Axolotl

Newsletter of the Ambystoma Genetic Stock Center
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Video: Example of circular progression in *Ambystoma mexicanum* sperm at 120 mOsm kg^{-1} Hanks' Balanced Salt Solution.

2022 Issue

AGSC Director's Note - Randal Voss

It's great to see axolotl research picking up again! So many interesting papers coming out, the 2022 Salamander Models meeting in Turkey only a few months away, and young investigators from our community being recruited by top institutions to lead their own labs. AGSC axolotls are in good health and supply, please let us know how we can help support your research efforts.

As many of you know, the Ambystoma Genetic Stock Center (AGSC) is a NIH P40 Research Resource that distributes laboratory axolotls (*Ambystoma mexicanum*) to researchers and educators nationally and internationally. The AGSC maintains wildtype, mutants, knockouts, and transgenics as living stocks because methods have not been optimized or standardized for cryopreservation of axolotl germplasm. This presents a critical barrier because we have finite resources to sustain our irreplaceable, captive-bred population for axolotl stock production, and import new stocks that may enhance research efforts. The AGSC is currently working to develop infrastructure and capacity for axolotl sperm cryopreservation and frozen stock management, including archiving and re-deriving stocks. An overview of this project is described below in this issue of the *Axolotl* Newsletter.

A number of new transgenic and knock-out lines have been developed in the last few years and we are working to make some of these available as new stocks. Prayag Murawala and James Monaghan kindly sent representative larvae of several transgenic lines, and new knockout lines and a spontaneous mutant will also soon be available. Contact us if you would like to get on the waiting list for any of these new AGSC stocks.

Axolotl Line	**Type**
tgSceI(*Mmu.B3Tub:memGFP*)^{ETNKA}	transgenic
tgSceI(*CAGGs:ER^{T2}-Cre-ER^{T2}-T2a-GFPnls*)^{ETNKA}	transgenic
tgSceI(*CAGGs:GFP*)^{ETNKA}	transgenic
tgSceI(*Xla.Caract:GFP*)^{ETNKA}	transgenic
tgSceI(*CAGGs:LoxP-GFP-LoxP-Cherry*)^{ETNKA}	transgenic
tgSceI(*Mmu.Prrx1:TFPnls-T2a-ER ^{T2}-Cre-ER^{T2})*^{ETNKA}	transgenic
tgSceI(*Mmu.Prrx1:TFPnls-T2a-Cre-ER^{T2})*^{ETNKA}	transgenic
tgSceI(*RARE:GFP)*^{PMX}	transgenic
tgSceI(*CAG*:*Clover-Geminin*(1-110)-*IRES-mKO2-Cdt*(30-120)^{JMONA}	transgenic
tm(*Kit*)^{SRV}	knockout
tm(*Prod1*)^{SRV}	knockout
tm(*Grhl3*)^{SRV}	knockout
sm(*Grhl3*)^{SRV}	mutant
tm(*Atrw*)^{SRV}	knockout
tm(*Mir214*)^{SRV}	knockout

Continuous development of research resources is needed to further research efforts using the axolotl. In this issue, a team of researchers from Mount Desert Biological Laboratory and Harvard University provide an update about a recent NIH grant they submitted to develop transgenic methodologies and new axolotl lines. Check it out along with an article from researchers at University of Kentucky that are working to make genomics data publicly available to the community.

The AGSC is funded by the Office of Research Infrastructure Programs at the National Institutes of Health. Please acknowledge use of AGSC stocks, including stocks derived from the AGSC axolotl population, in your publications by citing grant number (P40-OD019794).

Community News

Salamander Meeting in Turkey

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The 2022 Salamander Meeting will be held in Turkey at Istanbul Medipol University on 22-24 August 2022. The organization will be in hybrid form, which will host both on-site and online speakers and attendees. This year's theme is "Comparative Studies and Translational Opportunities in Salamander Research". There will be sessions for oral and poster presentations and slots are still available for conference talks. For more information and registration visit https://salamander2022.com .

Axobase Resource Updates

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We are happy to provide an update on behalf of the Axobase team. For many years, we as a community have discussed and dreamed of a unified web portal for axolotl-related resources. This was also highlighted as a critical need in the white paper that was issued after the 2019 salamander meeting. The first step of this dream became reality with the launch of www.axobase.org website in Aug 2021. The current website is generously funded by MDIBL and maintained by a team lead by Dr. Joel Graber (Director, Computational Biology core, MDIBL). The current website provides 1) links to all major web-resources of the axolotl community, 2) guidelines for gene and transgenic nomenclature, 3) a list of all available transgenic lines, 4) a list of antibodies that work in salamanders and 5) a comprehensive list of colleagues who are working in our field. While this was a good beginning, many more milestones remain to be accomplished.

Our ultimate goal is to have unified genomic and transcriptomic resources under one web portal, similar to Ensembl where one can find all available information for a gene of interest. Generation of such a site from the ground up would require significant effort, and would also be redundant, since much of the necessary functionality has already been developed by the GMOD (Generic Model Organism Database) community.

We therefore initiated discussions with the Xenbase team and reached an agreement in which they will provide necessary resources and backend support on a contract basis while we focus on data collection and axolotl-specific tool development.

Despite this agreement, development of the database is a major work and it will require both significant monetary and human resources, as well as support of the entire community. Hence to sustain this operation, it was necessary to find alternate funding source(s). To this end, we (James Godwin, Jessica Whited, Joel Graber, and Prayag Murawala) have written a joint R24 grant and submitted to ORIP, NIH with a subcomponent that includes funds to develop tools for Axobase. The grant notably does not support the development and maintenance database effort explicitly, but MDIBL will continue to support these efforts until alternative funding is secured. This grant was submitted to NIH on 26th January 2022, and we are hopeful for the success of it. We would like to thank entire community, who stood behind us with Letters of Support and encouraging emails.

The R24 grant not only focuses on development of computational tools for Axobase, but if successful, it will allow us to fill the necessary gap in transgenic resources. While Dr. Graber will focus on AxoBase tools development, three of our labs (Godwin, Whited, Murawala) will focus on improving transgenesis methodology, implementing novel transgenic tools and put efforts to develop Cre-zoo. We are committed to provide early access to all resources with a mutual consensus between users and developing team. We firmly believe that there are many fundamental questions of evolution, development and tissue regeneration that can be addressed using axolotl as a model organism and early access of these tools will help us all grow as a community.

We always welcome constructive comments, so please reach out to us, if you have any comments or questions.

Advances in Safeguarding the Genetic Resources of Axolotls

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Aquatic Germplasm and Genetic Resources Center, Louisiana State University Agricultural Center

The Aquatic Germplasm and Genetic Resources Center (AGGRC) at the Louisiana State University Agricultural Center in Baton Rouge, is working to establish comprehensive repository capabilities for the *Ambystoma* Genetic Stock Center (AGSC) and two other centers: the National *Xenopus* Resource and the National Resource for *Aplysia* (NIH grant R24OD028443). These repositories will be based on standardized, reproducible pathways and protocols developed at a centralized "Hub" facility (AGGRC) in collaboration with the USDA National Animal Germplasm Program (NAGP) in Ft. Collins, Colorado. Ultimately, we seek to establish a network that integrates all five NIH-funded national resource centers for aquatic biomedical models including the Zebrafish International Resource Center (ZIRC) and the *Xiphophorus* Genetic Stock Center (XGSC).

For *Ambystoma* sperm, challenges include the biological characteristics of axolotls such as large sperm size (>400 μm), morphology (e.g., an undulating membrane along the tail), genome size (>30 gB), and packaging in spermatophores. Collaborative work between the AGGRC and the AGSC indicates that

solution osmolality affects motility and can result in damage to sperm (e.g., coiled tails; see video at 120 mOsmol kg^{-1}). Preliminary trials have shown that freezing of axolotl sperm with 15% dimethyl sulfoxide or dimethyl formamide as cryoprotectants results in recovery of undular membrane activity after thawing. Further work will determine the conditions for repeatable cryopreservation and recovery of the sperm. *Ambystoma* resources at the AGGRC such as outreach programs, community integration, training, and freezing information will be available soon in a new on-line platform for the stock centers and their user communities.

Accessing Data from the Axolotl Genome Browser

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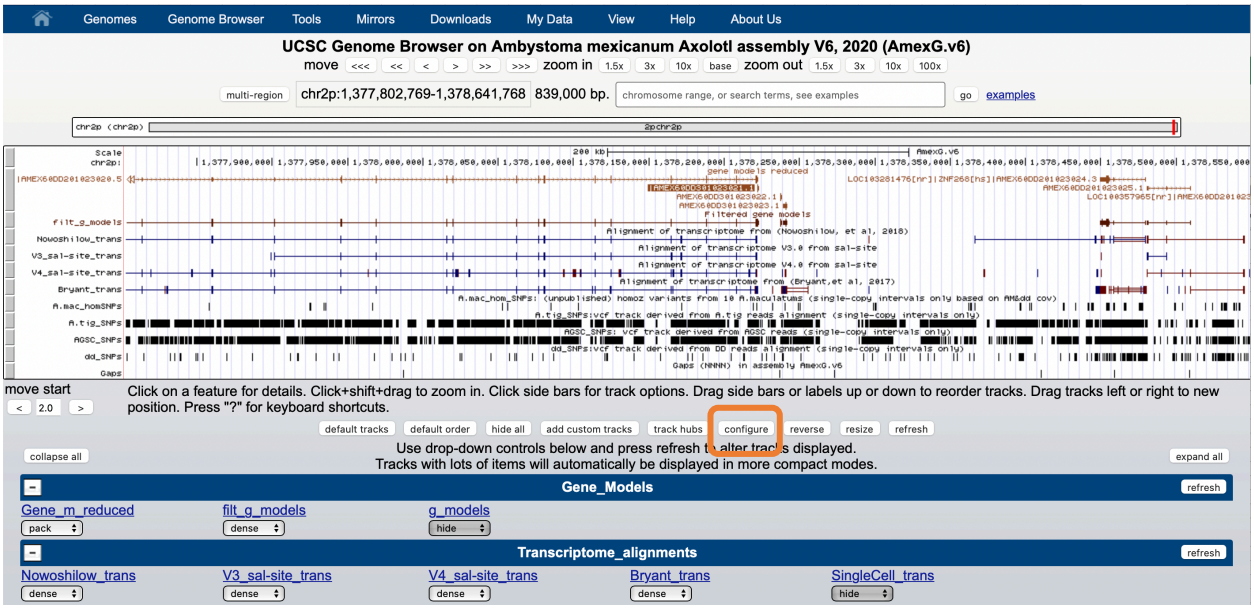
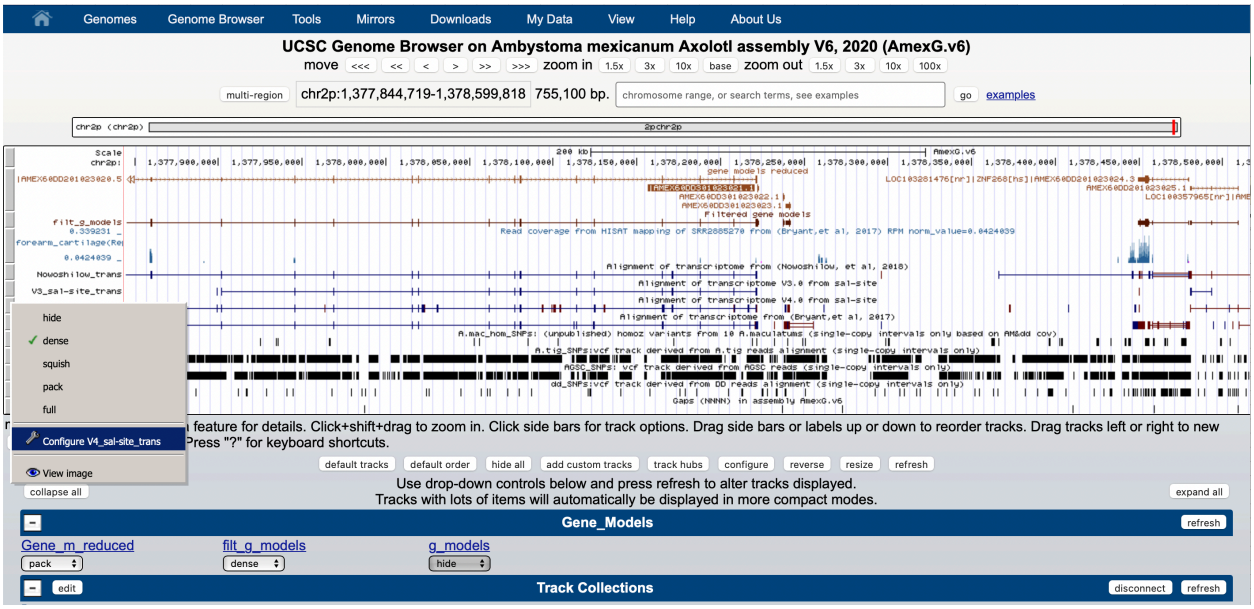
Here we describe various data tracks maintained on a UCSC Browser Assembly Hub that is hosted through ambystoma.uky.edu portal and publicly accessible from the Genome Resources page on Sal-Site or by this direct link.

The goal of the hub is to provide an abundance of genomic resources that can be analyzed visually and simultaneously. Data from the genome hub are accessed directly through the UCSC genome browser public interface, https://genome.ucsc.edu, providing immediate access to browser updates. The underlying reference sequence for the hub was constructed by adding mitochondrion sequence (NC_005797.1) to the AmexG_v6.0-DD assembly published in (Schloissnig et al., 2021) and available at (https://www.axolotl-omics.org/assemblies).

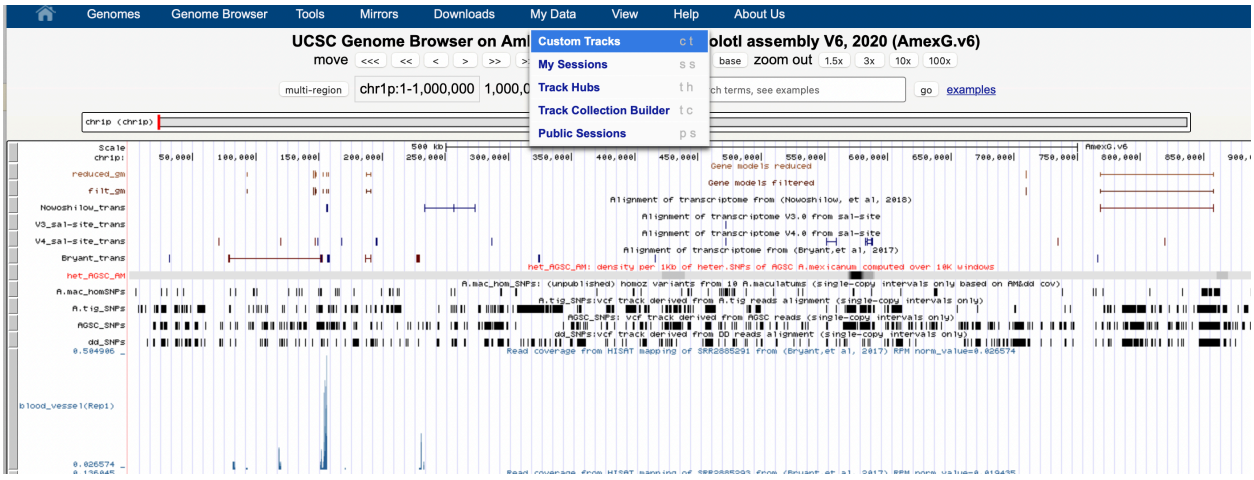
Hub tracks are combined in groups by data projects or data features. They include gene annotations, transcriptomes, variants, epigenetics studies, comparative tracks for *A mexicanum* from AGSC colony, *A tigrinum* and *A maculatum* species, and alignments of RNA-seq data from 12 various projects.
All groups and corresponding tracks can be viewed by pressing the “configure” button located under the track browser window.

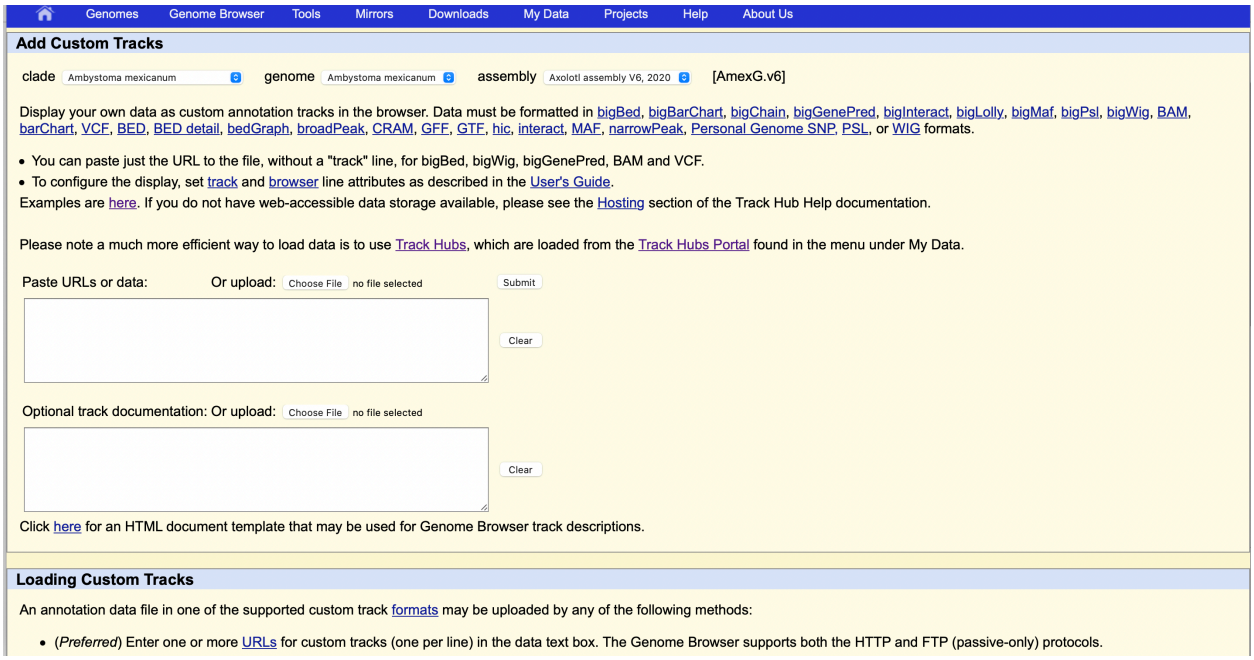


Users can visualize different tracks that are hidden by default by changing the status of a track from “hide” to “pack”, “dense” or “full” and pushing the “refresh” button on the right side of the group title. Users also can change track configuration – color, height, display, and many other options; create collections of tracks (example), and add their own custom tracks.



To add new custom data to the browser one should first generate a track file in an appropriate format. UCSC browser supports a large number of custom annotation track formats that can be uploaded directly or, in the case of binary files, referred via URL. To see all possible options, go to My Data menu, and choose Custom Tracks.

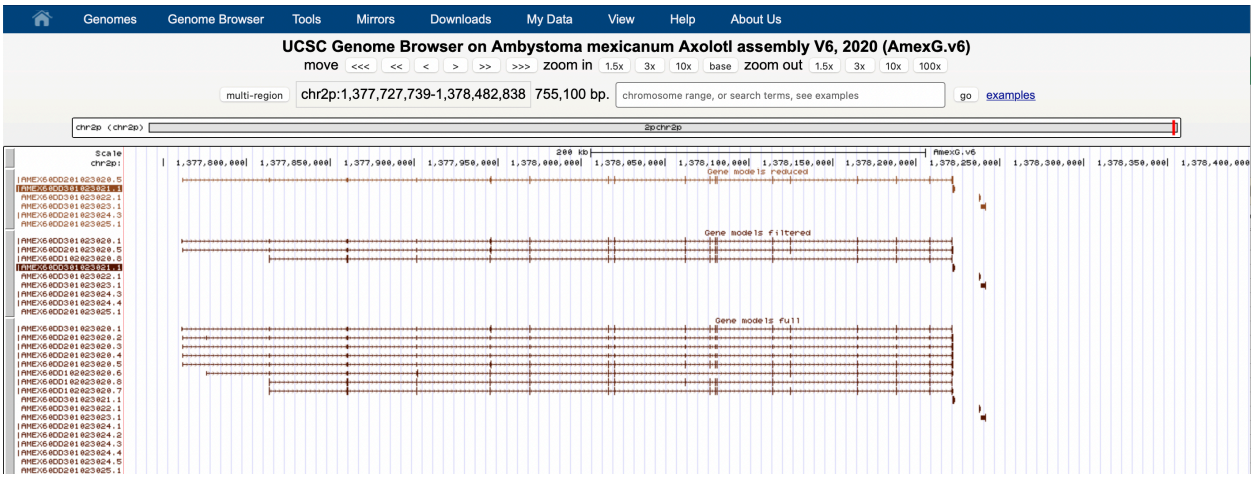




More information on how to use the USCS browser can be found on http://genome.ucsc.edu/training.

Description by groups

Gene Models group includes several gene annotation tracks that differ from each other by the level of redundancy. The **full_gm** track corresponds to the entire published annotation file AmexT_v47.FULL.gtf acquired from (https://www.axolotl-omics.org/assemblies, Schloissnig et al., 2021). The **filt_gm** and **reduced_gm** tracks present gene models that were filtered of some redundant sequences (these retain either the most abundantly expressed gene per annotation sub-version: reduced_gm, or the single highest expressed transcript per locus). Files underlying these tracks are indexed to allow search by gene name. Other filtration methods are also being implemented that provide further evidence-based assessment of gene model completeness.



Tracks in the **Transcriptomes** group present alignments of published transcriptomes to the reference genome assembly. Transcriptome sequences were aligned to the reference assembly using minimap2 with parameters --secondary=no -G 200k -ax splice.

Coverage tracks. Short read sequencing data were aligned with bowtie2-2.3.4.3 and normalized depth of coverage (mean coverage over 1 kb intervals) was calculated from primary alignments calculated with bedtools. These and most of the coverage tracks are stored in a BigWig format that can be downloaded and converted to BED format. This group includes **cov_AGSC_AM** track with coverage by *A mexicanum* reads submitted to NCBI under accession number **SRX800915** providing 20X modal coverage**; cov_A.tig** track presents coverage by *A tigrinum* reads (SRX5119016, SRX910869), 5X coverage; **cov_dd** – coverage by reads from sequencing of *A mexicanum* strain:d/d (PRJNA644663), 30X coverage; **cov_A.mac** – coverage by reads combined from the *unpublished* sequencing data of ten *A maculatum* individuals, with total coverage close to 20X.

Sequence Variants group includes tracks based on vcf files that report SNPs and small indels called in single copy intervals based on the alignments of short reads from the same studies outlined for the **Coverage tracks** group. Variants were called using bcftools and samtools mpileup with options -B -A -x -Q 0.

Tracks in **SNPs density** group provide an opportunity to assess levels of polymorphism and variation across broader intervals and are useful in identifying regions with high or low divergence in sequenced animals, such as the multi-megabase region on chr1 and albino introgression regions that vary across and within laboratory stocks (Timoshevskaya et al, 2020). The number of variants was calculated for each 10 Kb window and reported as a corresponding number of variants per 1 kilobase. Some tracks count only homozygous or heterozygous variants or variants that are observed in two species. The detailed description of each track can be found in track settings (click on the left sidebar of the track).

RNA-seq alignment tracks for numerous studies are grouped according to their NCBI project number and labeled with the corresponding SRR number and sample description. Reads were mapped to the reference using HISAT2 aligner v.2.2.0 (Kim et al., 2019). Depth of coverage metrics are normalized by reads per million as computed using bedtools v2.27.1 (Quinlan and Hall, 2010) and are converted to browser compatible BigWig format using KentUtils (Kent et al, 2002).

Several published and unpublished tracks of epigenetics data are hosted on this public hub: ATAC sequence profiles from axolotl upper forearm tissues at the homeostatic stage and seven regenerative stages from PRJNA682840; unpublished pilot CHIP-seq sequencing tracks for H3K9, H3K27 and RNA_PolII

epigenetic modifications from day zero embryo tail amputation; unpublished methylation profiles for 0, 6, 12, 24 hours post-amputation. For embargo/fair use of epigenetics and other unpublished data please refer to Sal-Site. ATAC-seq reads were aligned with HiSat2 and only unambiguously mapped reads with mapping quality > 30 were used for coverage calculation.

We plan to continue efforts to generate uniform tracks for published and pilot studies, and plan to make these tracks openly available for future browser updates or migrations. We are also happy to assist other groups seeking to generate analogous tracks for new datasets.