare L.	Barley	Ho
8	AUS165	Australian Grains Ge...	AGG 400271 BARL	ANATOLIAN BLACK	Cih 2970, VDB 2254.1	N/A	Hordeum vulgare L.	Barley	Ho
9	AUS165	Australian Grains Ge...	AGG 400302 BARL	ARMELLE	PI 410855, VDB 2384.1	N/A	Hordeum vulgare L.	Barley	Ho
10	AUS165	Australian Grains Ge...	AGG 400278 BARL	APAM	PI 467737, VDB 817.1	N/A	Hordeum vulgare L.	Barley	Ho
11	AUS165	Australian Grains Ge...	AGG 400297 BARL	ARIAE	Cih 2465, NWB 266,...	N/A	Hordeum vulgare L.	Barley	Ho
12	AUS165	Australian Grains Ge...	AGG 400296 BARL	ARIMAR	Cih 13626, Arizona ...	N/A	Hordeum vulgare L.	Barley	Ho
13	AUS165	Australian Grains Ge...	AGG 400272 BARL	ANOIDIUM	Cih 7269, VDB 261.3	N/A	Hordeum vulgare L.	Barley	Ho
14	AUS165	Australian Grains Ge...	AGG 400305 BARL	ASA	PI 266124, VDB 629.1	N/A	Hordeum vulgare L.	Barley	Ho
15	AUS165	Australian Grains Ge...	AGG 400276 BARL	ANTARES	PI 584865, VDB 2337.1	N/A	Hordeum vulgare L.	Barley	Ho
16	AUS165	Australian Grains Ge...	AGG 400260 BARL	ALGERIAN	NSB 152	N/A	Hordeum vulgare L.	Barley	Ho
17	AUS165	Australian Grains Ge...	AGG 400273 BARL	ANSGAR	PI 584821, WW 6156,...	N/A	Hordeum vulgare L.	Barley	Ho
18	AUS165	Australian Grains Ge...	AGG 400299 BARL	ARIVAT	Cih 7534, NSB 316	N/A	Hordeum vulgare L.	Barley	Ho
19	AUS165	Australian Grains Ge...	AGG 400269 BARL	AMARILLO	PI 39530, Cih 1073, ...	N/A	Hordeum vulgare L.	Barley	Ho
20	AUS165	Australian Grains Ge...	AGG 400266 BARL	ALPHA	NWB 781	N/A	Hordeum vulgare L.	Barley	Ho

View

Passport Data Genotype Data

Filters

Total Accessions: 178265

Apply Filter Reset Filter

Filter Mode: Passport Filter

Active Filters

Institute Code: AUS165

Text Search

Date

Holding Institute

Crops

Taxonomy: Genus

Taxonomy: Genus Species

Taxonomy: Species

Origin Of Material

Donor Institute

Biological Status Of Accession

Type Of Germplasm Storage

Available for distribution

Curation type

FIGS set

Subsets

☐ Check for genotype

#	Institute
1	AUS165
2	AUS165
3	AUS165
4	AUS165
5	AUS165
6	AUS165
7	AUS165
8	AUS165
9	AUS165
10	AUS165
11	AUS165
12	AUS165
13	AUS165
14	AUS165
15	AUS165
16	AUS165
17	AUS165
18	AUS165
19	AUS165
20	AUS165

More Results (356)

Genolink provides passport data (default) and genotype data

Three filter modes could be accessed through the drop down

Passport Filter (default): for searching any term in the passport data

Accession Filter: for searching a specific accession number
e.g., AGG 423634 BARL

Genotypeld Filter: for searching a specific genotype ID
e.g., AGG423634BARL1-B00026-2-79

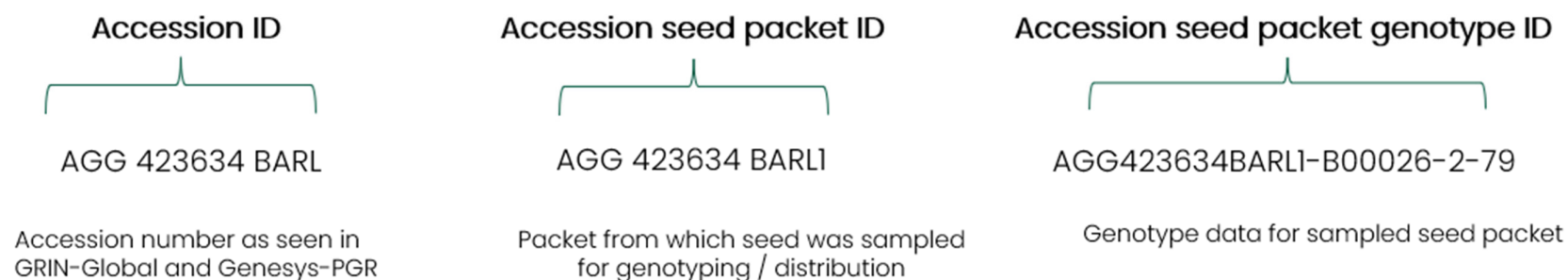
Under passport filter, various search options could be combined to refine searches

It is possible to apply a filter to limit the display to only accessions that have been genotyped by ticking this box

Accession and Genotype IDs

In this exercise, we will search for some known varieties of barley to assess their diversity in the context of global diversity.

The Genotype ID provided uniquely identifies the DNA sample that was genotyped in the AGG Strategic Partnership for that accession. Genesys-PGR tracks passport data at the accession level, while the AGG digital tool ecosystem captures genotype data at the level of DNA isolated from a specific seed packet.



Accession IDs can be found from several sources:

- AGG GRIN-Global database (<https://ausgenebank.agriculture.vic.gov.au/gringlobal/search>)
- Genolink (<https://genolink.plantinformatics.io/>)
- Genesys-PGR (<https://www.genesys-pgr.org/>)
- Publications

Task 5: Searching passport data for specific accession names

First, we will search for barley accessions called 'Osiris' in the AGG.

1. Search for Osiris in the 'Text Search' bar

2. Select 'barley' in the 'Crops' dropdown menu

3. Click on 'Apply Filter'

The interface shows a 'Passport Data' tab with a table of accessions. The 'Active Filters' section includes 'Institute Code: AUS165', 'Text Search', 'Date', 'Holding Institute', and 'Crops'. The 'Crops' dropdown menu is open, showing a list of crops with 'barley 28237' selected. The 'Apply Filter' button is highlighted in the right-hand view.

#	Institute Code
1	AUS165
2	AUS165
3	AUS165
4	AUS165
5	AUS165
6	AUS165
7	AUS165
8	AUS165
9	AUS165
10	AUS165
11	AUS165
12	AUS165
13	AUS165
14	AUS165
15	AUS165
16	AUS165

Now we can review passport data for the 11 accessions returning a match for 'Osiris'.

The number of accessions meeting the filter criteria are indicated

The screenshot shows the 'Passport Data' tab in the Genesys-PGR system. On the left, a 'Filters' panel shows 'Total Accessions: 11' and 'Filter Mode: (Passport Filter)'. The 'Active Filters' section lists: Text: Osiris, Institute Code: AUS165, and Crop: barley. The main table displays 11 accessions, all from AUS165, with names like OSIRIS 3, OSIRIS, and OSIRIS. A green callout points to the 'Total Accessions: 11' text. Below the table is an 'Export All Passport Data' button. To the right, a detailed table shows the biological status, donor institute, provenance, region, and sub-region for each accession.

Biological status of accession	Donor Institute	Provenance of Material	Region	Sub-Region
Breeding/Research Material	Victoria Department of...	N/A	N/A	N/A
Traditional cultivar/Landrace	Victoria Department of...	Algeria	Africa	Northern Africa
Advanced/improved cultivar	THE UNIVERSITY OF W...	N/A	N/A	N/A
Advanced/improved cultivar	INSTITUTE OF PLANT ...	Germany	Europe	Western Europe
Advanced/improved cultivar	INSTITUTE OF PLANT ...	Germany	Europe	Western Europe
Advanced/improved cultivar	DENMARK	Algeria	Africa	Northern Africa
Advanced/improved cultivar	ICARDA, NURSERIES	Syria	Asia	Western Asia
Traditional cultivar/Landrace	ICARDA	Algeria	Africa	Northern Africa
Genetic stock	ICARDA	Algeria	Africa	Northern Africa
Genetic stock	THE UNIVERSITY OF A...	Australia	Oceania	Australia and Ne...
Breeders Line	N/A	N/A	N/A	N/A

Note!
The passport data retrieved from Genesys-PGR shows many differences for the accessions called 'Osiris'. It is unclear from passport data alone whether these accessions might be the same line or completely unrelated to one another.

Sometimes it is not clear why an accession has been returned from the search. Clicking on the accession link will take you back to Genesys-PGR, where you can see the full passport data. For example, the search term may appear in the pedigree, rather than the accession name.

Note!

Search result for 'Osiris' shows an accession called 'FFM221115'.

#	Institute Co...	Holding Institute	Accession Number	Accession Name	Aliases
1	AUS165	Australian Grains ...	AGG 40165 BARL	OSIRIS 3	VDB 734.2
2	AUS165	Australian Grains ...	AGG 401679 BARL	OSIRIS	Ciho 1622, selection from P...
3	AUS165	Australian Grains ...	AGG 401672 BARL	OSIRIS	WUB 341, P 8708
4	AUS165	Australian Grains ...	AGG 411853 BARL	OSIRIS	CI 1622, HOR 9487
5	AUS165	Australian Grains ...	AGG 411854 BARL	OSIRIS	N/A
6	AUS165	Australian Grains ...	AGG 495136 BARL	OSIRIS	N/A
7	AUS165	Australian Grains ...	AGG 495204 BARL	Osiris	N/A
8	AUS165	Australian Grains ...	AGG 495188 BARL	OSIRIS	selection from PI 39590, CL...
9	AUS165	Australian Grains ...	AGG 495182 BARL	OSIRIS	N/A
10	AUS165	Australian Grains ...	AGG 495289 BARL	Osiris	N/A
11	AUS165	Australian Grains Genebank, Agriculture Victoria	AGG 1041142 BARL	FFM221115	IC817-RA-281-OTR-OMR-OMR-2MR, ZCB23 74

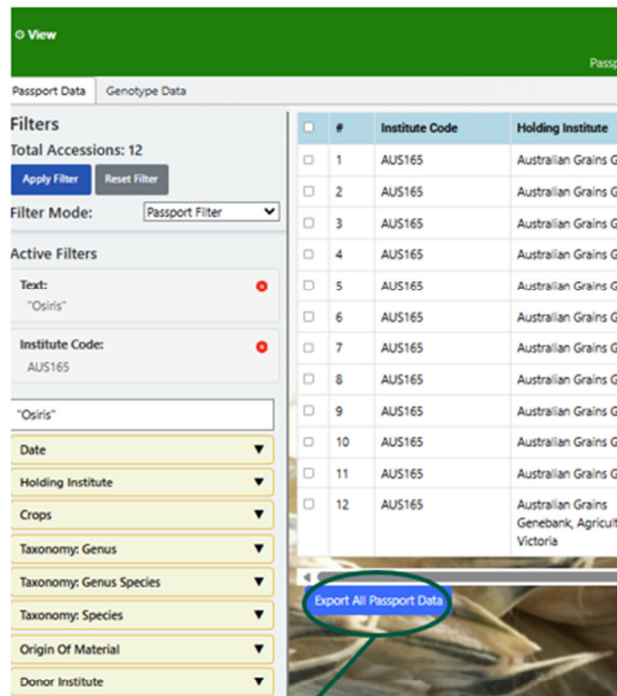
[Export All Passport Data](#)

4. Click the accession link to its Genesys-PGR record

Genesys	
Accession data	Directory
Resources	My List
Login	
Accession data > Passport data > AGG 1041142 BARL	
Accession details	
Passport data and everything else	
AGG 1041142 BARL	
Add AGG 1041142 BARL to my list	
SHOW CHANGES	
SIMILAR	
DOI	10.18730/1R1V5K
Accession number	AGG 1041142 BARL
Holding Institute	Australian Grains Genebank, Agriculture Victoria — Australia
Institute code	AUS165
Acquisition Date	January 18, 2023
Biological status of accession	Breeders Line
Ancestral information (pedigree)	RHN-03//LIGNEES27/NK1272/3//LIGNEES27/CHN-01//ALANDA-OSIRIS/BB89.2027/CHAMICO//TRADITION
Type of germplasm storage	Long term seed collection
Curation type	Partly curated
Availability for distribution	Available for distribution
ITPGRFA MLS	Accession is part of the Multi-lateral system of ITPGRFA
Accession URL	https://ausgenebank.agriculture.vic.gov.au/gringlobal/accessionetail?id=333370

Accession was retrieved by *Genolink* since Osiris is recorded as a part of its pedigree

Desired passport data fields can be exported as a text file.



5. Click on 'Export All Passport Data' to open download options menu



6. Either 'Select All Fields' or choose from individual fields to download passport data and select 'Download'

7. Click 'Close' to exit the download menu

Next, we will explore how the search can be refined using exact phrase matching.

1. Search for La Trobe in the 'Text Search' bar

#	Institute Code	Holding Institute	Accession Number	Accession Name	Aliases
1	AUS165	Australian Grains Ge...	AGG 401255 BARL	LA MESTA	NSB 350
2	AUS165	Australian Grains Ge...	AGG 401254 BARL	LA ESTANZUELA FO...	VD8 2235.1
3	AUS165	Australian Grains Ge...	AGG 401253 BARL	LA ESTANZUELA 750...	VD8 2112.1
4	AUS165	Australian Grains Ge...	AGG 401256 BARL	LA PREVISION 19	VD8 374.1
5	AUS165	Australian Grains Ge...	AGG 403091 BARL	FLUKA	PI 506423
6	AUS165	Australian Grains Ge...	AGG 402993 BARL	ALOUETTE	FRANRAGVS 55396
7	AUS165	Australian Grains Ge...	AGG 402994 BARL	TORKEL	FRANRAGVS 115548
8	AUS165	Australian Grains Ge...	AGG 405923 BARL	SMASH	PI 483454
9	AUS165	Australian Grains Ge...	AGG 405924 BARL	PRECOCE LARITE	MPH 1267, PI 584908...
10	AUS165	Australian Grains Ge...	AGG 405920 BARL	MOGADOR	PI 584910
11	AUS165	Australian Grains Ge...	AGG 405922 BARL	PRECOCE LEPEUPLE	PI 315956, Cho 13169
12	AUS165	Australian Grains Ge...	AGG 405932 BARL	NEFTA	PI 584858
13	AUS165	Australian Grains Ge...	AGG 405921 BARL	PASTORALE	N/A
19	AUS165	Australian Grains Ge...	AGG 406364 BARL	MALTINE	CFH 4914
20	AUS165	Australian Grains Ge...	AGG 406363 BARL	PRESSION	CFH 4572

2. Search for La Trobe in the 'Text Search' bar using double quotes

#	Institute Code	Holding Institute	Accession Number	Accession Name	Aliases
1	AUS165	Australian Grains Ge...	AGG 419881 BARL	LA TROBE	N/A
2	AUS165	Australian Grains Ge...	AGG 1042268 BARL	ICBM18-0140-OMR-0...	FFM2300151, ZBF24 ...
3	AUS165	Australian Grains Ge...	AGG 414119 BARL	OzBarley_MAXIMUS ...	OzB_062

Why did these accession match the search query?

Searching "La Trobe" accession returns 2 matches compared with 110 results for La Trobe.

Note!
Genolink applies 'OR' logic between search terms.

Task 6: Accessing genotype data from *Genolink*

Next, we will assess whether the Osiris accessions have been genotyped and find the Genotype ID. Note that the Genotype ID is used in the genotype data within *Genolink* itself, but also in the digital tool *Pretzel* and the original genotype data files released on Dataverse.

Passport Data Genotype Data

Passport data sourced from Genesys-Plot. Use of this service means you agree

Filters

Total Accessions: 11

Apply Filter Reset Filter

Filter Mode: Passport Filter

Active Filters

Text: Osiris

Institute Code: AUS165

Crops: barley

Osiris

Date

Holding Institute

Crops

#	Institute Code	Holding Institute	Accession Number	Accession Name
1	AUS165	Australian Grains Ge...	AGG 401680 BARL	OSIRIS 3
2	AUS165	Australian Grains Ge...	AGG 401679 BARL	OSIRIS
3	AUS165	Australian Grains Ge...	AGG 401672 BARL	OSIRIS
4	AUS165	Australian Grains Ge...	AGG 411853 BARL	OSIRIS
5	AUS165	Australian Grains Ge...	AGG 411854 BARL	OSIRIS
6	AUS165	Australian Grains Ge...	AGG 495136 BARL	OSIRIS
7	AUS165	Australian Grains Ge...	AGG 495204 BARL	Osiris
8	AUS165	Australian Grains Ge...	AGG 495188 BARL	OSIRIS
9	AUS165	Australian Grains Genebank, Agriculture Victoria	AGG 495182 BARL	OSIRIS
10	AUS165	Australian Grains Ge...	AGG 495289 BARL	Osiris
11	AUS165	Australian Grains Ge...	AGG 1041142 BARL	FFM221115

Export All Passport Data

1. Repeat the search for Osiris in the Passport Data

2. Scroll the bottom bar to the right to access passport data

Genotype Status	GenotypeID
Completed	AGG401680BARL1-B...
Completed	AGG401679BARL1-B...
Completed	AGG401672BARL1-B...
Completed	AGG411853BARL1-B...
Completed	AGG411854BARL2-B...
Completed	AGG495136BARL1-B...
Completed	AGG495204BARL1-B...
Completed	AGG495188BARL1-B...
Completed	AGG495182BARL1-B00026-8-22
TBC	N/A
TBC	N/A

Note!

Completed:
genotype data available

Pending:
genotyping scheduled

TBC:
genotyping not scheduled

Excluded:
genotyping is not scheduled

Genotype data can be accessed from *Gigwa* directly through *Genolink* for specified accessions. Genotype data is available as raw or as filled-in genotypes (where missing data has been imputed).

3. Tick box in header to select all accessions

4. Click on 'Genotype Data'

	Institute Code	Holding Institute	Accession Number
☒	AUS165	Australian Grains Genebank, Agriculture Victoria	AGG 401680 BARL
☒	AUS165	Australian Grains Ge...	AGG 401679 BARL
☒	AUS165	Australian Grains Ge...	AGG 401672 BARL
☒	AUS165	Australian Grains Ge...	AGG 411853 BARL
☒	AUS165	Australian Grains Ge...	AGG 411854 BARL
☒	AUS165	Australian Grains Ge...	AGG 495136 BARL
☒	AUS165	Australian Grains Ge...	AGG 495204 BARL
☒	AUS165	Australian Grains Ge...	AGG 495188 BARL
☒	AUS165	Australian Grains Ge...	AGG 495182 BARL
☒	AUS165	Australian Grains Ge...	AGG 495289 BARL
☒	AUS165	Australian Grains Ge...	AGG 1041142 BARL

5. Click on 'Lookup Data'

Server found: gigwa.plantinformatics.io

6. Select raw genotype calls from the gigwa server

7. Select 'PositionRange' filter type from the drop-down list

Genotype data for specific genomic regions can be shown through filtering by position (chromosome and base-pair range), or by marker identifiers (e.g. AVRIG03881, AVRIG03883) (Marker IDs are provided in 2026_ABTS_SupplementaryFile1.txt).

Passport data sourced from Genesys-PGR. Use of this service means you agree to their [Terms and Conditions](#) and acknowledge Genesys-PGR as the data provider.

Passport Data | Genotype Data

Genotype Data

Search Genotype **Reset**

Search Summary

Server: gigwa.plantinformatics.io
 9 of 11 accessions have sample name mappings.
 9 of 11 accessions have genotypes in Gigwa.

Select Dataset:

Server: gigwa.plantinformatics.io
 AGG_BARLEY519260430_AGG-
 Barley_MorexV3_Raw_phased_anchors_unique
 AGG_BARLEY5260430_AGG-
 Barley_MorexV3_Filled_in_phased_anchors_unique

PositionRange
 Start Position End Position
 chr3H

8. Select 'chr3H' from the drop-down list

9. Click on 'Search Genotype'

Note!
 Two accessions have different genotype calls relative to the other accessions.

	CHROM	POS	ID	REF	ALT	OSIRISGAG 401679 BA	OSIRISGAG 401672 BA	OSIRISGAG 411853 BA	OSIRISGAG 495188 BA	OSIRISGAG 495182 BA	OSIRISGAG 495136 BA	OSIRISGAG 411854 BA	OSIRIS 38AGG 401680 BA	OSIRISGAG 495204 BA
79	chr3H	16915708	AVRIG03881	G	A	0	0	0	0	0	0	0	0	0
80	chr3H	17502903	AVRIG03883	T	C	0	0	0	0	0	0	0	0	0
81	chr3H	17559027	AVRIG03884	G	T	0	0	0	0	0	0	0	0	0
82	chr3H	18382066	AVRIG03886	G	A	2	2	2	2	2	0	2	0	2
83	chr3H	18382704	AVRIG03887	A	G	0	0	0	0	0	0	0	0	0
84	chr3H	18382915	AVRIG03888	T	G	2	2	2	2	2	0	2	0	2
85	chr3H	18383692	AVRIG03889	C	T	0	0	0	0	0	0	0	0	0
86	chr3H	18400469	AVRIG03890	T	C	2	2	2	2	2	0	2	0	2
87	chr3H	18433346	AVRIG03885	A	C	0	0	0	0	0	0	0	0	0
88	chr3H	18527501	AVRIG03891	G	A	0	0	0	0	0	0	0	0	0
89	chr3H	18530823	AVRIG03892	A	G	2	2	2	2	2	0	2	0	2
90	chr3H	18531210	AVRIG03893	G	A	0	0	0	0	0	0	0	0	0
91	chr3H	18654178				0	0	0	0	0	0	0	0	0

First Prev 1 2 3 Next Last

Note!
 Filtered genotype calls can be exported as a vcf.

10. Scroll down chromosome to view genotype calls

After visualising different genome regions, two accessions (AGG 401680 BARL1 and AGG 495136 BARL1) appear quite different from the other seven accessions.

FairyBread tasks

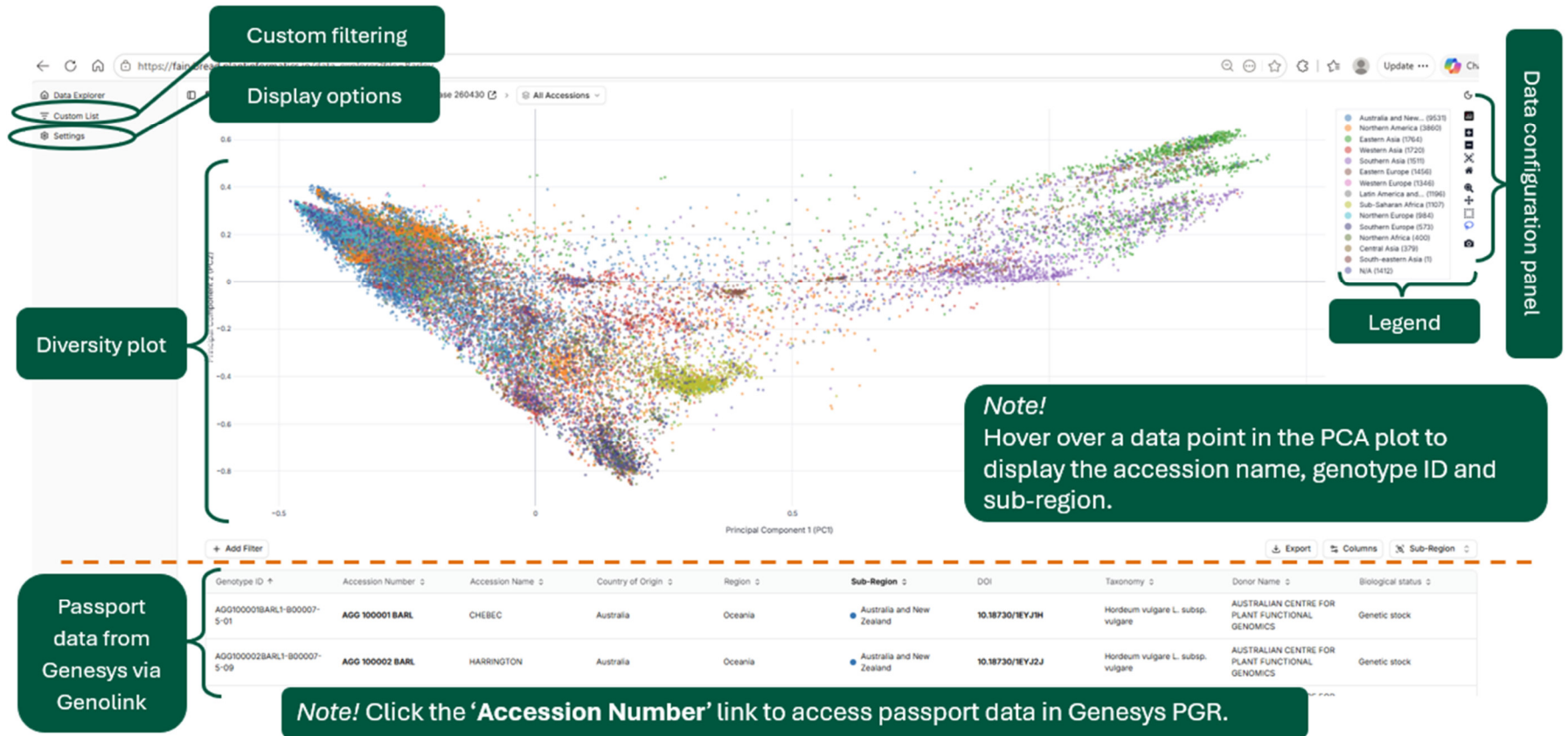
In these tasks, we will investigate the diversity of the various Osiris accessions relative to global diversity and explore some of the *FairyBread* functionalities.

Task 7: Navigating the *FairyBread* interface

Go to *FairyBread* (<https://fairybread.plantinformatics.io>). The diversity data for wheat is loaded by default. The first step is to load barley.

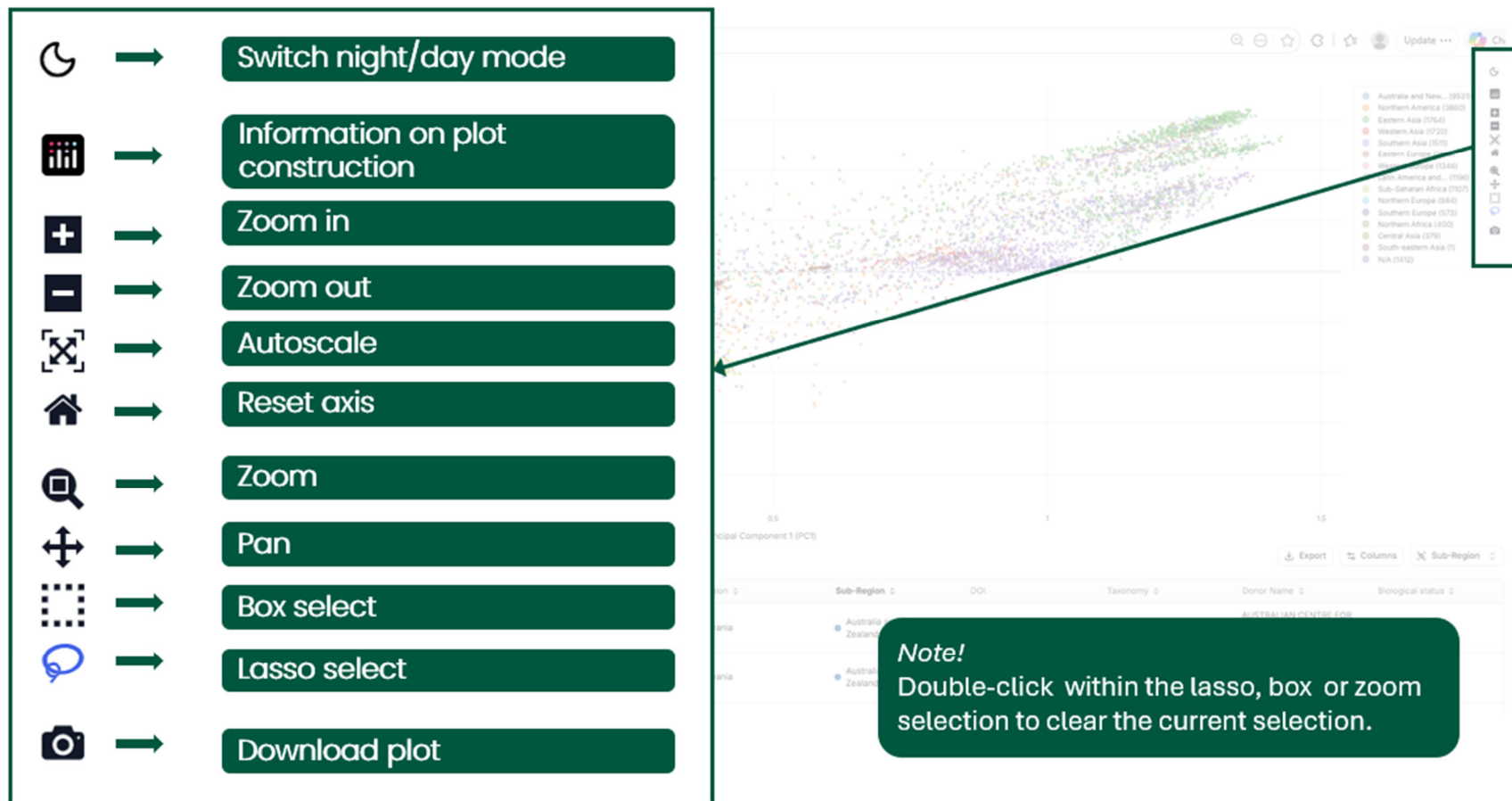


The *FairyBread* interface is described below.



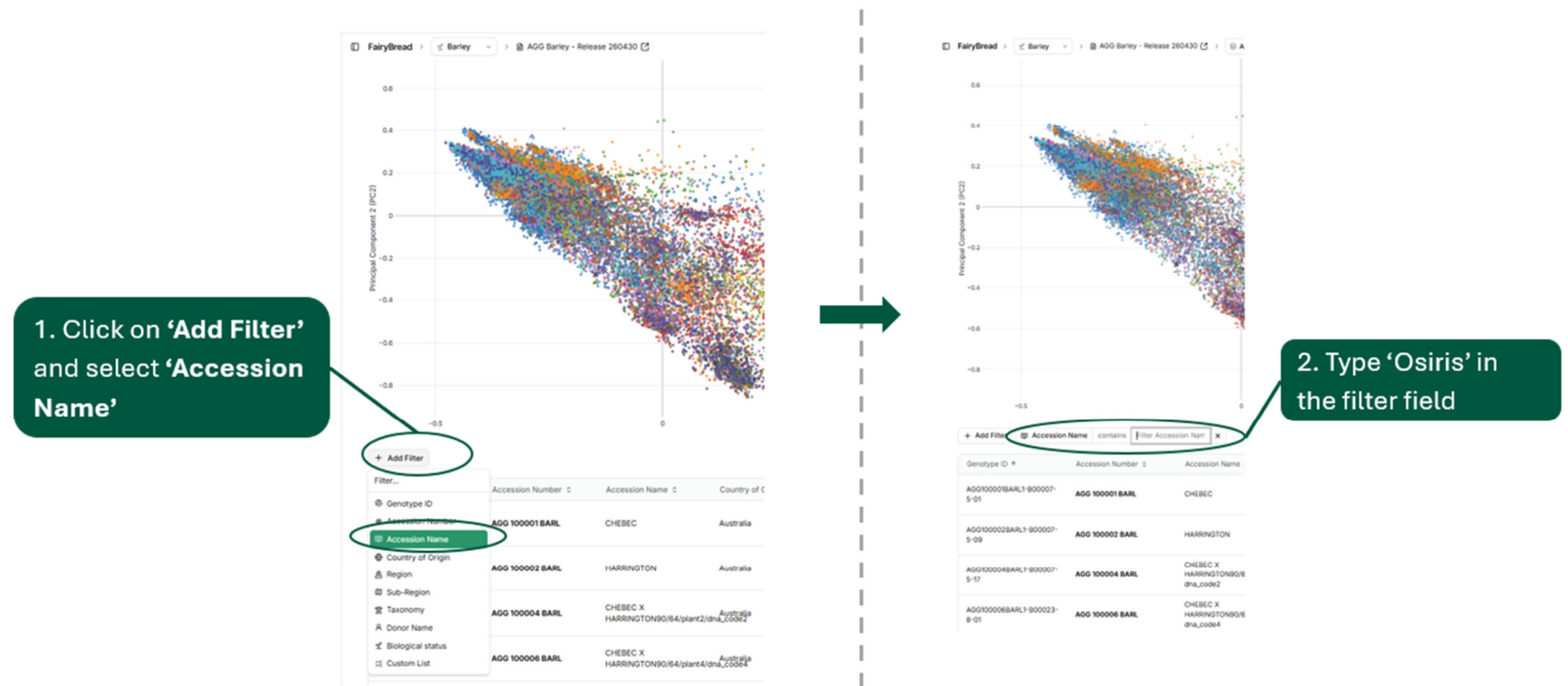
Data configuration panel

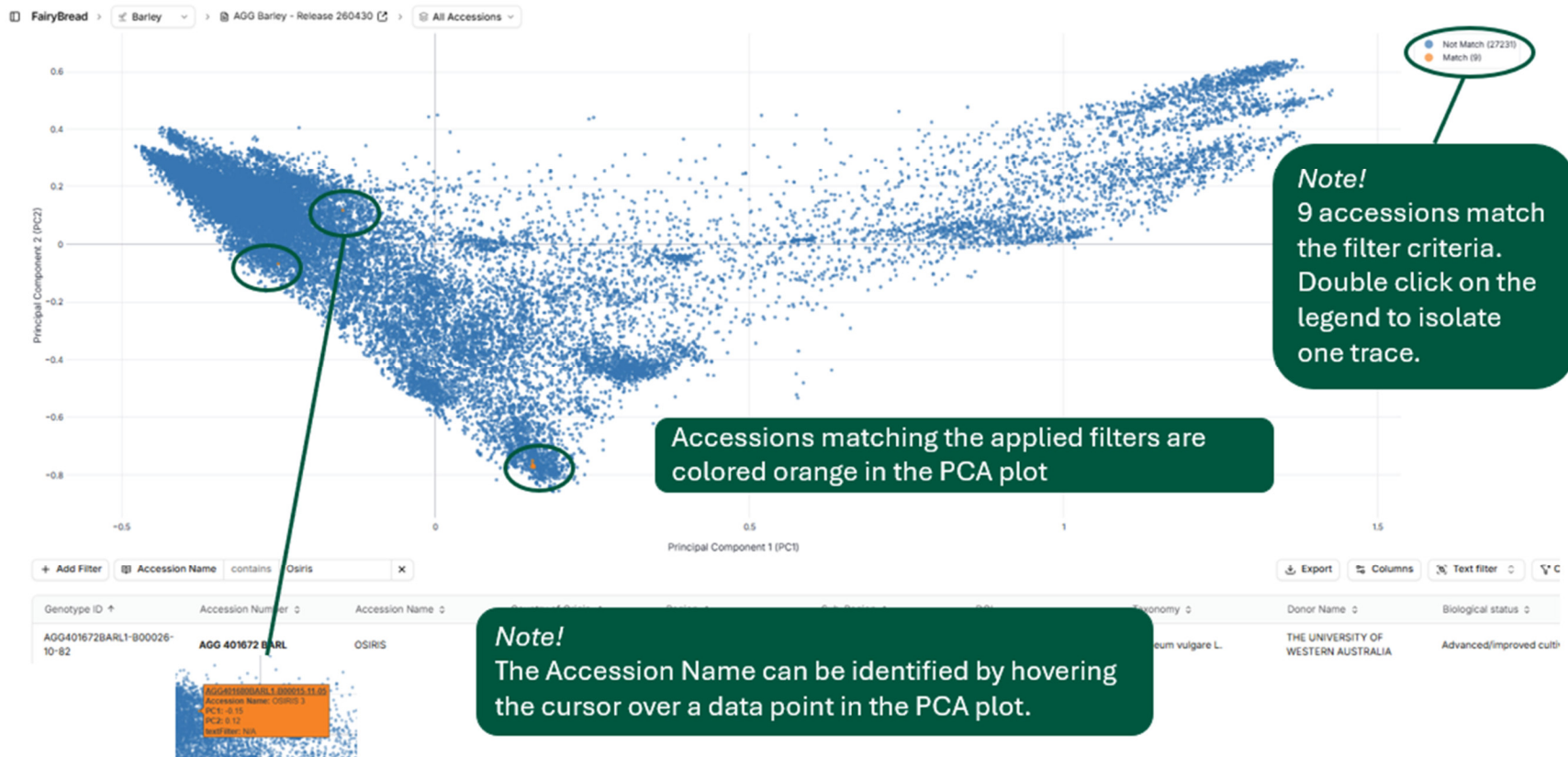
Options for manipulation of the plot are available in the data configuration panel, on the right-hand side of the screen.



Task 8: Visualise the accession 'Osiris' in the context of the entire diversity of the AGG barley collection

Use the passport filters to find the accessions called 'Osiris' in *FairyBread*.





Nine accessions matching the search criteria are displayed in orange, while non-matches are shown in blue. Seven are clustered together towards the bottom while the other two accessions are clearly separated, suggesting they are outliers.

By using the lasso tool, users can limit display of passport data for only the selected samples that meet filter criteria.

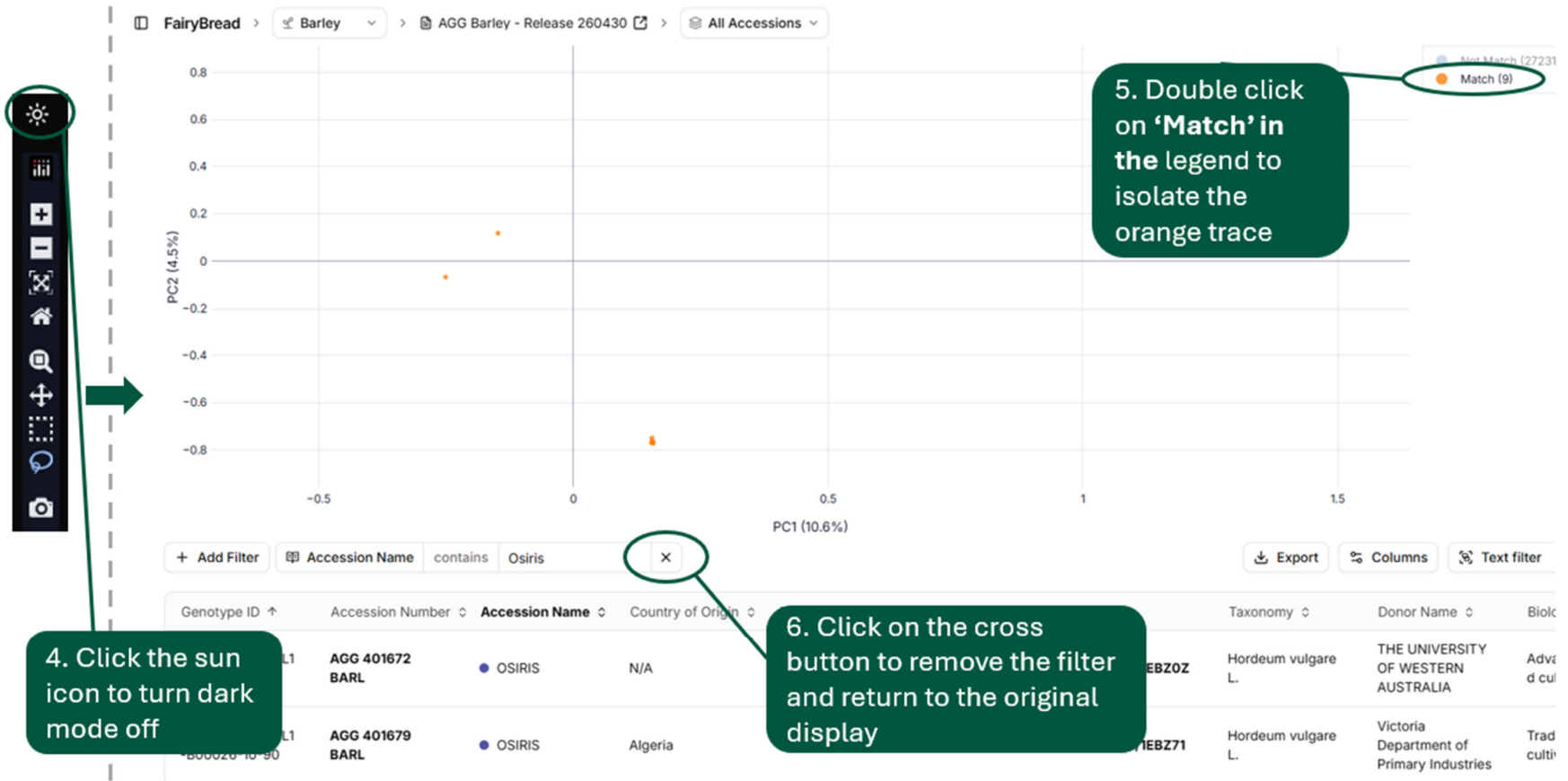


The two sample outliers in the table are the same samples observed to have different genotype calls in *Genolink*, confirming their status as outliers. Double click the cursor on the plot to release the lasso tool selection.

Due to the density of data points, it can be helpful to alter the display to more easily visualise samples in the plot matching the filter(s) applied. Firstly, dark mode can be switched on to increase contrast, which can enable the orange matched samples to be more easily identified.



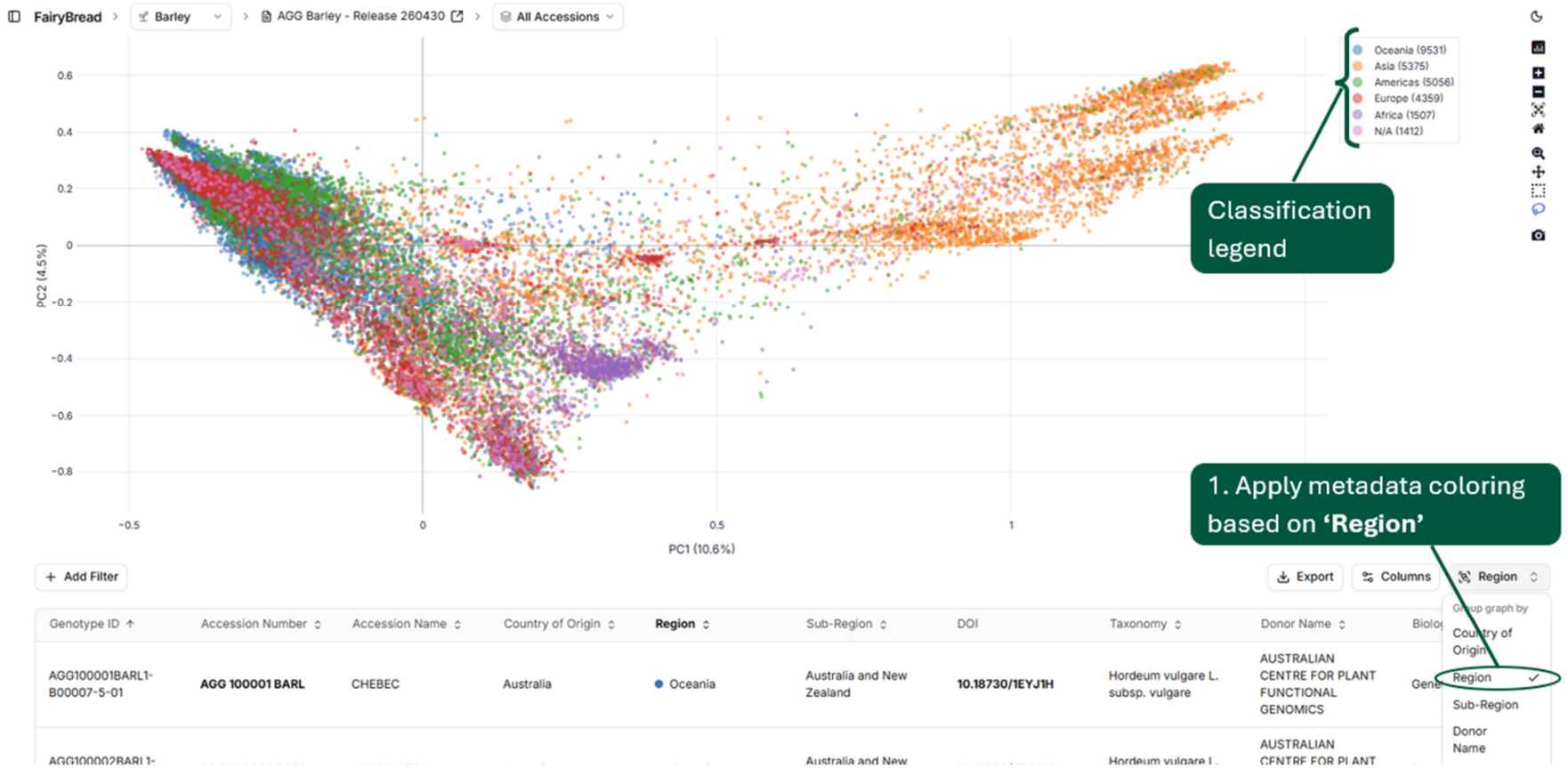
Secondly, samples not matching the filter can be hidden from view.

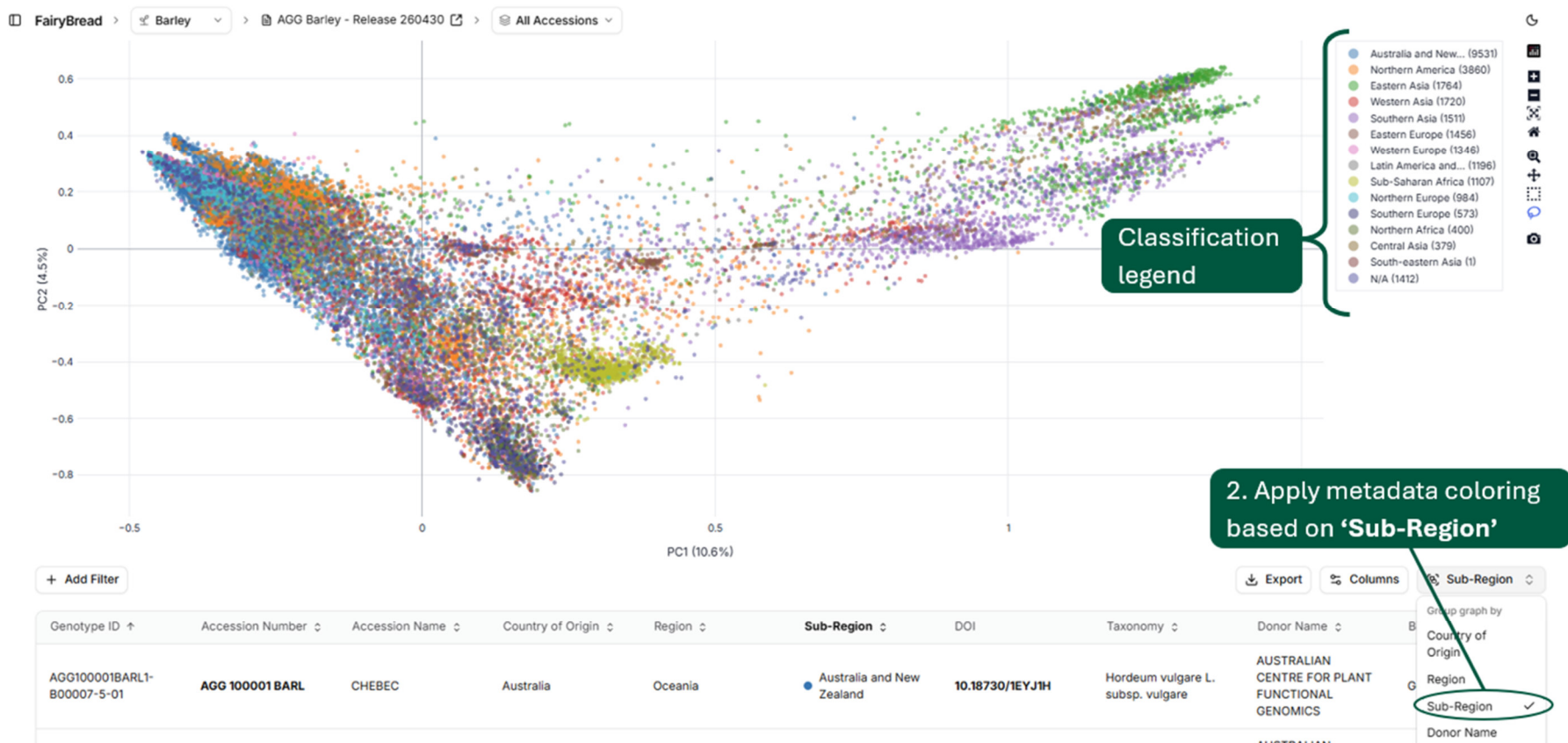


Task 9: Explore accessions based on passport data

Germplasm in the AGG can be coloured according to different fields in the passport data, including their geographic origin. Colouring based on region (e.g. Europe) or sub-region (e.g. Northern Europe) is often more useful than colouring by country due to the large number of countries represented in the AGG. Note that the data contained in 'region' and 'sub-region' fields has been implemented in *Genolink* specifically and is not part of the passport data from Genesys-PGR.

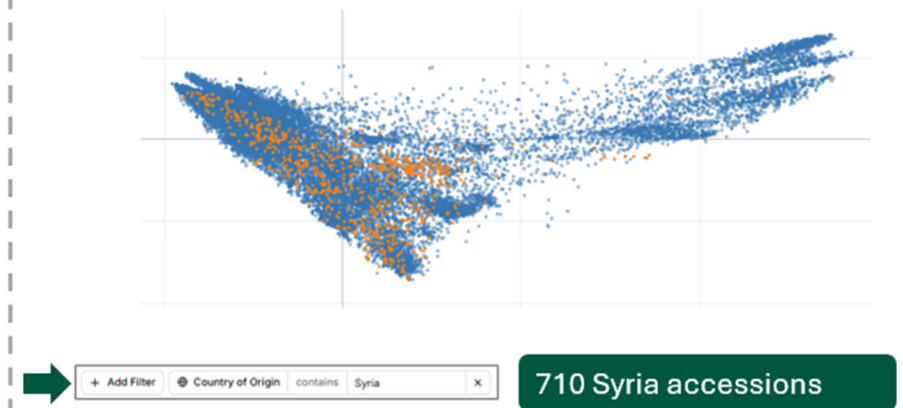
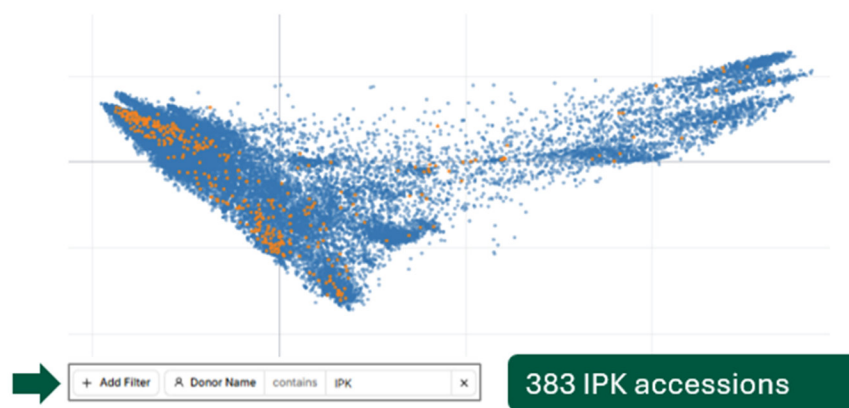
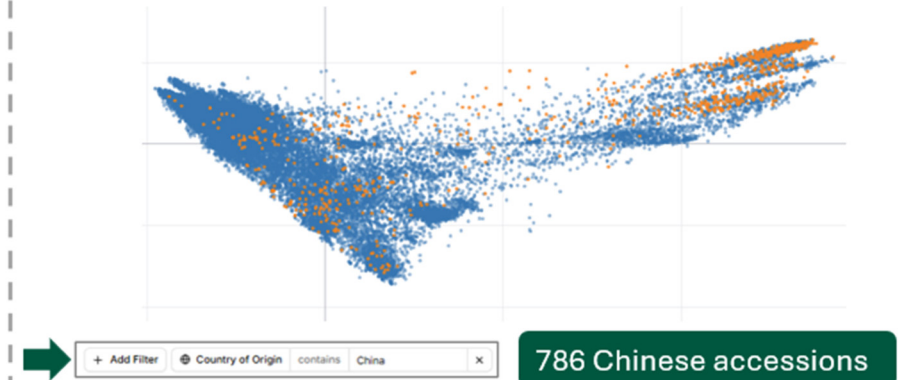
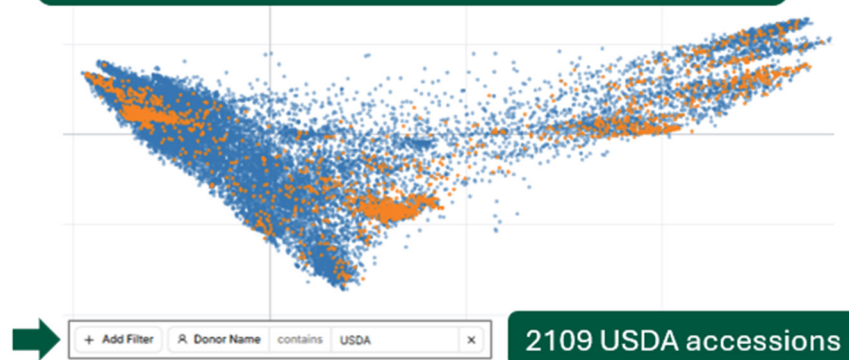






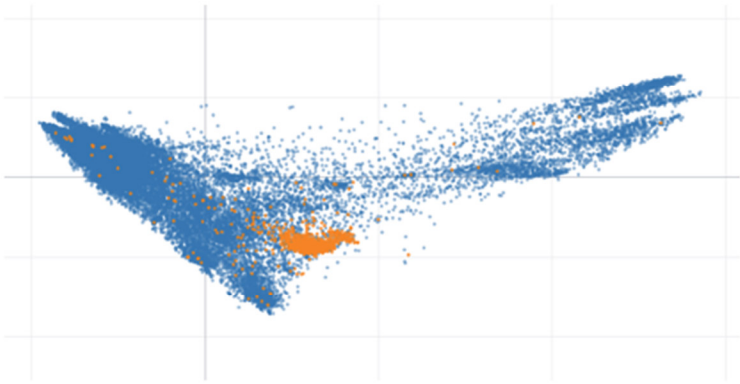
Samples can also be identified in the plot through filtering for a range of passport fields.

3. Search by '**Donor Name**' or '**Country of Origin**' as shown in these examples



Task 10: Investigate genetic clustering patterns using 3D PCA plot

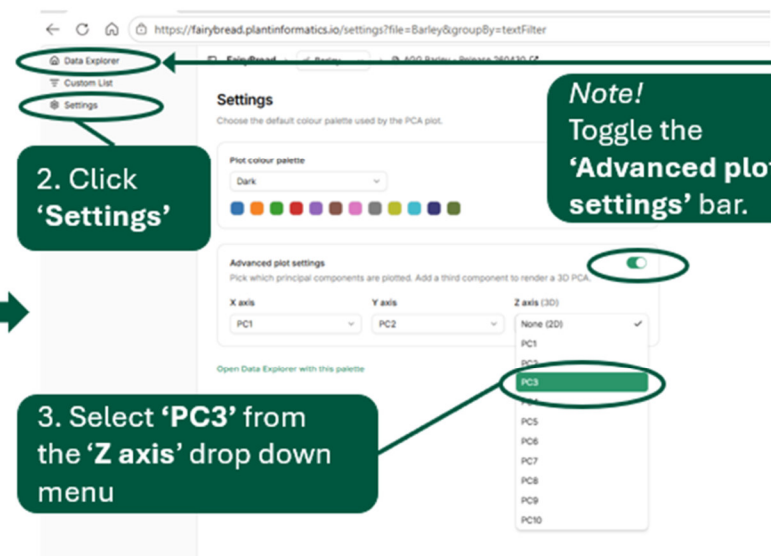
Sometimes genetic relatedness can be misleading when looking at a 2D PCA plot. In the example below, lines from Ethiopia do not look to be as genetically distinct as reported previously in the IPK barley genebank (Milner et. al., 2019). In this example, we will visualise the plot in 3D to get a better understanding of genetic diversity.



➔

1. Filter for accessions originating from 'Ethiopia' using the 'Country of Origin' filter

➔

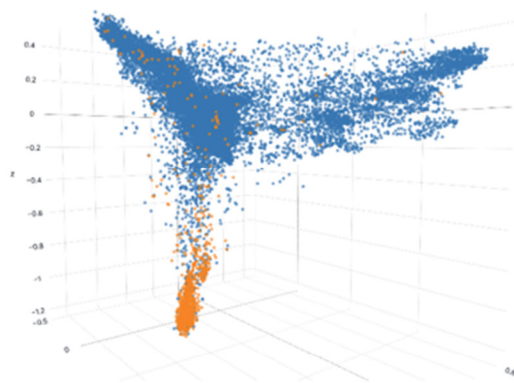


2. Click 'Settings'

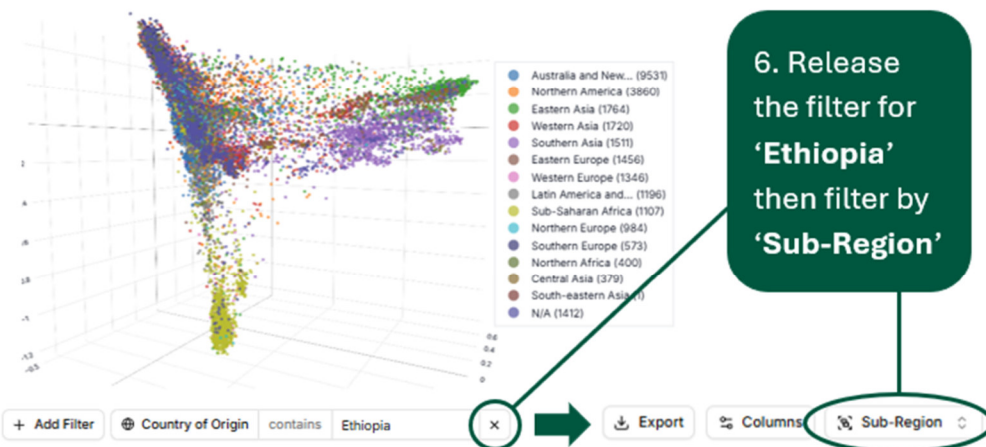
3. Select 'PC3' from the 'Z axis' drop down menu

4. Select 'Data Explorer' from the left side panel to return to the plot

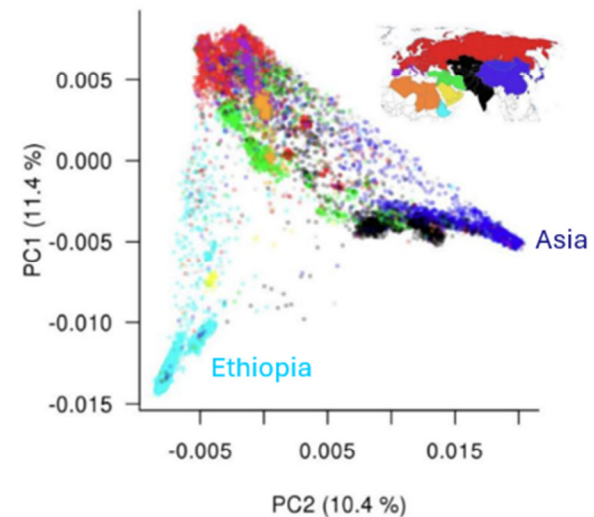
Note! Toggle the 'Advanced plot settings' bar.



5. Rotate the axis with the cursor until most of the 892 accessions from Ethiopia are shown on the left-hand plot



6. Release the filter for 'Ethiopia' then filter by 'Sub-Region'



Supplementary Figure 3 from Milner et al. 2019
19,778 barley accessions from the IPK genebank genotyped with GBS

Once the data is plotted in 3D, the separation of the Ethiopian samples can be visualised much more clearly. This plot now closely resembles that reported by Milner et. al. (2019) for barley accessions in the IPK genebank.

Pretzel tasks

Navigating the *Pretzel* interface

The *Pretzel* screen consists of three main sections:

The screenshot shows the Pretzel web interface at <https://agg.plantinformatics.io/mapview>. The interface is divided into three main sections by vertical dashed lines:

- 1 Datasets**: The left sidebar contains a search bar, filters for Group and Crop, and a list of datasets. It also includes an 'Upload new data' button and a 'Search' function.
- 2 Canvas**: The central area is a large empty space for data visualization.
- 3 Metadata**: The right sidebar displays details for the selected dataset, 'Cheng_2024', including its namespace, parent, creation date, and a list of features.

The overall layout is labeled **Pretzel main interface**.

Feature Search

Selected Features 2

Features Input 1

Rht-B1

☒ Match aliases

Search

Q DNA Sequence Blast Search

Sequence Input

DNA Sequence Input: *FASTA*

```
>AY423375.2
GGGAGAAAGAAAACCGAGAGAGAGCAAAAATGGTTGTA
GAAGAAACCCCCAAATCCATCATCACCAGCATCAAC
ATCACAACAAACCCCTAATCGCGTTATCGAAGCAGCA
ACAATCTTGAAGGAAGAGAGATGCTCGATGAAGAAC
ATTCTTCGCGCATCTCCAAACCGCGTGTCACCAAC
GTACCTCTTCGAGAAATCTTGAGCATTTCTGGTTTGA
TAGCCGCGACCAAAATCGCAACACGCGCTGGG
GTACCTCAATGCGACCACTCATCATCTTCTCCACTGT
TGAGAGATTTGGGACATTTACAATAATCATCATCT
TGCGAATGATTGGAGAAACATTTGATCATGAGATGA
AATTTCCGGAGCGGTTGTGGAATGTAAAGGGGTAGGCG
...

```

Clear Search

61095778	AVR/G18001	0.00	T	A	G
61912405	AVR/G18002	0.00	C	T	A
62347067	AVR/G18003	0.00	G	A	G
62348602	AVR/G18004	0.00	T	G	A
63024340	AVR/G18006	0.00	C	T	A
63026377	AVR/G18007	0.00	C	A	G
63027295	AVR/G18008	0.00	C	A	G
63118861	AVR/G33890	0.17	G	A	G
63368745	TaSNPp08b1-5	0.00	T	A	G
63741955	TaSNPp08b1-10	0	A	A	G
63744493	TaSNPp08b1-4	0.00	T	G	A
64315845	AVR/G18011	0.00	A	G	A
64316212	AVR/G18012	0.00	A	G	A
65316477	AVR/G18014	0.00	A	G	A
65434899	AVR/G18015	0	G	A	G
66199320	AVR/G18017	0.00	C	A	G
66523728	AVR/G18019	0.00	G	A	G
587854116	AVR/G21638	0.20	G	A	G
587992256	AVR/G21639	0.21	A	G	G

Background information relevant to workshop tasks

The exercises undertaken in this workshop focus on *Rrs2*, a major gene for resistance to barley scald (*Rhynchosporium commune*). *Rrs2*, located on chromosome 7HS (Hanemman *et. al.*, 2009), is a well-characterised and effective source of resistance (Marzin *et. al.*, 2016; Hautsalo *et. al.*, 2021), making it an attractive target for introducing novel resistance into barley breeding germplasm. While *Rrs2* has been extensively studied, a key challenge for breeders is determining where this resistance occurs within broader germplasm collections and identifying additional material that may carry the same favourable resistance haplotype. Extensive resources already exist for *Rrs2*, including diagnostic markers, candidate genes, and candidate carrier lines (Marzin *et. al.*, 2016; Hautsalo *et. al.*, 2021). However, translating this knowledge into practical germplasm discovery requires the integration of genomic data, diversity analyses, and visualisation tools.

Using *Genolink*, *FairyBread*, and *Pretzel*, the tasks in this workshop demonstrate how existing knowledge of *Rrs2* can be combined with AGG genomic resources to identify candidate sources of scald resistance, discover promising germplasm, and improve the likelihood of successfully introducing *Rrs2* into barley pre-breeding programs. These examples show how genomic information can support more informed germplasm selection for both research and breeding.

Aims:

- Where is the *Rrs2* gene located in the barley genome?
- Can markers on the Wheat Barley Infinium 40K SNP beadchip detect *Rrs2*?
- What is the frequency of the *Rrs2* haplotype in the AGG?

Task 11: Identify a likely accession in the AGG that can be used in subsequent steps to identify a haplotype for *Rrs2*

Firstly, we need to identify accessions known to carry the *Rrs2* gene.

- Twelve accessions were reported to carry *Rrs2* in Marzin et. al. (2016) (see the table on the right-hand side).

We will use *Genolink* to search the passport data to determine whether any of these accessions are in the AGG and whether we have genotype data for them. We will start by looking for 'Osiris'.

Variety	Information	Resistance	PE1	PE2	PE3	PE4	PE5	PE6	Gene
Anakin	Freising (DNA)	not analyzed	x	H1	H1	H1	H1	H1	Rrs2+
Atlas	Acc.495017	resistant	H1	H1	H1	H1	H1	H1	
Atlas 46	Acc.HOR3876	resistant	H2	H1	H1	H1	H1	H1	
Atlas 54	(CI 9556) Acc.HOR9547	resistant	H1	H1	H1	H1	H1	H1	
CI2235	Freising Acc.HOR10575	resistant	H1	H1	H1	H1	H1	H1	
CI452395	Freising	not analyzed	H1	H1	H1	H1	H1	H1	
Digger	Acc.18560	resistant	H1	H1	H1	H1	H1	H1	
Gloriacome	Freising	not analyzed	H1	H1	H1	H1	H1	H1	
Escaladura	Freising	resistant	H1	H1	H1	H1	H1	H1	
Forrest (AUS)	Acc.400180, HOR3631	resistant	H1	H1	H1	H1	H1	H1	
Hobbit	Freising (DNA)	not analyzed	x	H1	H1	H1	H1	H1	
Landi	Freising (DNA)	not analyzed	x	H1	H1	H1	H1	H1	
Livet (AUS)	Acc.408567	not analyzed	H1	H1	H1	H1	H1	H1	
Osiris	Freising	resistant	H1	H1	H1	H1	H1	H1	
Pewter	Freising	resistant	H1	H1	H1	H1	H1	H1	Rrs2-
PI 452395	Freising	resistant	H1	H1	H1	H1	H1	H1	
Turk x Atlas	(CI 7189) Acc.HOR8881	resistant	H1	H1	H1	H1	H1	H1	
Wisconsin winter x Glabron	(CI 8162) Acc.HOR8885	resistant	x	H1	H1	H1	H1	H1	
Abyssinian (HOR 5041)	(CI 1237) Acc.HOR5041	resistant	H2	H2	H2	H3	H1	H2	
Alexis	Freising	susceptible	H1	H2	H2	H2	H1	H3	
Atlas 68	Acc. BCC814, Genbank IPK	resistant	H2	H2	H2	H3	H1	H2	
Barke	Freising	susceptible	H1	H2	H2	H2	H1	H3	
Brier	Acc.HOR3106	susceptible	H4	H3	H3	H6	H1	H4	
CI 11258	Freising	not analyzed	?	H4	H4	H5	H1	H5	
Gairdner	Acc.408175	resistant	H1	H5	H2	?	H1	H2	
Golden Promise	(GZ) Genbank IPK	susceptible	H3	H6	H5	H4	H1	H6	
Harrington	Freising	resistant	H2	H2	H2	H3	H1	H2	
Hendrix	Freising	susceptible	H3	H6	H5	H4	H1	H6	
Hispoint	(CI8828) Acc.HOR9535	susceptible	H1	H5	H2	?	H1	H2	
Hudson	Acc. BCC880, Genbank IPK	intermediate	H2	H2	H2	H3	H1	H2	
Jet	(CI 967) Acc.HOR5032	resistant	?	H6	H5	H4	H1	H6	
Morex	Genbank IPK	susceptible	H4	H3	H3	H6	H1	H4	
Nigrindum	(CI 11549) Acc.HOR4844	resistant	H3	H6	H6	H4	H1	H6	
Onslow	Acc.406008	resistant	H1	H5	H2	?	H1	H2	
Opal	Freising	susceptible	H5	H4	H4	H5	H1	H5	
Prestige	Freising	susceptible	H2	H2	H2	H3	H1	H2	
Quinn	(CI 1024) Acc.HOR2397	not analyzed	H3	H6	H5	H4	H2	?	
Steffi	Freising	susceptible	H3	H6	H5	H4	H1	H6	
Steudelli	(CI 2226) Acc.402008	not analyzed	H2	H2	H2	H3	H1	H2	
Turk 8886	(CI 14400) Acc.HOR8886	susceptible	H1	H2	H2	H2	H1	H8	
Cebada Atlas	Acc.HOR19750	resistant	H6	H7	H7	H7	H1	H7	

In this task, we will begin by finding a specific accession in the AGG that might represent the accession 'Osiris' reported to contain *Rrs2* in Marzin *et. al.* (2016).

1. Use the Passport Filters to search for 'Osiris' barley accessions in the AGG

11 relevant accessions are retrieved, with 9 having been genotyped

Filters

Total Accessions: 11

Apply Filter Reset Filter

Filter Mode: Passport Filter

Active Filters

Text: Osiris

Institute Code: AUS165

Crop: barley

Osiris

Date

Holding Institute

#	Institute Code	Holding Institute	Accession Number	Accession Name	Provenance of Material	GenotypeID
1	AUS165	Australian Grains Ge...	AGG 401680 BARL	OSIRIS 3	N/A	AGG401680BARL1-B...
2	AUS165	Australian Grains Ge...	AGG 401679 BARL	OSIRIS	Algeria	AGG401679BARL1-B...
3	AUS165	Australian Grains Ge...	AGG 401672 BARL	OSIRIS	N/A	AGG401672BARL1-B...
4	AUS165	Australian Grains Genebank, Agriculture Victoria	AGG 411853 BARL	OSIRIS	Germany	AGG411853BARL1-B00004-2-30
5	AUS165	Australian Grains Ge...	AGG 411854 BARL	OSIRIS	Germany	AGG411854BARL2-B...
6	AUS165	Australian Grains Ge...	AGG 495136 BARL	OSIRIS	Algeria	AGG495136BARL1-B...
7	AUS165	Australian Grains Ge...	AGG 495204 BARL	Osiris	Syria	AGG495204BARL1-B...
8	AUS165	Australian Grains Ge...	AGG 495188 BARL	OSIRIS	Algeria	AGG495188BARL1-B...
9	AUS165	Australian Grains Ge...	AGG 495182 BARL	OSIRIS	Algeria	AGG495182BARL1-B...
10	AUS165	Australian Grains Ge...	AGG 495289 BARL	Osiris	Australia	N/A
11	AUS165	Australian Grains Ge...	AGG 1041142 BARL	FFM221115	N/A	N/A

After reviewing passport data retrieved, we can see that 9 of the 11 accessions in the AGG have been genotyped. Based on the information in the table on the previous page, we know the 'Osiris' accession we are interested in originated from Freising, so we will begin by prioritising one of the accessions (AGG 411853 BARL) with 'Germany' in the 'Provenance of Material' field. Of note, this is one of the accessions in the 'Osiris' cluster in *FairyBread*, not one of the outliers identified in Task 8.

Next, we will use information reported in the literature to determine a location in the genome to analyse. Relevant information includes:

- In 2009, *Rrs2* was localised to a distal region of chromosome 7HS, and a SNP was identified that tracks a resistance associated haplotype on 7HS, potentially resulting from an introgression or inversion event (Hanemann *et. al.*, 2009).
- In 2021, a KASP marker was developed, facilitating high-throughput genotyping and incorporation of scald resistance into modern barley breeding germplasm (Hautsalo *et. al.*, 2021).
 - **KASP marker: FJ974009**
 - **Prior to this workshop, the position of this marker was identified in the Morex v3 genome by searching for KASP primer sequences**
- In 2025, significant marker-trait associations were identified on chromosome 7HS using the barley 50K SNP array (JHI-Hv50k-2016-441289 and JHI-Hv50k-2016-445855), providing independent evidence that this chromosomal region remains a key source of scald resistance in diverse barley germplasm (Noe *et. al.*, 2025).
 - **50K marker IDs: JHI-Hv50k-2016-441289, JHI-Hv50k-2016-445855**
 - **The 50K markers are already loaded in Pretzel, so no background work is required to locate their positions in Morex v3.**

Task 12: Upload a dataset into *Pretzel* to track marker position

An Excel sheet has already been pre-loaded with information about the KASP marker 'FJ974009' reported by Hautsalo *et. al.* (2021). Before uploading it to *Pretzel*, some editing is required to make the dataset name unique. After uploading the custom alignment file, the data will remain private by default. Refer to the Appendix to find more information about the Pretzel upload file. The following instructions show how to prepare the metadata. More details on the Excel upload file and meta data is available in the Appendix.

The screenshot shows an Excel spreadsheet with the following data:

Field	Alignment Barley_KASP_XX
shortName	Barley_FJ974009_KASP
displayName	Hordeum vulgare - MorexV3 - Markers - Rrs2 - KASP - XX
type	Alignment
Crop	Barley
Species	Hordeum vulgare
Data Source	Primers sequences were based on FJ974009 in Hanemann et al. 2009
Licensing of original data	NA
Curator	Name
Publication	Hanemann et al. 2009. Fine mapping, physical mapping and development of diagnostic markers for the Rrs2 scald resistance gene in barley. Theoretical and Applied Genetics 110:1507-1522; Hautsalo et al. 2021. A KASP marker for the Rrs2 scald resistance gene in barley. Theoretical and Applied Genetics 123:1507-1522
Comments	Primer positions were determined using isPcr against Morex V3.
Genome_id	Barley_MorexV3
Alignment type	Marker
Marker type	KASP
parentName	Barley_MorexV3

The spreadsheet is titled 'Pretzel_Workshop_barley_Alignment_Upload_XX.xlsx' and the worksheet is named 'Metadata'. The 'Field' column is highlighted in blue, and the 'Alignment|Barley_KASP_XX' column is highlighted in green. The 'Curator' field is highlighted in red.

1. Save the Excel file with a unique name incorporating your initials to replace 'XX' e.g. 'Pretzel_Workshop_barley_Alignment_Upload_KF.xlsx'
2. Rename the 'Field' by incorporating your initials to make it unique (i.e. replace 'XX' with your initials) e.g. 'Alignment|Barley_KASP_KF'
3. Rename 'displayName' by incorporating your initials to make it unique e.g. 'Hordeum vulgare - MorexV3 - Markers - Rrs2 - KASP - KF'
4. Add the dataset 'Curator' name
5. Rename worksheet by incorporating your initials, ensuring it EXACTLY matches the 'Field' name above in step 2. e.g. 'Alignment|Barley_KASP_KF'

Next, the position of marker 'FJ974009' will be uploaded into *Pretzel* as a private dataset, which means only you can see it.

The image is a two-panel screenshot of the Agg Plant Informatics web application, separated by a vertical dashed line. The left panel shows the 'Upload' tab selected in the top navigation bar. Below the navigation bar, there is a 'Data Specification' section with a toggle switch. Underneath, there is a 'fileDrop' section with buttons for 'CSV' and 'JSON'. A green callout box with the text '6. Select the 'Upload' tab' points to the 'Upload' button in the navigation bar. Below the 'fileDrop' section, there is a 'Replace Dataset with upload' checkbox, which is checked. A green callout box with the text '7. Select the 'Choose File' to upload the Alignment file to Pretzel' points to the 'Choose File' button. The right panel shows the 'Upload' tab selected in the top navigation bar. Below the navigation bar, there is a 'Data Specification' section with a toggle switch. Underneath, there is a 'fileDrop' section with buttons for 'CSV' and 'JSON'. A green callout box with the text '8. 'Success' notification confirms data was uploaded' points to a green success notification box that says 'Success' and 'Dataset 'Barley_KASP_KF' Uploaded successfully'.

6. Select the 'Upload' tab

7. Select the 'Choose File' to upload the Alignment file to Pretzel

8. 'Success' notification confirms data was uploaded

Task 13: Load the KASP marker position in the Morex v3 genome assembly

Next, we will find the KASP dataset in *Pretzel* and load the position of the marker within the Morex v3 genome assembly.

The screenshot shows the Pretzel web interface. The top navigation bar has tabs for Explorer, View, Search, and Upload. The Explorer tab is selected. Below the navigation bar, there are filters for Group (All), Crop (Barley), and Category (All). The search bar contains the text 'KASP'. Below the search bar, there are buttons for Case (insensitive) and Match (all). The left panel shows a list of datasets, with 'Hordeum vulgare - MorexV3 - Markers - Rrs2 - KASP - KF' highlighted. The right panel shows a list of datasets, with 'Hordeum vulgare - MorexV3 - Markers - Rrs2 - KASP - KF' highlighted.

1. Select the 'Explorer' tab
2. Select drop down for Crop to filter the data and select 'Barley'
3. Type 'KASP' in the Search bar

Note!
Search has been done in a case insensitive manner and to match all words in search bar.

Note!
Filtered datasets will appear in the left panel.

4. Click on the black '+' icon on the left side of the dataset name to show available chromosomes

Refer to the Appendix for more details on meta data fields.

The screenshot displays the AGG digital tool interface, which is divided into several panels. On the left, the 'Datasets' panel shows a search for 'KASP' and a list of datasets. A green arrow points to a '+' icon next to 'Hordeum vulgare - MorexV3 - Markers - Rrs2 - KASP', with a callout box stating: '5. Click on the green '+' icon on the left side of the chr7H to load the dataset. It changes to a '-' symbol once loaded'. Another green arrow points to a padlock icon next to 'Hordeum vulgare - MorexV3 - Markers - Rrs2 - KASP - KF', with a callout box stating: 'Note! Padlock icon indicates a private dataset.' A third green arrow points to the text 'chr7H' in the dataset list, with a callout box stating: 'Note! Check that the metadata for the dataset is correct.' The central panel shows a genomic map of 'Barley_MorexV3 : chr7H' with a scale from 0 to 600M. On the right, the 'Dataset' panel shows the details for 'Barley_KASP_KF', including its parent, creation date, and group. A green arrow points to the 'Barley_KASP_KF' label, with a callout box stating: 'Field' for dataset name. Below this, the 'Meta' section shows a list of 13 metadata fields, including 'shortName', 'displayName', 'type', 'crop', 'species', 'data source', 'licensing', 'curator', 'publi', 'comments', 'genome_id', 'alignment type', and 'marker type'.

5. Click on the green '+' icon on the left side of the chr7H to load the dataset. It changes to a '-' symbol once loaded

Note! Padlock icon indicates a private dataset.

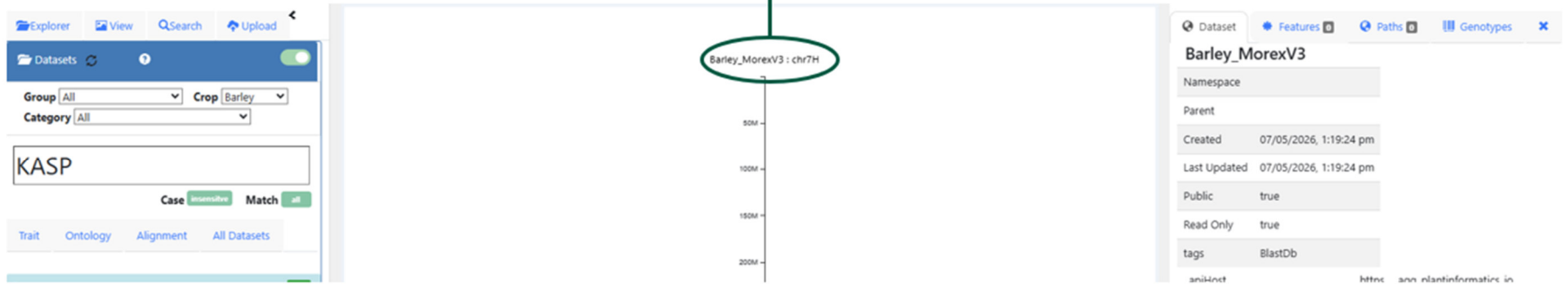
Note! Check that the metadata for the dataset is correct.

'Field' for dataset name

Metadata (13)

- shortName: Barley_F3974009_KASP
- displayName: Hordeum vulgare - MorexV3 - Markers - Rrs2 - KASP - KF
- type: Alignment
- crop: Barley
- species: Hordeum vulgare
- Data Source: Primers sequences were based on F3974009 in Hanemann et al. 2009
- Licensing of original data: NA
- Curator: Kerrie Forrest
- Publi: Hanemann et al. 2009. Fine mapping, physical mapping and development of diagnostic catio markers for the Rrs2 scald resistance gene in barley. Theoretical and Applied Genetics 119:1507-1522 Mautsalo et al. 2021. Pyramiding of scald resistance genes in four spring barley MAGIC populations. Theoretical and Applied Genetics 134:3829-3843.
- Comments: Primer positions were determined using isPcr against Morex V3.
- Genome_id: Barley_MorexV3
- Alignment type: Marker
- Marker type: KASP

6. Click on the chromosome name on the top of the axis to display the axis title menu



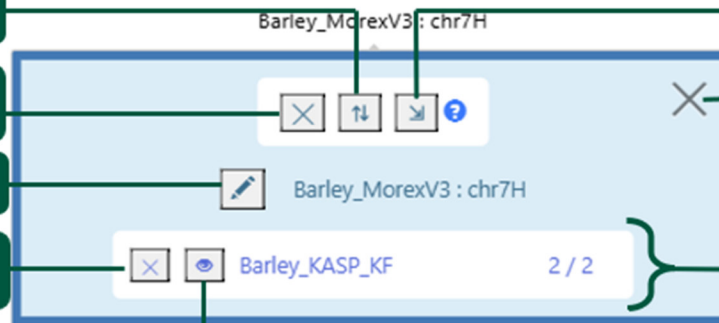
Flip Axis

Remove all datasets from view

Rename axis title

Remove selected dataset from view

Display/hide features of a dataset

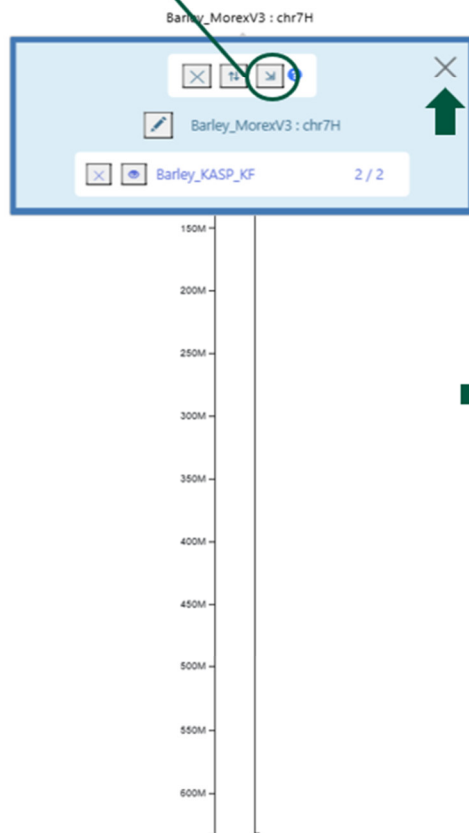


Split Axis

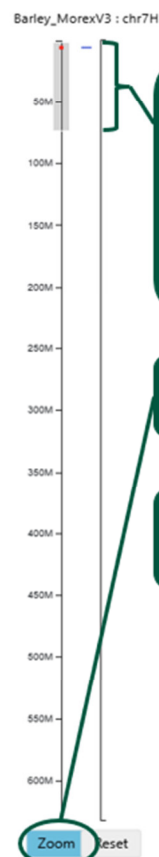
Close axis title menu

Requested features v/s Total features

7. Click on the '**Split Axis**' and close the Axis Title menu

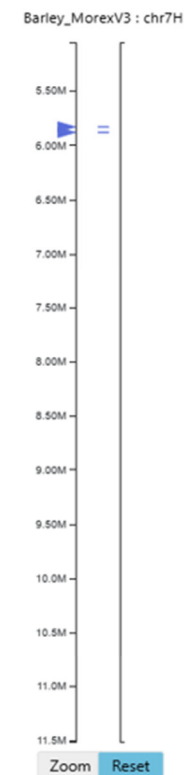


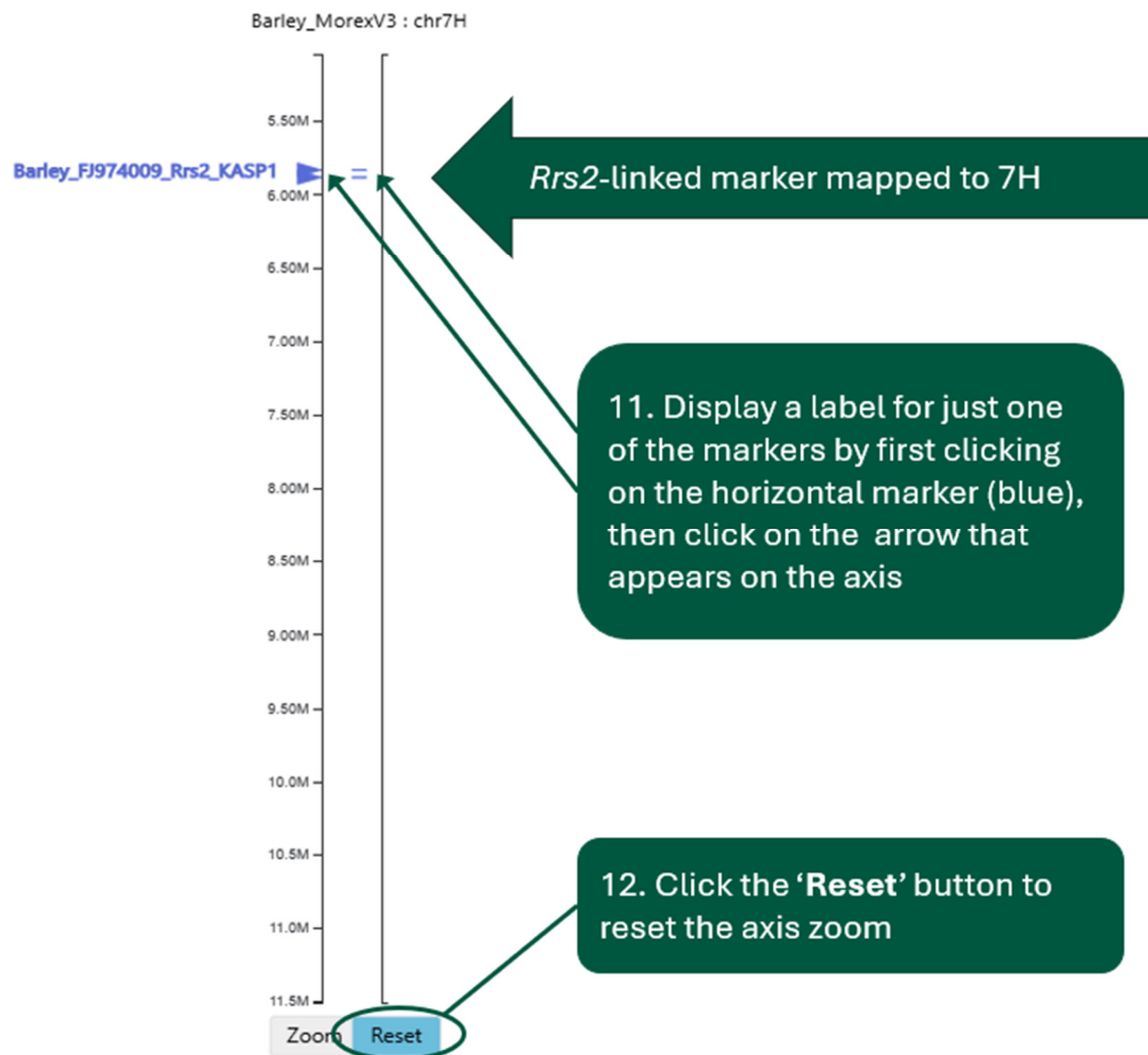
8. With your mouse pointer, click and drag along the axis to highlight the portion of the chromosome surrounding the marker. Selected feature will turn red



9. Click on the '**Zoom**' button

10. Repeat steps 9-10 to zoom in on markers

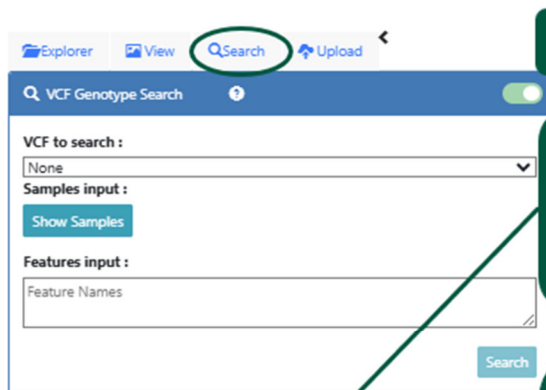




You have now identified the position of the *Rrs2*-linked KASP marker in chromosome 7H of the Morex v3 genome assembly.

Task 14: Search for an existing marker in *Pretzel*

Next, we will find the position of two barley 50K markers reported to track scald resistance on 7HS in Noe et. al. (2025) for further evidence of the genome location of *Rrs2*. Since the 50K markers are already loaded into Pretzel as a public dataset, we can go straight to Feature Search to find if the markers have been mapped to the Morex v3 genome assembly. Markers names can be typed manually or copied from the provided file '2026_ABTS_SupplementaryFile1.txt'.

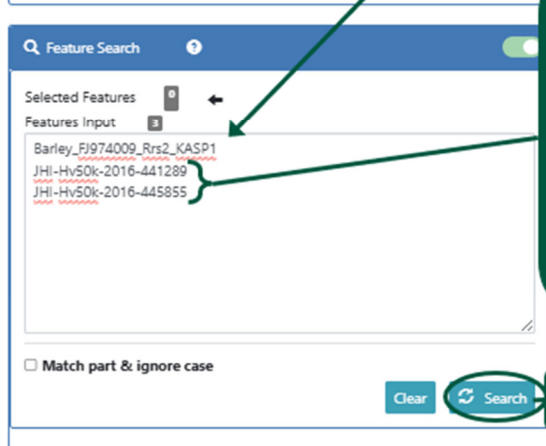


1. Click on the 'Search' tab

Note!
The marker labelled in the previous step will also appear in the 'Feature Search' field

2. Type the two markers below in the 'Features Input' field within 'Feature Search', or copy from the provided file '2026_ABTS_Supplementary File1.txt'.
JHI-Hv50k-2016-441289
JHI-Hv50k-2016-445855

3. Click on 'Search'

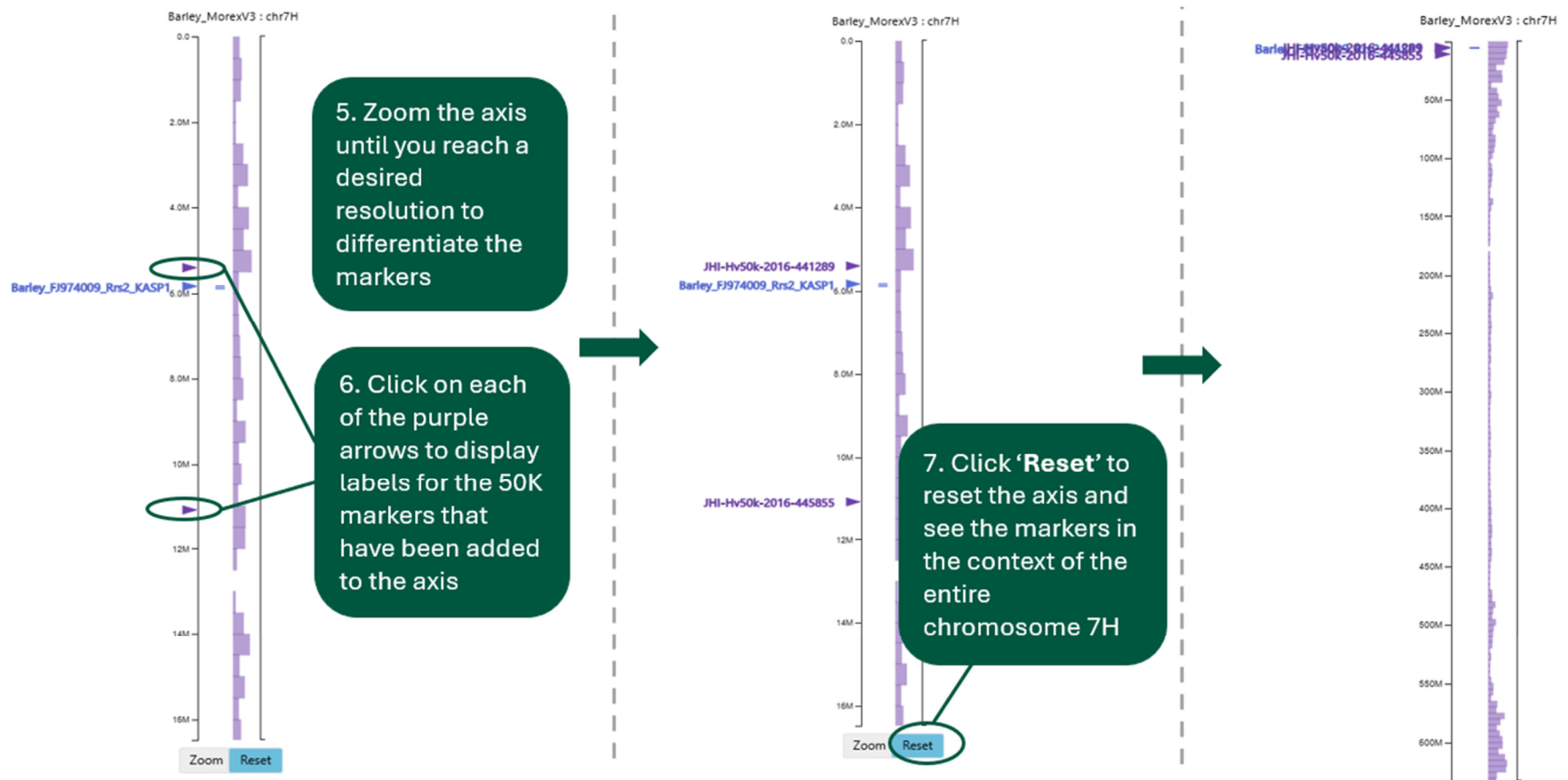


4. Click on the "+" button to load the dataset 'MorexV3_50k' to display the position of features of interest

Note!
All Pretzel datasets containing a feature with requested ID are listed below.

Feature	Count
MorexV3_50k chr7H	2
Barley_FJ974009_KASP chr7H	1
Barley_KASP_KF chr7H	1

Task 15: Compare the position of markers from different literature sources



Once labels are applied, you can see that the two 50K markers are in close proximity to the KASP marker at the top of chromosome 7H, suggesting they are interrogating the same locus.

Task 16: Compare the positions of trait-linked markers to positions of the Wheat Barley 40K markers

Since we want to relate this information to the genotype data for the AGG barley collection, we want to know which Wheat Barley 40K markers are close to the trait-linked markers. Note that we will be loading marker positions to begin with, not genotype data.

1. Select the 'Explorer' tab

2. Select 'Barley' from the 'Crop' filter

3. Type '40k' in the Search bar

4. Click on the black '+' icon on the left side of the dataset name to show available chromosomes

5. Click on the green '+' icon beside chr7H to load the dataset. It changes to a '-' symbol once loaded

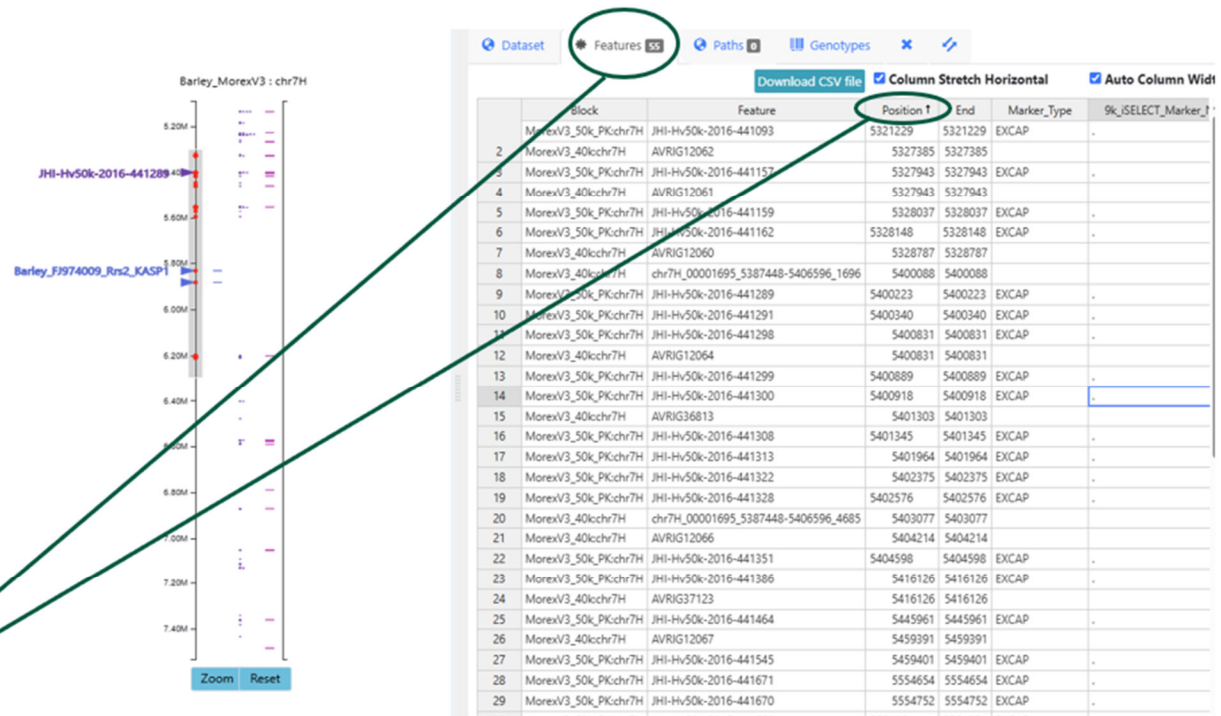
Since we want to relate this information to the genotype data for the AGG barley collection, we want to know which wheat barley 40K markers are close to the trait-linked markers.

Note!

40k markers have now been added to the axis as a new track and are coloured magenta

6. With your mouse pointer, click and drag along the axis to highlight a portion of the chromosome surrounding the trait-linked markers. Selected feature will turn red

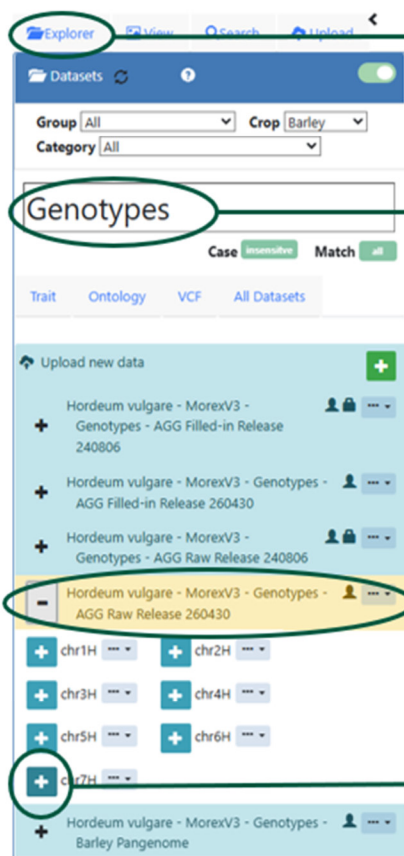
7. Click on the 'Features' tab to see the markers, then click 'Position' header to sort by position along the chromosome.



We can now see which 40K markers are in the vicinity of the markers linked to *Rrs2*.

Task 17: Load AGG genotype data for barley

Next, we will load the genotype data generated by the AGG Strategic Partnership (<https://doi.org/10.7910/DVN/LXU0WD>) so we can begin searching for a haplotype to track the *Rrs2* gene.

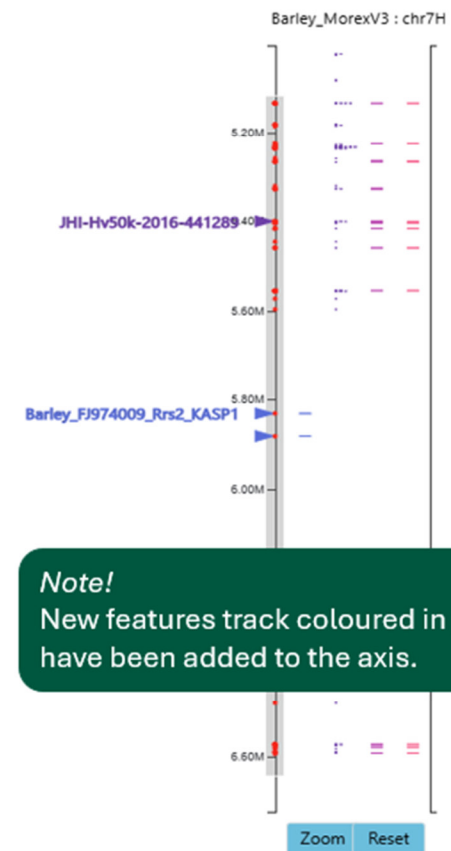


1. Select the 'Explorer' tab

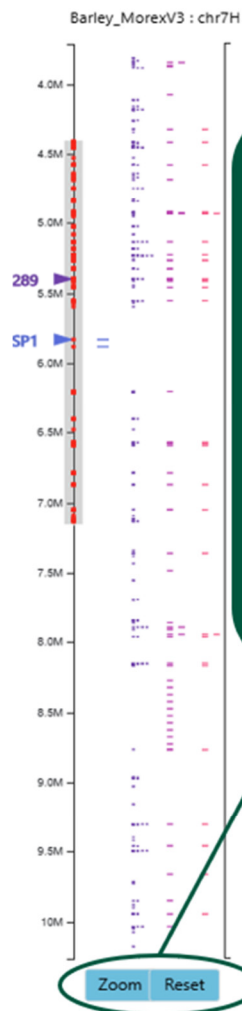
2. Type 'Genotypes' in the Search bar

3. Click on the black '+' icon on the left side of the dataset name 'Hordeum vulgare – MorexV3 – Genotypes – AGG Raw Release 260430' to show available chromosomes

4. Click on the green '+' icon on the left side of the chr7H to load the dataset. It changes to a '-' symbol once loaded



Note!
New features track coloured in pink have been added to the axis.



5. Reset the axis, then highlight the axis and zoom until you have a region covering about 4.5 – 7.0 Mbp. With your mouse pointer, click and drag along the axis to highlight the markers (they will turn red).

6. Click on the 'Genotypes' tab to display base pair positions, features and Ref/alt/null nucleotides for the selected markers

7. Adjust the height of the header row by hovering your pointer here until the cursor changes to a double-ended arrow, then drag upwards or downwards

8. Click on the cog wheel to display the 'Genotypes' menu

Chr	Barley_MV3_VCF-AGG-row	Barley_KASP_kf	MorexV3_50k	MorexV3_40k	Position	Name	MAF	Ref	Alt	Null
chr7H					4417009	AVRIG12045	0.17	T	G	N
chr7H					4578990	Xcget616	0.00	C	T	N
chr7H					4924614	AVRIG12049	0.04	T	G	N
chr7H					4925070	AVRIG12050	0.35	A	G	N
chr7H					4929652	AVRIG37122	0.10	C	T	N
chr7H					4931908	AVRIG12053	0.15	G	A	N
chr7H					4933346	AVRIG12054	0.21	C	T	N
chr7H					4933398	AVRIG12055	0.33	G	T	N
chr7H					5134964	AVRIG12057	0.16	T	G	N
chr7H					5226241	AVRIG12058	0.15	T	C	N
chr7H					5265323	AVRIG12062	0.00	C	T	N
chr7H					5266725	AVRIG12060	0.20	A	G	N
chr7H					5401303	AVRIG36813				
chr7H					5404214	AVRIG12066				
chr7H					5416126	AVRIG37123				
chr7H					5459391	AVRIG12067				
chr7H					5555008	AVRIG12068				
chr7H					6572577	AVRIG12070				
chr7H					6579121	AVRIG12072				
chr7H					6591726	AVRIG12073				
chr7H					6871484	2_0534				
chr7H					7053370	AVRIG12076				

Note! Nucleotides have been defined, but the actual genotype calls are not yet loaded

Next, we will display the genotype calls in this region for the accession 'Osiris', since we know from Task 11 that this accession is likely to carry *Rrs2*, and has been genotyped. The Accession ID is provided in 2026_ABTS_SupplementaryFile1.txt. Afterwards we will use the genotype calls from Osiris to define a haplotype.

9. Type the accession ID for Osiris **AGG411853BARL** into the 'Filter by Name' field

10. Click on the genotype ID that appears in the box below, so it gets highlighted in grey

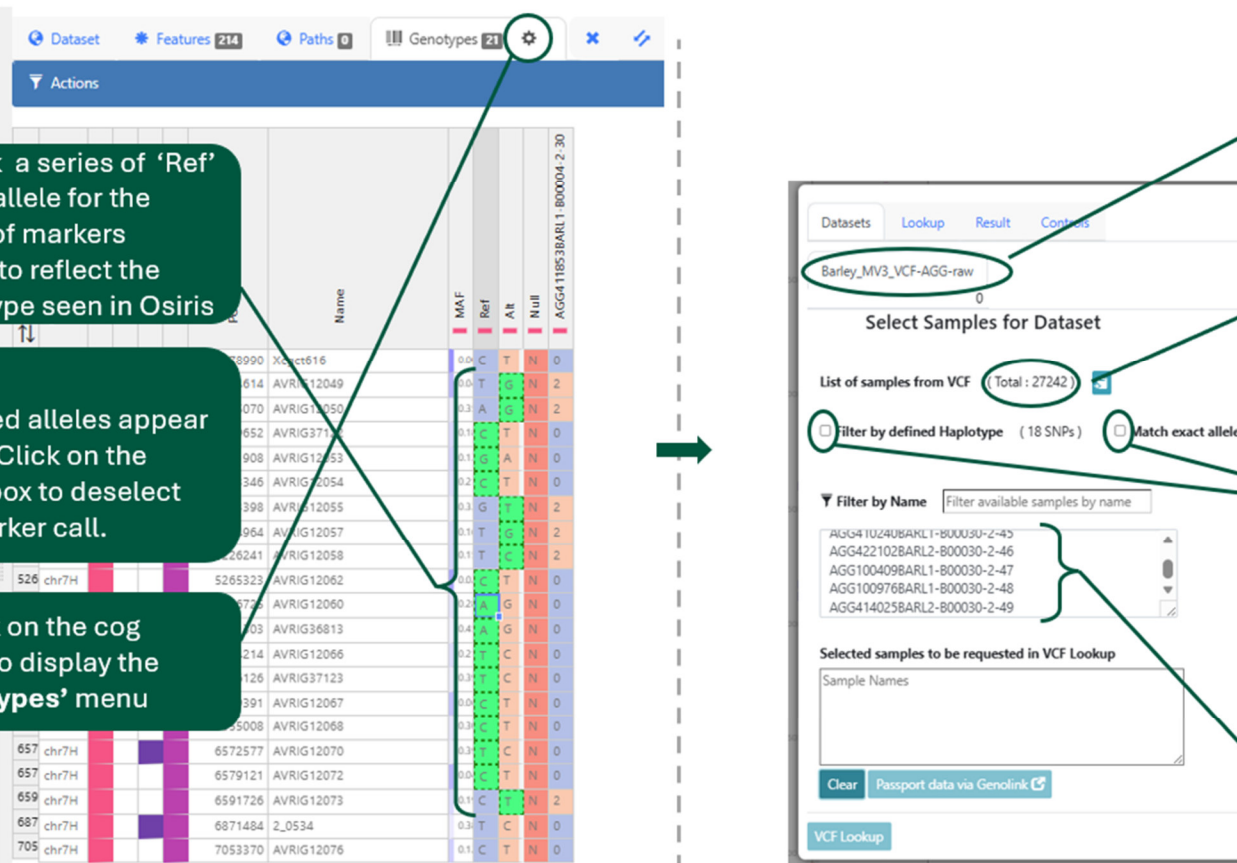
11. Click on the 'VCF Lookup' button to load the genotype calls for the Osiris accession

12. Toggle button and adjust row height to increase space for genotype calls displayed

Note: Genotype calls are now displayed for Osiris

Task 18: Search for AGG accessions using the haplotype filter

Next, we will define the haplotype around the region of interest based on the alleles observed in Osiris, then search for how many other barley accessions in the AGG contain the same haplotype.



1. Click a series of 'Ref' or 'Alt' allele for the series of markers shown to reflect the haplotype seen in Osiris

Note! Selected alleles appear green. Click on the green box to deselect the marker call.

2. Click on the cog wheel to display the 'Genotypes' menu

Note! Name of the vcf file loaded is shown in this tab.

Note! The total number of samples in the vcf file is displayed by default.

3. Tick the 'Filter by defined Haplotype' and 'Match exact alleles' to filter the samples and to avoid heterozygous genotype calls

Note! Genotype IDs for all the samples in the vcf file are shown by default.

Note!

The number of samples having the defined haplotype is shown here.

4. Click on any sample in the box indicated then click 'Ctrl' + 'A' on your keyboard to select all samples

Note!

All 289 sample IDs will now appear in this box.

Datasets Lookup Result Controls

Barley_MV3_VCF-AGG-raw

289

Select Samples for Dataset

List of samples from VCF (Filtered : 289)

☒ Filter by defined Haplotype (18 SNPs) ☒ Match exact alleles

Filter by Name Filter available samples by name

AGG404087BARL1-B00002-2-64
AGG401782BARL2-B00001-10-34
AGG401751BARL1-B00001-10-41
AGG402025BARL1-B00001-10-72
AGG401990BARL1-B00001-10-87

Selected samples to be requested in VCF Lookup (289)

AGG404087BARL1-B00002-2-64
AGG401782BARL2-B00001-10-34
AGG401751BARL1-B00001-10-41
AGG402025BARL1-B00001-10-72
AGG401990BARL1-B00001-10-87

Clear Passport data via Genolink

VCF Lookup

Note!

You can remove any unwanted samples by highlighting them and pressing 'Backspace' on your keyboard.

5. Click on the 'Lookup' tab

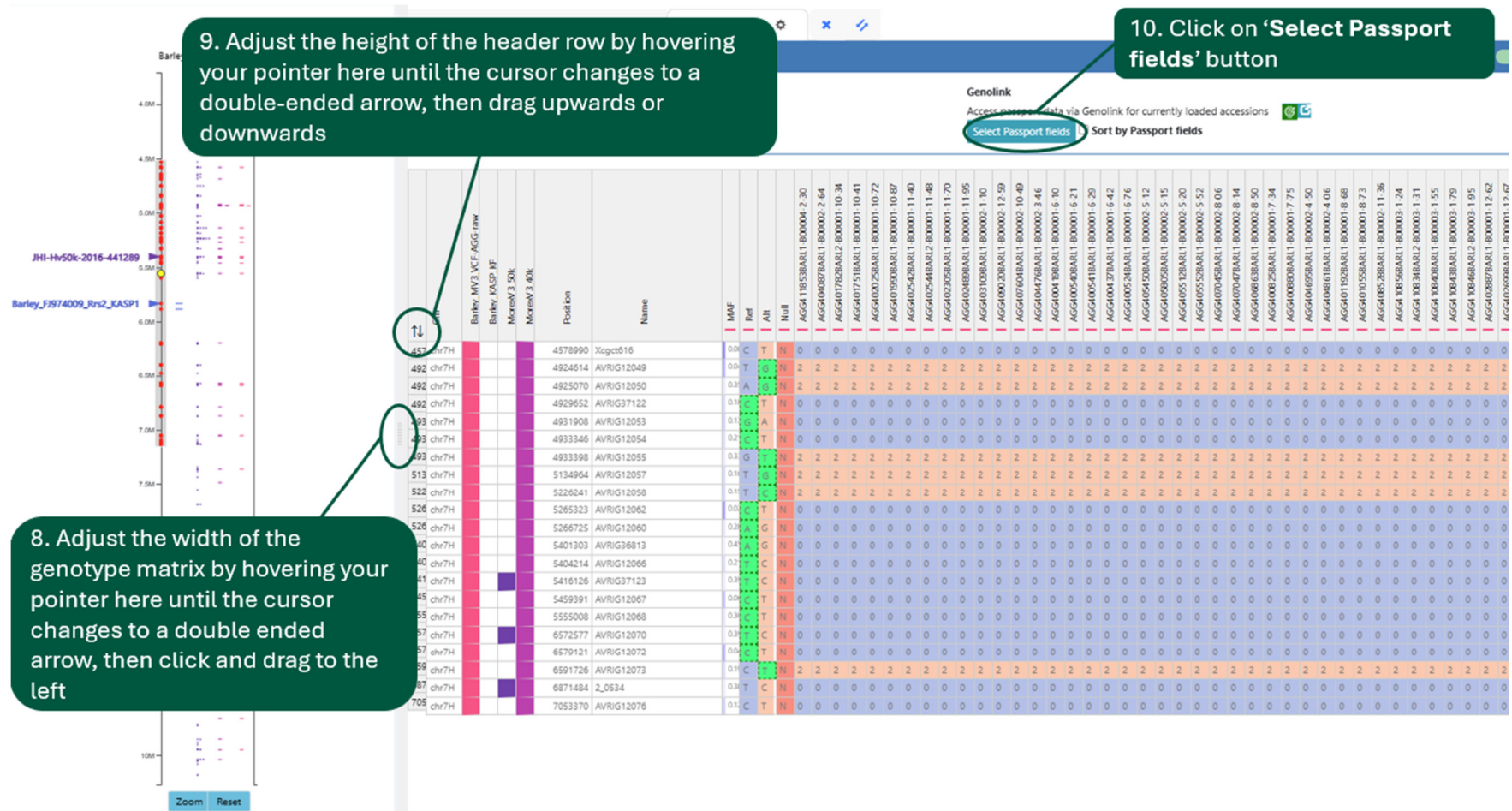
6. Increase the maximum number of samples that can be displayed in *Pretzel* by typing '300' into the 'Samples Limit' field

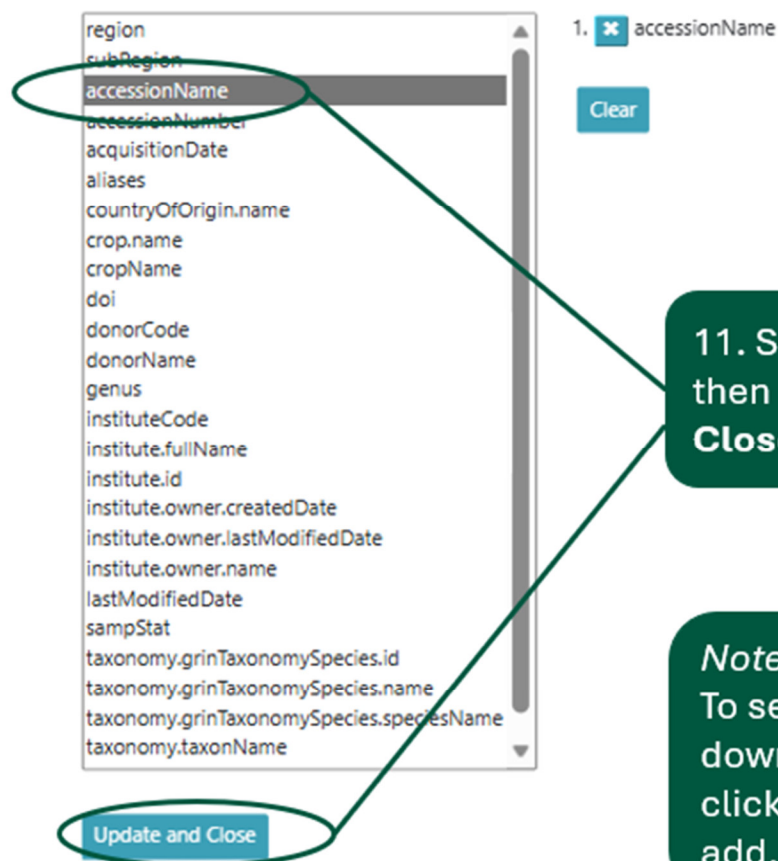
7. Click the 'VCF Lookup' button to load the genotypes into *Pretzel*

The screenshot shows the 'Lookup' tab in the Pretzel software interface. The 'Format' is set to '012'. The 'Auto lookup - all datasets' checkbox is checked. The 'Rows' slider is set to 300. The 'Samples Limit' field is set to 300. The 'VCF Lookup' button is highlighted.

VCF Genotype for brushed interval	4446203
Brushed Features	20

The genotypes for all 289 accessions with a matching haplotype are now displayed. Next, we will load passport data from *Genolink* to help identify the accessions loaded.





11. Select '**accessionName**' then click on the '**Update and Close**' button

Note!

To select multiple fields, hold down the Ctrl button while clicking each field you want to add.

Conclusion

We were able to connect research knowledge to genotype data from the AGG Strategic Partnership to investigate *Rrs2* in barley:

- Where is the *Rrs2* gene located in the barley genome?
 - **Around 5-6 Mb on chromosome 7H of Morex v3**
- Can markers on the wheat barley Infinium 40K SNP beadchip detect *Rrs2*?
 - **While not validated, a SNP haplotype was identified in multiple accessions known to carry *Rrs2*, suggesting we can increase our chance of detecting *Rrs2***
- What is the frequency of the *Rrs2* haplotype in the AGG?
 - **Only 289 accessions of the 27,242 barley accessions in the AGG carry the haplotype we defined**

Additional tasks - *Rrs2* locus for resistance to scald

Task 19: Analyse genetic diversity in *FairyBread* using a custom list of accessions

Next, we will compare the overall genetic diversity of the 289 lines found to carry the same haplotype as Osiris in the *Rrs2* region of 7H using *FairyBread*. The genotype IDs are also contained in **2026_ABTS_SupplementaryFile2.txt**.

The image shows a two-step process in the FairyBread web interface. On the left, the main dashboard has tabs for Dataset, Features (219), Paths (0), and Genotypes (21). A green circle highlights the settings gear icon next to the Genotypes tab. A green callout box with an arrow points to this icon, containing the text: "1. Click on the cog wheel to display the 'Genotypes' menu". On the right, the "Select Samples for Dataset" dialog is open for the dataset "Barley_MV3_VCF-AGG-raw" (289 samples). It shows a list of samples from VCF, filtered to 289. A green circle highlights a "Copy" button (represented by a document icon) next to the list. A green callout box with an arrow points to this button, containing the text: "2. Click on the 'Copy' button to copy a list of filtered sample identifiers to the clipboard". The list of samples includes identifiers like AGG404087BARL1-B00002-2-64, AGG401782BARL2-B00001-10-34, AGG401751BARL1-B00001-10-41, AGG402025BARL1-B00001-10-72, and AGG401990BARL1-B00001-10-87. Below the list, there is a section for "Selected samples to be requested in VCF" with the same identifiers and buttons for "Clear" and "Passport data via Genolink".

1. Click on the cog wheel to display the 'Genotypes' menu

2. Click on the 'Copy' button to copy a list of filtered sample identifiers to the clipboard

Next, we will go to *FairyBread* (<https://fairybread.plantinformatics.io>) and follow the steps below to display the 289 accessions relative to the whole AGG barley collection.

3. Select 'Custom List'

4. Click 'Reset' to clear the displayed Genotype IDs, then paste in the 289 Genotype IDs copied from Pretzel by clicking in the box and pressing 'Ctrl' + 'V' on the keyboard

5. Click the 'Parse' button

Matched Genotypes IDs will be displayed

6. Click on 'Data Explorer' to return to the plot view

Custom Genotype List

Paste a list of genotype IDs to find their records within the selected crop dataset. Accepts plain line-separated lists, CSV, or TSV

Crop Dataset: AGG Barley - Release 260430

Barley

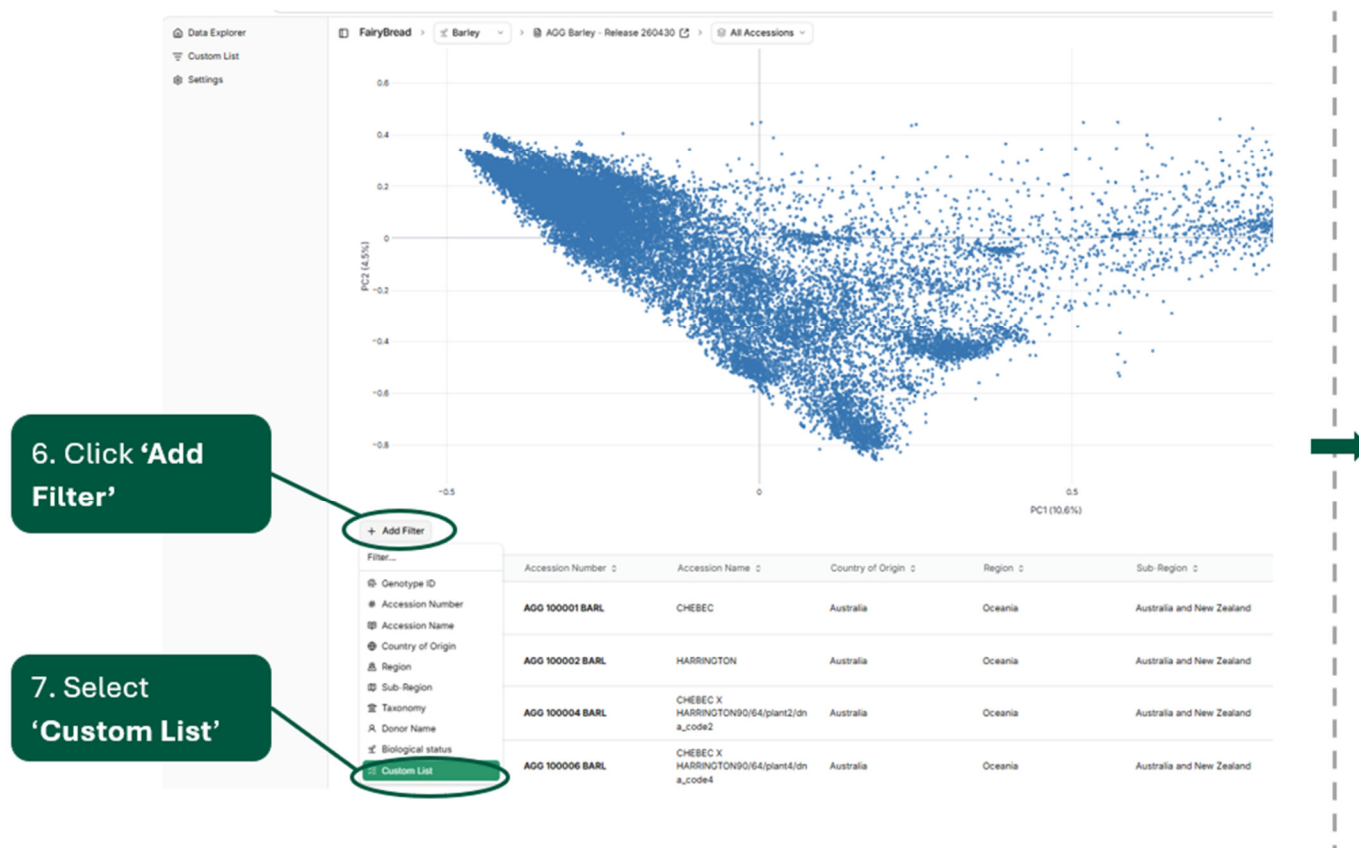
Genotype IDs One per line, or CSV/TSV

AGG490026BARL1-B00027-10-41
 AGG495019BARL1-B00027-10-43
 AGG404179BARL1-B00030-4-66
 AGG407172BARL1-B00030-11-34
 AGG414020BARL2-B00030-2-17
 AGG414023BARL2-B00030-2-41
 AGG414005BARL2-B00030-2-42
 AGG414025BARL2-B00030-2-49

Parse **Reset**

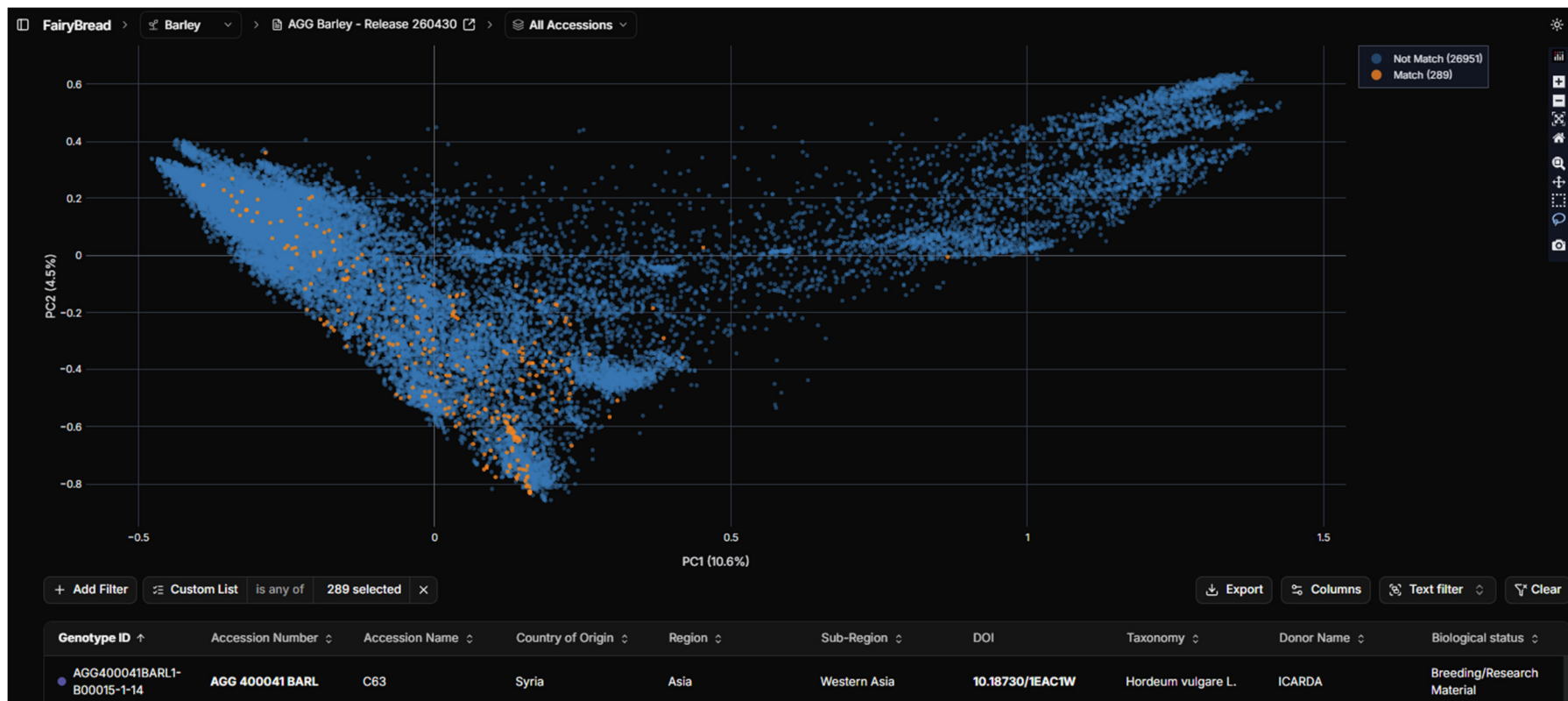
Matched 289

Genotype ID	Accession Name	Accession Number	Co
AGG405707BARL1-B00018-8-27	MXB.88 (F4 SEL.)	AGG 405707 BARL	Me



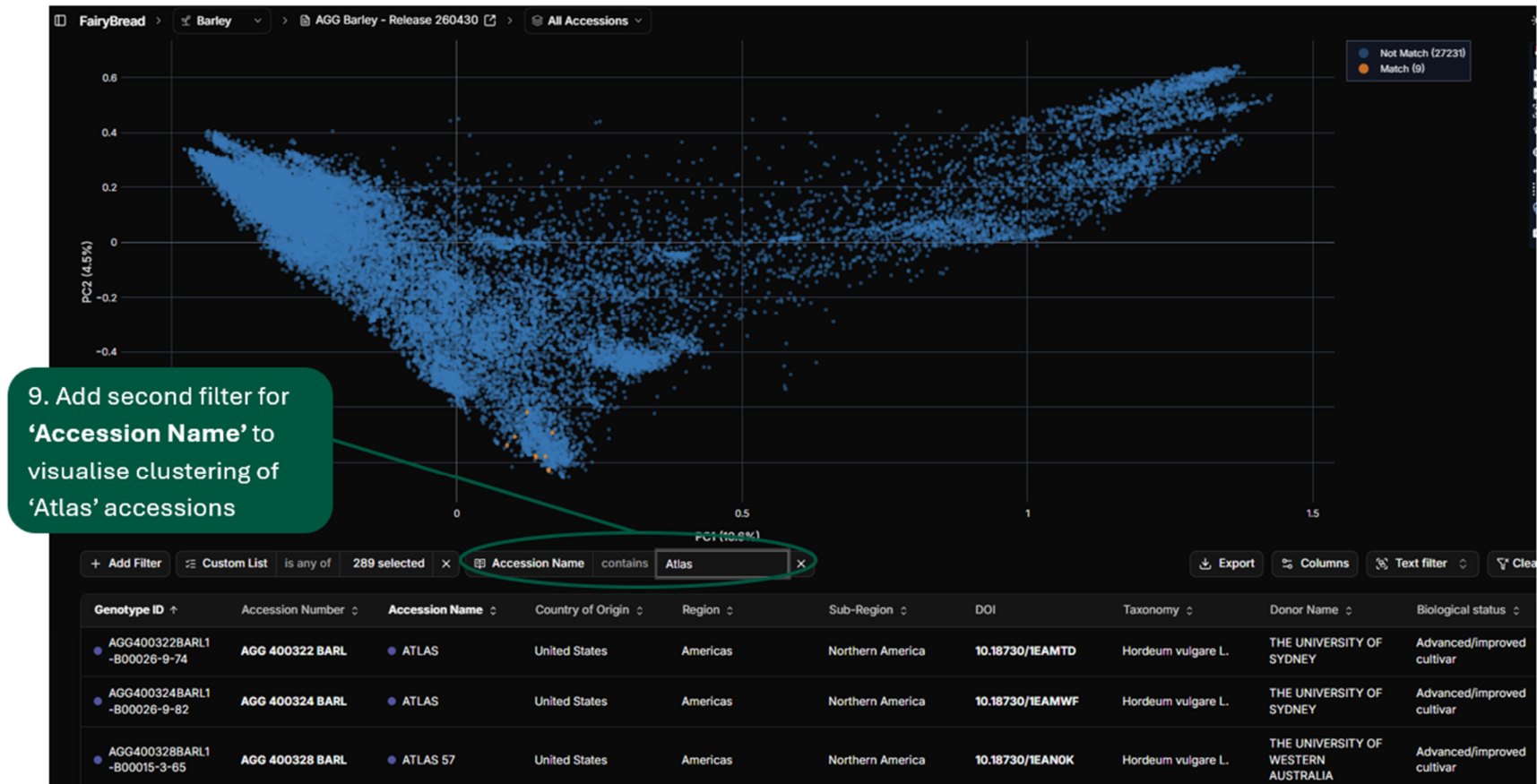
8. Click button to convert to dark mode

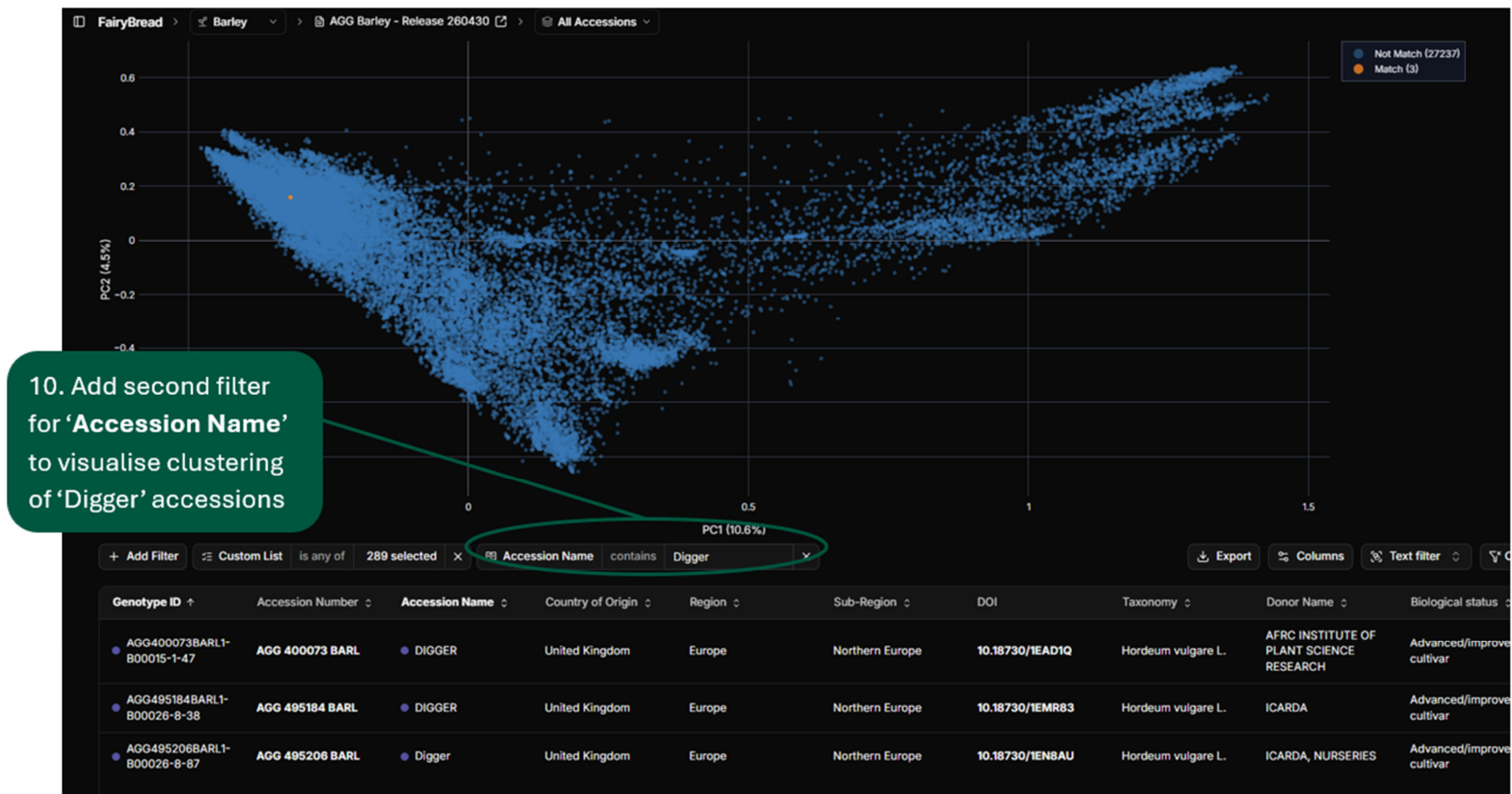




Based on this plot, we can see that the 289 accessions carrying the 'Osiris' haplotype around the *Rrs2* locus are otherwise quite genetically diverse overall.

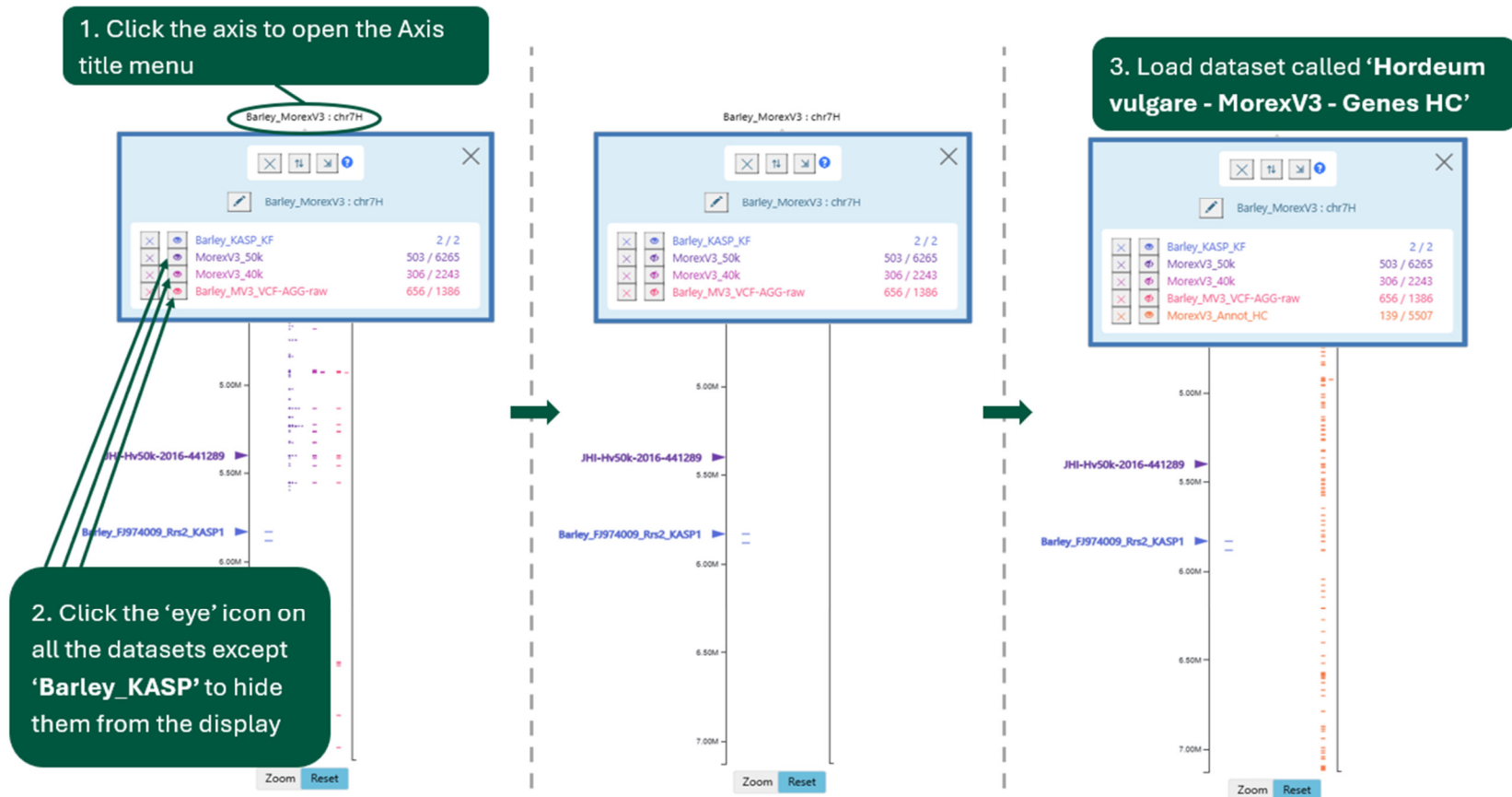
Try exploring the results further by adding another filter for 'Digger' or 'Atlas', then try using the lasso tool to select accessions from the plot.





Task 20: Explore annotated genes in the *Rrs2* locus

Load the high-confidence annotated genes for Morex v3 (**Hordeum vulgare - MorexV3 - Genes HC**) and investigate whether you can identify any potential candidate genes in the region containing *Rrs2*. For example, Marzin *et. al.* (2016) reported that PECTIN ESTERASE INHIBITOR genes co-segregate with *Rrs2*. Gene information can be found in the '**Features**' table. Other feature types will be hidden from view to simplify the amount of data presented in the '**Features**' table.

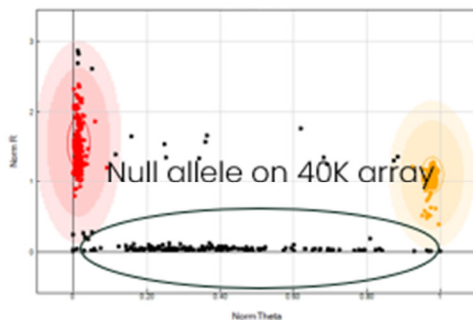


Additional tasks on the *nud* gene conferring hulless barley

Task 21: Finding haplotypes in the *nud* gene locus

In this task, we will search for a haplotype tagging the nudum (*nud*) locus that confers a hulless (or 'naked') caryopsis. Taketa *et. al.* (2008) reported a 17kb deletion on chromosome 7H containing the *nud* gene was identified in Kobinkatagi (a Japanese landrace) and *nud*-Bowman (a near isogenic line), relative to hulled variety Haruna Nijo, and identified a candidate gene for the *Nud* (hulled) gene as an ethylene response factor (ERF) family transcription factor. Jayakodi et al (2020) identified the same 17kb deletion in sequence data for 36 naked varieties.

This example demonstrates the power of capturing information about 'null' genotype calls, when a marker is deleted or highly divergent. This can be seen as a lack of fluorescence when using SNP BeadChips, or as missing sequence from a genome assembly. Null calls are now captured in the AGG genotype data standard and are recognised by *Pretzel* to enable investigation using null calls.



Genotype Call	Description
0/0:0	Homozygous REF (two reference alleles)
1/1:0	Homozygous ALT (two alternate alleles)
0/1:0	Heterozygous (one reference allele and one alternate allele)
./.:1	Null
./.:0	Missing data

We will begin by finding the location of the *Nud* gene (conferring the hulled trait) in Morex v3.

The image shows a two-step process in the Morex v3 web interface. The first step involves the 'VCF Genotype Search' panel, where the 'Search' tab is selected. The second step involves the 'Feature Search' panel, where the gene 'HORVU.MOREX.r3.7HG0719680' is entered in the 'Features Input' field. The 'Search' button is clicked, and the '+ MorexV3_Annot_HC chr7H' button is used to load the dataset. The final result is a chromosome plot for 'Barley_MorexV3 : chr7H' showing the position of the feature on the chromosome axis.

1. Click on the 'Search' tab

2. Type the gene below in the 'Features Input' field within 'Feature Search', or copy from the provided file '2026_ABTS_Supplementary File1.txt' *HORVU.MOREX.r3.7HG0719680*

3. Click on 'Search'

4. Click on '+' button to load the dataset 'MorexV3_50k' to display the position of features of interest. Button will turn '-' once selected

Feature has been added to the axis

Next, we will load into *Pretzel* the *in-silico* genotype calls for Wheat Barley 40k markers from the 76 accessions making up barley pangenome v2 (Jayakodi *et al.*, 2024), produced using the digital tool *Brioche*, to see if we can detect a haplotype to track the hulless trait at the *nud* locus.

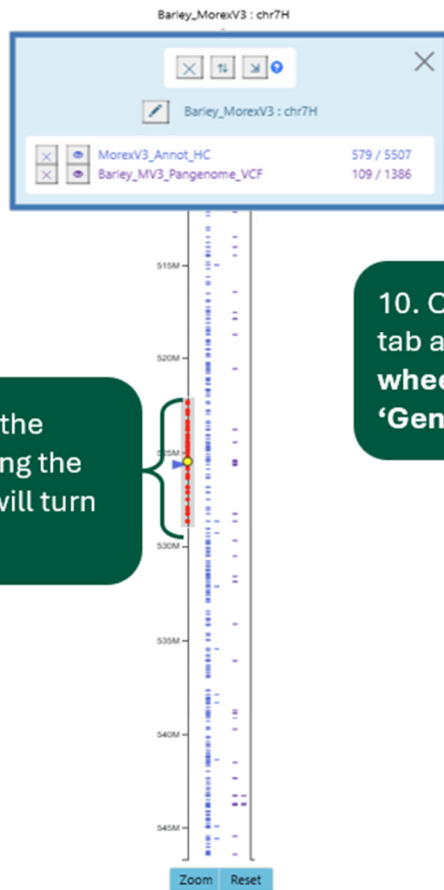
5. Select the 'Explorer' tab

6. Type 'pangenome' in the Search bar

7. Click on the black '+' icon on the left side of the dataset name 'Hordeum vulgare - MorexV3 - Genotypes - Barley - Pangenome' to show available chromosomes

8. Click on the green '+' icon on the left side of the chr7H to load the dataset. It changes to a '-' symbol once loaded

9. Highlight a portion of the chromosome surrounding the gene. Selected feature will turn red.



10. Click the 'Genotypes' tab and click the 'cog wheel' to display the 'Genotypes' menu

Dataset Features 59 Paths 0 Genotypes 14

Actions

VCF Lookup VCF Download

Chr	Barley_MV3_Pangenome_VCF	Position	Name	MAF	Ref	Alt	Null
523	chr7H	523782965	AVRIG13419	0.1	G	T	N
524	chr7H	524457647	AVRIG13421	0.2	G	T	N
525	chr7H	525401394	AVRIG13428	0.3	A	G	N
525	chr7H	525461988	AVRIG13425	0.1	A	G	N
525	chr7H	525469162	AVRIG13423	0.1	C	N	
525	chr7H	525470080	AVRIG13422	0.1	T	C	N
525	chr7H	525621635	AVRIG13429	0.3	A	G	N
525	chr7H	525621881	AVRIG13430	0.4	T	C	N
525	chr7H	525624308	AVRIG13431	0.1	G	A	N
525	chr7H	525628278	AVRIG13432	0.4	G	A	N
525	chr7H	525683936	AVRIG13433	0.4	A	G	N
528	chr7H	528242963	AVRIG13434	0.3	G	T	N
528	chr7H	528244802	AVRIG13435	0.3	T	C	N
528	chr7H	528637990	AVRIG13436	0.0	A	G	N

The screenshot shows a web interface with tabs: Datasets, Lookup, Result, and Controls. The 'Lookup' tab is active. At the top, a text box contains 'Barley_MV3_Pangenome_VCF' with a count of 76. Below this is a section titled 'Select Samples for Dataset'. It includes a label 'List of samples from VCF (Total : 76)' and a checkbox 'Filter by defined Haplotype'. A 'Filter by Name' section has a text input 'Filter available samples by name'. Below this is a list of sample IDs, with the first five highlighted in blue. At the bottom of this list is a 'VCF Lookup' button. Below the list is a section 'Selected samples to be requested in VCF Lookup (76)' containing a list of sample IDs and a 'Clear' button. At the very bottom is a 'Passport data via Genolink' button.

Barley_MV3_Pangenome_VCF 76

Select Samples for Dataset

List of samples from VCF (Total : 76)

☐ Filter by defined Haplotype

Filter by Name Filter available samples by name

GCA_951817035.1_Hvulgare_WBDC_103_BPGv2_genomic
GCA_951817075.1_Hvulgare_WBDC_103_BPGv2_genomic
GCA_951817375.1_Hvulgare_WBDC_348_BPGv2_genomic
GCA_951817435.1_Hvulgare_WBDC_349_BPGv2_genomic
GCF_904849725.1

Selected samples to be requested in VCF Lookup (76)

GCA_949782835.1_Hvulgare_cv_Barke_BPGv2_genomic
GCA_949782845.1_Hvulgare_cv_Bonus_BPGv2_genomic
GCA_949782985.1_Hvulgare_cv_Aizu6_BPGv2_genomic
GCA_949783005.1_Hvulgare_10TJ18_BPGv2_genomic
GCA_949783095.1_Hvulgare_cv_Akashinriki_BPGv2_genomic

Clear Passport data via Genolink

VCF Lookup

Note!
All 76 sample IDs will now appear in this box.

Note!
You can remove any unwanted samples by highlighting them and pressing 'Backspace' on your keyboard.

Note!
Name of the vcf file loaded is shown in this tab.

Note!
The total number of samples in the vcf file is displayed by default.

11. Click on any sample in the box indicated then click 'Ctrl' + 'A' on your keyboard to select all samples

12. Click 'VCF Lookup'

Note!
Genotype calls are now displayed for selected samples.

[illegible]

13. Click a series of 'Null' alleles for the markers shown to sort the samples from left to right according to haplotype

Ref	Alt	Null	GCA_949783005.1	Hudjase	30178	BF62	genomic
GCA_949783005.1	Hudjase	o/ Martinelli	BF62	genomic			
GCA_94978325.1	Hudjase	o/ Li, Huang	ZM001467	BF62			
GCA_949783125.1	Hudjase	HCX_7172	BF62	genomic			
GCA_949783485.1	Hudjase	HCX_2779	BF62	genomic			
GCA_949783575.1	Hudjase	HCX_7385	BF62	genomic			
GCA_951815065.1	Hudjase	HCX_7552	BF62	genomic			
GCA_951817035.1	Hudjase	HCX_9043	BF62	genomic			
GCA_949783465.1	Hudjase	HCX_3323	BF62	genomic			
GCA_951816945.1	Hudjase	HCX_9972	BF62	genomic			
GCA_949782835.1	Hudjase	o/ Baile	BF62	genomic			
GCA_949782895.1	Hudjase	o/ Benus	BF62	genomic			
GCA_949783105.1	Hudjase	o/ Bauman	BF62	genomic			
GCA_949783115.1	Hudjase	o/ Martins	BF62	genomic			
GCA_949783135.1	Hudjase	o/ KGT	Paved	BF62	genomic		
GCA_949783165.1	Hudjase	o/ Golden	Mexico	BF62	genomic		
GCA_949783185.1	Hudjase	o/ Golden	Promise	BF62	genomic		
GCA_949783265.1	Hudjase	o/ Baile	BF62	genomic			

Four markers close to the *nud* gene (AVRIG13429, AVRIG13430, AVRIG13431 and AVRIG13432) show a null allele ('N') haplotype. This null haplotype is present in varieties Du Li Huang and Akashinriki known to have the hullless trait.

Next, we will load AGG accessions that are likely to be hulless based on passport data. These can be found by searching for 'naked' in Genolink, or by copying examples contained in the supplementary file '2026_ABTS_SupplementaryFile1.txt'. This will enable direct connectivity between the pangenome, and genotype calls generated from the wheat barley 40k SNP array for barley accessions in the AGG.

14. Load the raw AGG genotype calls dataset 'Hordeum vulgare – MorexV3 – Genotypes – AGG Raw Release 260430'

Explorer
View
Search
Upload

Datasets
?

Group All
Crop Barley

Category All

raw

Case insensitive
Match all

Trait
Ontology
VCF
All Datasets

Upload new data
+

+

Hordeum vulgare - MorexV3 - Genotypes - AGG
Raw Release 240806

-

Hordeum vulgare - MorexV3 - Genotypes - AGG
Raw Release 260430

+ chr1H

+ chr2H

+ chr3H

+ chr4H

+ chr5H

+ chr6H

+ chr7H

14. Highlight features surrounding the *nud* gene, then click the cog button to load Genotype Table Controls

Barley_MorexV3 : chr7H

Dataset
Features
Paths
Genotypes

Actions
VCF Lookup

Chr	Barley_MV3_Pangenome_VCF	Barley_MV3_VCF-AGG-raw	MorexV3_Annot_HC	Position	Name	MAF	Ref	Alt	Null	MAF
523	chr7H			523782965	AVRIG13419	0.1	G	T	N	0.01
524	chr7H			524457647	AVRIG13421	0.2	G	T	N	0.01
525	chr7H			525401394	AVRIG13428	0.3	A	G	N	0.1
525	chr7H			525461988	AVRIG13425	0.1	A	G	N	0.01
525	chr7H			525469162	AVRIG13423	0.1	T	C	N	0.01
525	chr7H			525470080	AVRIG13422	0.1	T	C	N	0.01
525	chr7H			525621635	AVRIG13429	0.3	A	G	N	0.01
525	chr7H			525621881	AVRIG13430	0.4	T	C	N	0.01
525	chr7H			525624308	AVRIG13431	0.1	G	A	N	0.01
525	chr7H			525628278	AVRIG13432	0.4	G	A	N	0.1
525	chr7H			525683936	AVRIG13433	0.4	A	G	N	0.1
528	chr7H			528242963	AVRIG13434	0.3	G	T	N	0.01
528	chr7H			528244802	AVRIG13435	0.3	T	C	N	0.01

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15. Paste Genotype IDs for 'naked' AGG accessions taken from the file '2026_ABTS_SupplementaryFile1.txt' into the box indicated, then click 'VCF Lookup'

16. Click 'Select Passport field' and select 'AccessionName' to load passport data from Genolink

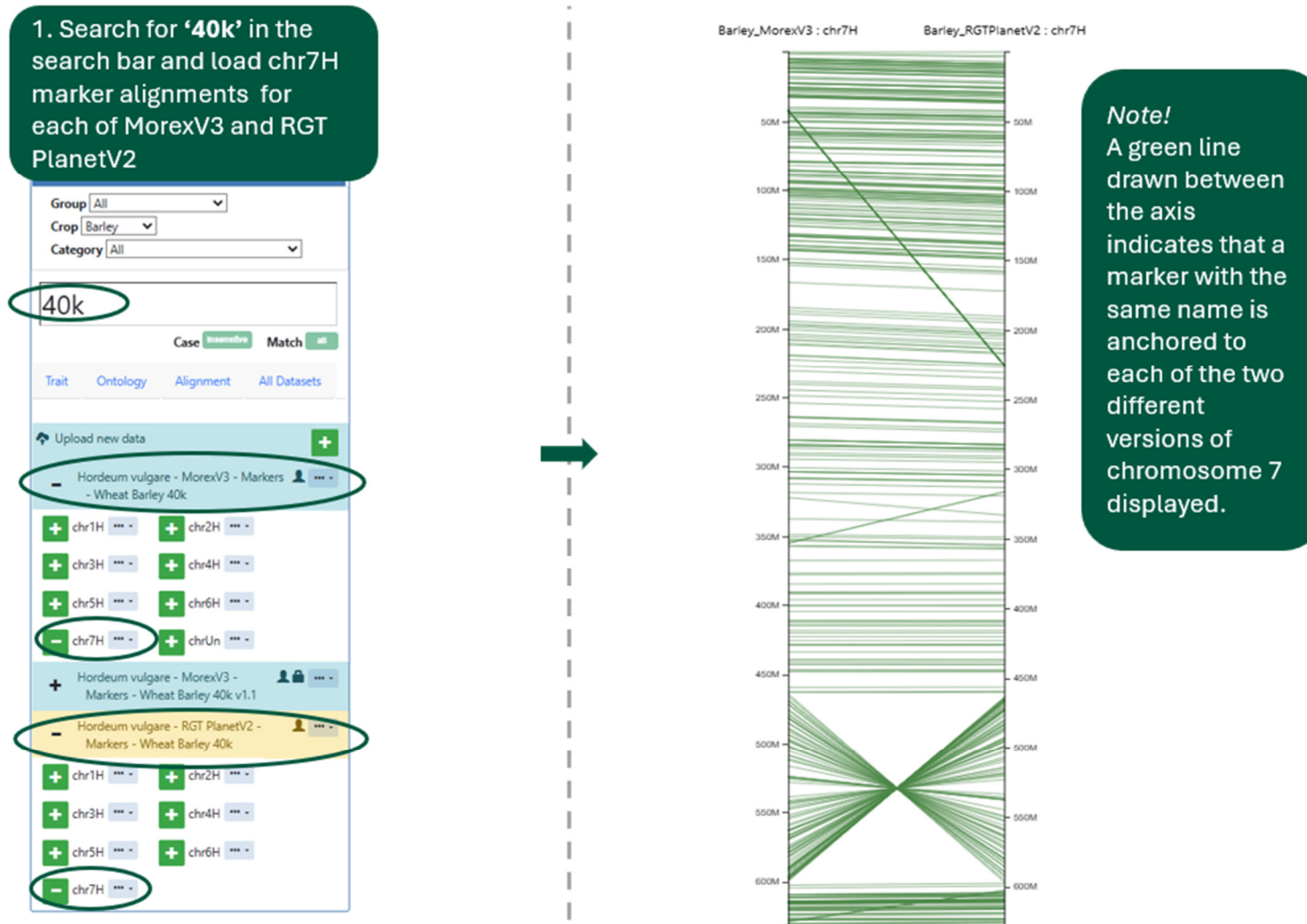
Select Passport fields | Sort by Passport fields

AccessionName	Chr	Position	Name	MAF	Ref	Alt	Null	MAF	Ref	Alt	Null
AGG401954BARL1-800001-10-70	chr7H	523782965	AVRIG13419	0.1	G	T	N	0.0	2	2	2
AGG401982BARL1-800001-10-71	chr7H	524457647	AVRIG13421	0.2	G	T	N	0.0	2	2	2
AGG402025BARL1-800001-10-72	chr7H	525401394	AVRIG13428	0.3	A	G	N	0.1	2	2	2
AGG401763BARL1-800001-10-73	chr7H	525461988	AVRIG13425	0.1	A	G	N	0.0	2	2	2
AGG401796BARL1-800001-10-74	chr7H	525469162	AVRIG13423	0.1	T	C	N	0.0	2	2	2
AGG423022BARL2-800005-12-83	chr7H	525470080	AVRIG13422	0.1	T	C	N	0.0	2	2	2
AGG4230251BARL1-800001-11-29	chr7H	525621635	AVRIG13429	0.3	A	G	N	0.0	2	2	2
AGG423026BARL3-800003-7-12	chr7H	525621881	AVRIG13430	0.2	T	C	N	0.0	2	2	2
AGG423027BARL2-800025-12-62	chr7H	525624308	AVRIG13431	0.1	G	A	N	0.0	2	2	2
AGG407969BARL1-800027-4-10	chr7H	525628278	AVRIG13432	0.4	G	A	N	0.1	2	2	2
AGG407969BARL1-800027-4-10	chr7H	525683936	AVRIG13433	0.4	A	G	N	0.1	2	2	2

After loading passport data, we can see that multiple AGG barley accessions containing the word 'naked' in their Accession Name possess a haplotype with two null alleles (markers AVRIG13429 and AVRIG13429). It is worth noting that *in-silico* genotype calls derived from genome assemblies do not always perfectly correlate with genotype calls derived from SNP arrays due to hybridisation kinetics. In this case, the two null markers are sufficient to track the *nud* haplotype across both datasets.

Task 22: Compare structural variation between Morex and RGT Planet at the chromosome level using *Pretzel*

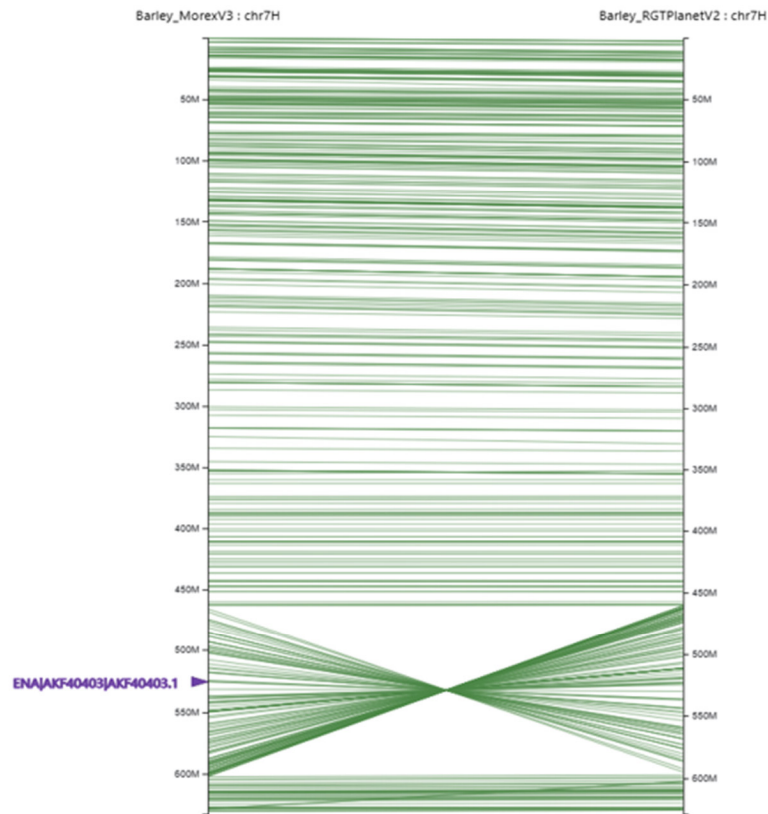
Outputs from *Brioche* can also be used to compare marker ordering for chromosomes from different genome assemblies.



Task 23: Search for the *Nud* gene in the Morex v3 genome using BLAST

DNA sequence for the *Nud* gene (AKF40403) is contained in the file '2026_ABTS_SupplementaryFile1.txt'. Instructions on how to perform a BLAST search in *Pretzel* can be found in the documentation page: <https://docs.plantinformatix.io/Use-Cases/Finding-a-marker-or-gene-on-a-genome-using-dna-sequence/>.

The screenshot shows the 'DNA Sequence Blast Search' interface. The 'Reference to search' dropdown is set to 'Hordeum vulgare - MorexV3 - Genome'. The 'DNA Sequence Input' is set to 'FASTA'. The input sequence is:
>ENAI|AKF40403|AKF40403.1 Hordeum
vulgare NUD
ATGGTACAGTCCAAGAAGAAGTTTCGCGCGTC
AGGCAGCGCCACTGGGGCTCCTGGGTCTCCGAG
ATCAGGCATCTCTCTGAAGAGGAGGGTGTGG
TTGGGCACCTTTGAGACGGCGAGGAGGCTGC
Below the input, there are 'Clear' and 'Search' buttons. Under 'Optional Inputs', there are sliders for 'Rows' (set to 500), 'Table Result Limit' (set to 0), 'Length of Hit' (set to 50), '% Identity' (set to 70), and '% Coverage' (set to 70).



References

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- Milner SG, Jost M, Taketa S *et. al.* (2019) Genebank genomics highlights the diversity of a global barley collection. *Nat Genet* 51: 319–326. <https://doi.org/10.1038/s41588-018-0266-x>.
- Noe SM, Åstrand J, Zakieh M *et. al.* (2025) Harnessing novel genetic markers for scald resistance from gene bank spring barley genotypes. *BMC Plant Biology.* 25: 781 <https://doi.org/10.1186/s12870-025-06813-2>.
- Taketa, S. *et al.* (2008) Barley grain with adhering hulls is controlled by an ERF family transcription factor gene regulating a lipid biosynthesis pathway. *Proc. Natl Acad. Sci. USA* 105: 4062–4067. <https://doi.org/10.1073/pnas.0711034105>.

Appendix

Pretzel data upload templates

Overview

Data can be uploaded into *Pretzel* using several methods, but the Excel upload method is recommended for most users. Three types of data (alignments, genetic maps and QTLs) can be independently uploaded to *Pretzel* as custom datasets, as described below. Note that it is also possible to upload genomes, BLAST databases and vcf files with assistance from the *Pretzel* development team.

Data type	Dataset Name Format	Description
Alignment	Alignment DataName	Refers to a feature with a defined position or interval, except for a QTL. This is commonly used for markers or genes, which are typically anchored to a specified genome assembly. Units are in base pairs (bp).
Map	Map DataName	Used for uploading genetic maps, where units for the defined position are in centiMorgans (cM).
QTL	QTL DataName	Used for uploading QTLs. The defined interval positions can relate to either a genome assembly (where coordinates are in bp), or a genetic map (where coordinates are in cM),

The Excel template file consists of two sheets, the '**Metadata**' sheet for defining parameters and providing meta data, and the '**Dataset**' sheet that contains data you wish to upload into *Pretzel*.

	A	B
1	Field	Alignment Barley_KASP_XX
2	shortName	Barley_FJ974009_KASP
3	displayName	Hordeum vulgare - MorexV3 - Markers - Rrs2 - KASP - XX
4	type	Alignment
5	Crop	Barley
6	Species	Hordeum vulgare
7	Data Source	Primers sequences were based on FJ974009 in Hanemann et al. 2009
8	Licensing of original data	NA
9	Curator	Name
10	Publication	Hanemann et al. 2009. Fine mapping, physical mapping and development of diagnostic markers for the Rrs2
11	Comments	scald resistance gene in barley. Theoretical and Applied Genetics 119:1507-1522; Hautsalo et al. 2021.
12	Genome_id	Barley_MorexV3
13	Alignment type	Marker
14	Marker type	KASP
15	parentName	Barley_MorexV3
16		
17		
18		
19		
20		
21		
22		
23		
24		
25		
26		
27		
28		
29		

'Metadata'
sheet

'Dataset'
sheet

< > Metadata Alignment|Barley_KASP_XX +

Metadata sheet

The figure and table below describe the fields in the **'Metadata'** sheet for uploading a custom QTL dataset into *Pretzel*. The mandatory fields are used by *Pretzel* and are case sensitive; they must match the relevant dataset in *Pretzel* exactly to link datasets effectively e.g. to link a QTL to a genome assembly. The optional parameters below are used to display meta data in the **'Dataset'** panel to align with FAIR (Findable, Accessible Interoperable, Re-usable) data standards. We recommend using the parameters below as best practise. Additional optional columns can be added if desired. The figure below shows where the mandatory parameters in the **'Metadata'** sheet get incorporated into *Pretzel*.

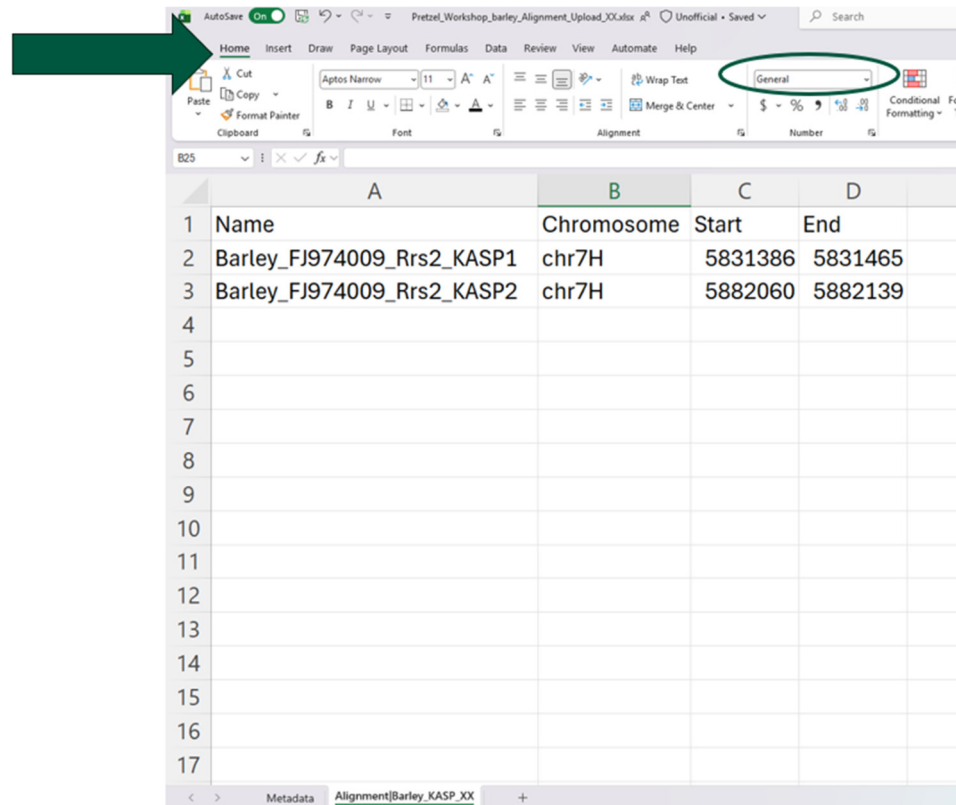
The diagram illustrates the integration of metadata from a spreadsheet into the Pretzel interface. A central vertical axis represents the genome assembly 'Barley_MorexV3 : chr7H'. To the left, the 'Upload new data' panel shows a list of datasets, with 'Hordeum vulgare - MorexV3 - Markers - Rrs2 - KASP - KF' highlighted. A green arrow labeled 'shortName of the parentName' points from this dataset to the central axis. Another green arrow labeled 'Field for dataset name' points from the central axis to the 'Barley_KASP_KF' dataset panel on the right. A third green arrow labeled 'parentName' points from the 'Parent' field in the dataset panel to the central axis. A fourth green arrow labeled 'displayName' points from the 'displayName' field in the metadata section to the central axis. The metadata section on the right contains a JSON object with fields like shortName, displayName, type, crop, species, data source, licensing, curator, pubId, comments, genome_id, alignment type, and marker type. A bracket labeled 'MetaData' groups these fields.

Parameter	Details	Usage		
		QTL	Alignment	Map
Field	Name of the custom dataset to be loaded into <i>Pretzel</i>. It is structured as the type of dataset on the LHS, a pipe symbol ' ' and the unique name of the dataset on the RHS. E.g. 'QTL Dataset_XX', 'Alignment Dataset_XX' or 'Map Dataset_XX' This dataset name must be unique, it cannot match any other datasets loaded in <i>Pretzel</i>, including datasets loaded by other users. Note that this dataset name is NOT used in the left-hand side Explorer panel, it is only displayed in the 'Dataset' panel (see Figure below). This must exactly match the dataset name on the RHS of the pipe symbol in the Excel worksheet name i.e. the portion in bold e.g. QTL Wheat_CSv2.1_7AL_QTLs-KF The name of the dataset is limited to 31 characters (including e.g. 4 characters used for 'QTL ') due to limitations set by MS Excel on the sheet name. Take care to ensure there are no spaces.	mandatory	mandatory	mandatory
Species	Taxonomy e.g. Triticum aestivum	mandatory	mandatory	mandatory
Crop	Used to filter the datasets in the ' Explorer ' panel based on the crop type e.g. Wheat	mandatory	mandatory	mandatory
displayName	Complete, descriptive name that shows up in the left-hand 'Explorer' panel	mandatory	mandatory	mandatory
parentName	Name of the genome assembly or genetic map that the feature (e.g. QTL, marker, gene) is being anchored to. This must exactly match the dataset name in <i>Pretzel</i> .	mandatory	mandatory	not applicable
shortName	Short dataset name displayed at the top of the chromosome axis in <i>Pretzel</i> . Required only for a genome or genetic map dataset.	not applicable	not applicable	mandatory
platform	Type of technology platform used to generate data e.g. 90k, 40k, SSRs, etc. If multiple types of markers were used e.g. in the case of a consensus map, then the use of 'multiple' is recommended.	not applicable	not applicable	mandatory
licensing	E.g. CC BY 4.0 (or 'none' if not applicable)	optional	optional	optional
Citation	Reference to a manuscript, 'personal communication (Researcher name)', 'unpublished' etc.	optional	optional	optional
DOI	DOI links the uploaded data to the source e.g. manuscript	optional	optional	optional
Comments	Longer description of the dataset, and/or information on the study, or how the data was compiled. This could include: - parents and filial generation of a mapping population - information about the germplasm (e.g. diverse landraces) - trait information - analysis and/or filtering steps taken	optional	optional	optional
Contact	Person / organisation who uploaded the data (e-mail address encouraged)	optional	optional	optional

Dataset sheet

The fields in the '**Dataset**' sheet are very specific to the type of data being uploaded. The names of the mandatory parameters in the LHS column below are case sensitive.

It is important that the numbers entered into the '**Start**', '**End**' and '**Position**' fields are formatted correctly. In excel, highlight the relevant cells and set the format to 'General', ensuring they do not contain any commas or thousand separators e.g. '678380602' not '678,380,602'.



Additional, optional columns can be added as desired to capture other information e.g. human-readable gene descriptions, gene identifiers.

Parameter	Details	Usage		
		QTL	Alignment	Map
Name	Name of the QTL or feature	mandatory	mandatory	not applicable
Marker	Name of the markers in the genetic map	not applicable	not applicable	mandatory
Chromosome	Name of the chromosome the feature is anchored to. You must check the name of the chromosome in the relevant dataset in <i>Pretzel</i> for the ' parentName '. E.g. if the chromosome in <i>Pretzel</i> is called 'Chr7A', then the use of e.g. '7A' will fail to anchor the feature to the chromosome.	mandatory	mandatory	mandatory
Trait	Name of the trait being investigated	mandatory	not applicable	not applicable
Start	Start position of the QTL. If you have anchored the QTL to a chromosome in a genome assembly, this should be the number in basepairs. If you have anchored the QTL to a genetic map, then the number should be in cM.	mandatory	mandatory	not applicable
End	End position of the QTL. If the QTL is associated with a SNP marker, then the Start and End positions should be the same number.	mandatory	mandatory	not applicable
Position	Position of the marker in the genetic map	not applicable	not applicable	mandatory
Ontology	Crop ontology term from https://cropontology.org/ e.g. CO_321:0000902. The data contained in this field enables <i>Pretzel</i> to colour-code QTLs based on crop ontology terms.	optional	not applicable	not applicable
Reference	Reference to a published manuscript / 'personal communication (Researcher name)', 'unpublished' etc. It can be especially helpful to list a reference for each entry if data has been compiled from multiple sources.	optional	optional	optional
Comments	Longer description of the dataset, and/or information on the study, or how the data was compiled.	optional	optional	optional

Troubleshooting

If you encounter a bug and *Pretzel* is no longer responding as expected, reset your session. The easiest way to do this is put your cursor in the URL field of your browser and delete characters back to the main URL: <https://agg.plantinformatics.io/>, then press 'Enter' on your keyboard.

If any software bugs are causing problems, please report them to the *Pretzel* development team: info@plantinformatics.io.

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