

Albert-Ludwigs University Freiburg
Department of Computer Science
Bioinformatics Group

Master Thesis

Predicting Hi-C contact matrices using machine learning approaches

Author:
Ralf Krauth

Examiner:
Prof. Dr. Rolf Backofen

Second Examiner:
Prof. Dr. Ralf Gilsbach

Advisors:
Anup Kumar, Joachim Wolff

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Abstract

Harhar!

Zusammenfassung

Hohoho!

Erklärung

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Bad Krozingen, den 10. März 2021

Ralf Krauth

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1 Introduction

In recent years, the three-dimensional organization of DNA has been shown to be a key factor for important processes in molecular biology. However, even with the most advanced experimental methods, it remains comparatively expensive to determine the spatial folding of DNA directly, so that current knowledge of three-dimensional DNA organization is still sketchy. In the last five years, several methods have thus been proposed to improve on this situation by determining DNA-DNA interactions *in-silico*. All of these are using existing experimental data which is easier to obtain than spatial data, but correlates with 3D chromatin structure in certain ways. However, most current *in-silico* approaches have disadvantages and shortcomings, and the current thesis attempts to improve on these.

1.1 Spatial structure of DNA

In the late 1970s, Watson and Crick discovered the now well-known double helix structure of DNA molecules [1]. However, this is not the only relevant spatial structure of genomes. At larger scales, DNA molecules can be wound around certain proteins, so-called histones, forming DNA-protein complexes named nucleosomes. Several of these can further be compacted into fibers, which in turn can be “supercoiled” into the also well-known chromosomes, fig. 1.

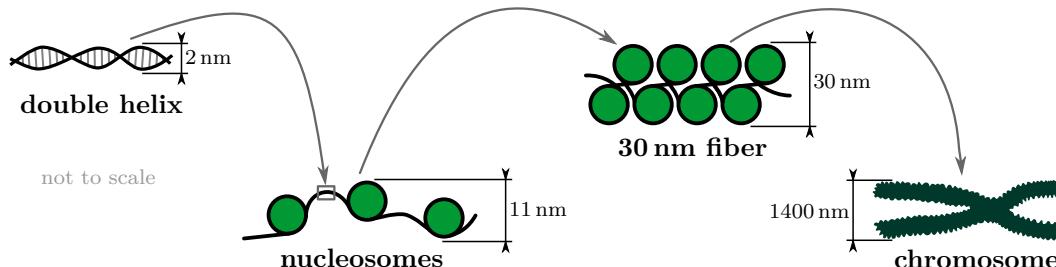


Figure 1: spatial chromatin structures (simplified, cf. [2])

While the spatial structure of chromatin outlined above brings along compaction (fig. 1 from left to right), e.g. to make chromosomes fit into eucaryotic cell nuclei, it also has other functional implications. One well known effect of spatial structure is the regulation of gene transcription by establishing or releasing contacts between gene enhancers and the relevant promoters [3, 4]. Since enhancers can be up to a million basepairs away from the promoters, the two can only interact by means of spatial DNA structure. Many effects of spatial structure are still under investigation, two recent studies have for example found dependencies between chromatin conformation and cell differentiation in *Drosophila Melanogaster* [5] and investigated spatial structure dynamics during phase transitions in murine cells [6].

While the driving mechanisms behind the formation of spatial chromatin structures are partially still under research, certain proteins like CTCF or modified histones are already well known to mediate or prevent DNA-DNA contacts [7, 8, 9].

In the last two decades, DNA-sequencing-based techniques have increasingly been utilized to capture the spatial structure of DNA experimentally. The method of choice within this thesis is called Hi-C and shall be explained in the following section.

1.2 The Hi-C process for determining spatial DNA structure

The Hi-C process is an elaborate biochemical procedure for investigating the spatial structure of DNA by detecting DNA-DNA interactions within and across chromosomes. The original Hi-C workflow has been developed by Lieberman-Aiden et al. in 2009 [10] and is depicted in simplified form in fig. 2.

The typical input (In) to Hi-C consists of several millions of cells, which are treated chemically to fix existing DNA-DNA contacts, commonly using formaldehyde, before they are lysed. Next, the DNA is extracted and cut into fragments by certain restriction enzymes (1), usually HindIII or DpnII, and the cut ends are repaired with nucleotides, some of which are marked by biotin (2). The free ends are then joined (3) under conditions which prefer ligations among open ends over ligations between different fragments. Originally, such conditions were achieved by high dilution of the fragments in solvents, but especially this part of the protocol has been replaced by more efficient methods in later works [11, 12]. The ligated fragments are then purified and cut into shorter sequences, some of which contain biotinylated nucleotides and some not (4). The fragments of interest – the ones containing biotinylated nucleotides – are then selected by pulling down biotin (5), for example using magnetic tags, and subjected to paired-end DNA-sequencing (6). In the end, the output of the Hi-C lab process is a large number of short genomic “reads”, which are subsequently processed in the bioinformatics part of the Hi-C protocol outlined in the following section.

On the software side of the protocol, the reads first need to be mapped to the corresponding reference genome. Here, only reads are kept where the “left” sequence (6)(a) uniquely maps to a different region of the reference genome than the “right” sequence (6)(b). These so-called chimeric reads are subjected to quality control, and those passing are counted as an interaction between the two genomic positions (a) and (b) to which the two ends belong. However, at reasonable read coverages, interactions cannot be counted per base pair. Instead, the reference genome is split into equally sized bins (or regions), and the reads are counted for those bins where they belong. Common bin size values $b \in \{1, 5, 10, 25, 50, 100, 1000\}$ kbp.

The final outcome of a Hi-C experiment is then a (sparse) square matrix M , henceforth referred to as “Hi-C matrix”, which records the interaction count for all possible pairs of regions in the reference genome. The individual elements $m_{i,j}$ of the matrices are counts of interactions between the bins with indices $i, j \in \{0, 1, \dots\}$, where each bin index i and j uniquely maps to a genomic region with defined start- and end position. For example, if region index i corresponded to “chr1, 25000...49999” and index j corresponded to “chr1, 250000...274999”, then a matrix entry $m_{i,j} = 22$ would mean that 22 interactions have experimentally been measured between the two

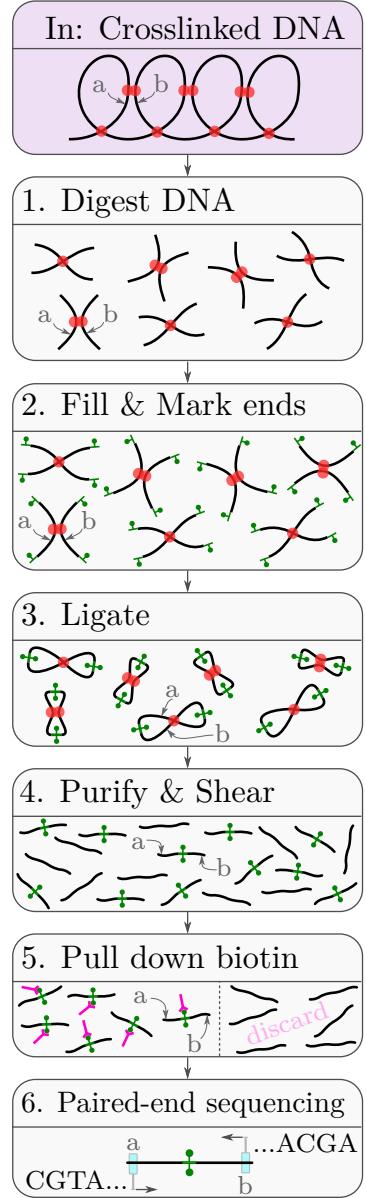


Figure 2: Hi-C lab process

mentioned genomic positions. Because interactions do not have a “direction”, Hi-C matrices are always symmetric and it thus holds that $m_{i,j} = m_{j,i}$.

In the bioinformatics part of the Hi-C protocol, often just a small fraction of all reads fulfill the selection criteria outlined above, for example due to reads not being chimeric or uniquely mappable. This makes Hi-C a comparatively inefficient, slow and thus expensive process. For example, the well-known dataset by Rao et al. [11] with matrix bin sizes down to 1 kbp, required several *billions* of reads being made.

Parts of fig. 2 and the process description above have been adapted from the preceding study project [13].

1.3 The ChIP-seq process for determining protein binding sites

The ChIP-seq process is a combination of Chromatin-Immuno-precipitation (ChIP) and DNA-sequencing, designed for investigating DNA-protein interactions [14, 15]. As with Hi-C, the input consists of a sufficient number of cells, which are first treated with formaldehyde to fix present proteins to DNA, fig. 3 (In). The cells are then lysed and the DNA-protein structure is extracted and cut into fragments, usually by sonication (1). Next, specific antibodies are added, designed to bind only to a certain protein of interest (2). These antibodies are additionally equipped with a tag, for example a magnetic one, so that the DNA-protein-antibody structures can be precipitated, while fragments without antibodies are discarded (3). The proteins and antibodies are then removed (4), the DNA is purified and finally sequenced (5). Typically, a control experiment is performed together with the ChIP-seq process, which comprises all steps described above, except the immunoprecipitation (2),(3).

The outcome of the ChIP-seq lab process is again a bunch of short genomic sequences, which are then fed into the bioinformatics part of the pipeline.

On the software side of the process, the reads are filtered for quality and mapped to an appropriate reference genome. The number of mapped reads per genomic position can then be simply be counted. It is common to process reads from the control experiment in the same way as reads from the ChIP-seq experiment and then use special software to call “peaks”, i. e. protein binding sites, at those genomic positions where the read count from ChIP-seq is statistically significant compared to the read count from the control group.

For the thesis at hand, the aligned reads from the ChIP-seq experiment have been used directly, because peak data was found to be too sparse for the machine learning approach used in the study project [13].

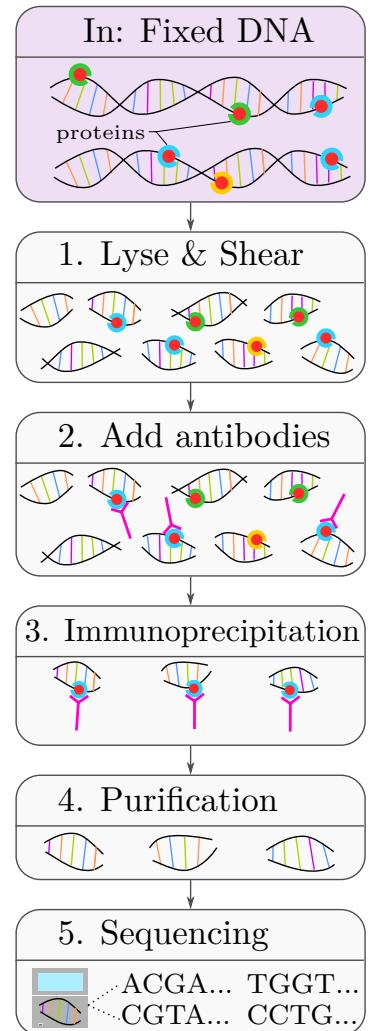


Figure 3: ChIP-seq lab process

Parts of fig. 3 and the process description above have been adapted from the study project [13].

1.4 Motivation and goal of the thesis

Due to the high effort, Hi-C experiments have been conducted only by few labs and for comparatively few organisms and cell lines so far. For example, as of January 2021, Hi-C assays are available for just 26 of more than 150 human cell lines listed in the Encyclopedia of DNA elements [16, 17], and these have been produced by only five university labs.

However, investigations and experiments with the available Hi-C data have shown correlations between the spatial DNA structure and the binding sites of certain transcription factors and histone modifications [11, 18]. Since these binding sites can be detected with the less costly ChIP-seq method, a computational method able to predict contact matrices from DNA-protein bindings would be very helpful.

The goal of this master thesis is thus to predict missing Hi-C data from existing ChIP-seq data, making use of the known correlations between the binding sites of transcription factors and histone modifications of the one hand and Hi-C interaction counts on the other hand. Because this is a wide field and lead time for a master thesis is limited, the present work will focus on machine learning techniques, which have proven a good choice for exploiting complex patterns and relationships, especially when it comes to the synthesis of 2D data from various kinds of input data [19]. More specifically, the goal of this thesis is to develop an easy-to-use machine learning approach for predicting Hi-C interaction matrices from ChIP-seq data, using standard in- and output formats with minimal pre- and postprocessing.

To underscore the usefulness of such an approach, table 1 to the side shows the first 16 human cell lines in ENCODE, sorted by total number of selected assays available. Although not necessarily all available experiments to date are included in ENCODE – for example, the experiments by Dixon et al. [9] for H1 cells are not listed – it is obvious that the public availability of ChIP-seq data is commonly much better than the one of Hi-C data, even for otherwise well investigated cell lines like HEK293 and MCF-7.

cell line	TF ChIP-seq	Histone ChIP-seq	Dnase-seq	Hi-C
K562	680	20	10	1
HepG2	668	15	3	1
A549	251	87	14	6
HEK293	231	6		
GM12878	211	15	3	7
MCF-7	155	18	8	
H1	88	55	3	
HeLa-S3	77	15	4	1
SK-N-SH	62	21	2	
IMR-90	16	34	2	2
HCT116	27	17	1	8
H9		35	7	
GM23338	15	13	1	
Ishikawa	25		4	
HEK293T	17		1	
GM23248	2	13	1	1

Table 1: availability of selected assays in ENCODE (extract)

2 Related work

In the last five years, several approaches have been presented to determine DNA-DNA interactions *in silico*, using existing data from various experiments. Section 2.1 gives an overview about these methods. Furthermore, some methods originally developed for image synthesis and similar tasks in computer vision might also be useful in the field of Hi-C matrix generation and are thus summarized in subsection 2.2. The section is then concluded by a short discussion of the existing work.

2.1 Methods for predicting DNA-DNA interactions and contact matrices

As of 2020, there is quite a body of existing work in the field of predicting DNA-DNA interactions, using various approaches and different types of input data.

Two conceptually similar methods have been proposed by Brackley et al. in 2016 and MacPherson et al. in 2018 [20, 21]. In both approaches, DNA is modeled as a “beads-on-a-string” polymer, and simulation techniques are employed to find energy-optimal spatial structures of these polymers. Apart from constraints derived from the molecule’s DNA sequence itself, the models also consider spatial contact constraints derived from ChIP-seq experiments of chromatin factors which are known to mediate such DNA-DNA contacts. The interaction matrices derived from the simulations look interesting, but the paper from Brackley et al. [20] is unfortunately lacking a comparison with “true” experimentally measured Hi-C matrices, and the results from MacPherson et al. [21] seem inferior to most other ones presented in this section.

Another simulation-based method has been developed by di Pierro et al. in 2017 [22] and later extended by Qi and Zhang [23]. In both cases, a convolutional neural network (CNN) is trained to learn different “open” and “closed” chromatin states from 11 chromatin factors, and the predicted chromatin states are then taken as constraints for beads-on-a-string models. The difference between [22] and [23] lies mainly in the number of states considered and the simulation methods applied; the results are mathematically convincing in both cases.

A further approach using chromatin states is due to Farrè and Emberly [24]. Here, the conditional probability of two genomic regions being in contact, given their distance and the chromatin state around them, is estimated using Bayes’ rule. In this case, the chromatin state – reduced to active or inactive – is derived from DNA adenine methyltransferase identification (DamID) signals of 53 chromatin factors using probabilistic methods [25]. The conditional probabilities on the right side of Bayes’ rule are either computed from training data or estimated with different probabilistic approaches, too. While the predicted contact matrices do not look like real Hi-C matrices with this approach, highly interacting regions are still often well identifiable.

Three further approaches in the field make use of random forests. 3DEpiLoop by Bkhetan and Plewczynski and Lollipop by Kai et al., both from 2018, use a random forest classifier to predict DNA loops, but differ in input data and preprocessing [26, 27]. While 3DEpiLoop is using only ChIP-seq data of histone modifications and transcription factors [26], Lollipop additionally takes ChIA-PET-, RNA-seq- and DNase-seq-data, CTCF motif orientation and loop length as inputs [27]. Both approaches show good coincidence of predicted loops with experimental data, but their output is binary and rather sparse. Contrary to these two, the third random-forest-based approach, HiC-Reg by Zhang et al. from 2019, allows predicting real-valued Hi-C interaction

matrices directly [28]. To this end, it employs random forest regression to predict interactions between two genomic “windows”, using ChIP-seq data of 13 chromatin factors and the genomic distance of the two windows. The published results for five human cell lines look interesting.

Another recent method which investigated decision-tree-based algorithms is due to Martens et al. (2020) [29]. Here, gradient boosted decision trees, logistic regression and neural networks were used to predict highly interacting chromatin regions and TAD domain boundaries from histone modifications and CTCF ChIP-seq data. However, in this setup, the neural network approach yielded the best, overall acceptable results of the three approaches, but again in form of a binary classifications. A neural-network approach with comparable input data, but without the restriction to binary classifications has been presented by Farrè et al. in 2018 [30]. Here, a one-dimensional convolutional filter is used to convert ChIP-seq data from 50 chromatin factors into a one-dimensional chromatin vector, which is then processed by a dense neural network (DNN). This allows predicting real-valued Hi-C interaction matrices, which resemble the general structure of experimentally derived matrices quite well.

While the approaches discussed so far have either modeled DNA as a “beads-on-a-string” polymer or not used it explicitly at all, there are also several machine-learning approaches which directly consider DNA sequence without a need for polymer modeling. In 2019, Singh et al. presented SPEID [31], an approach to predict promoter-enhancer interactions from DNA sequence, using a combination of CNNs, a recurrent network (LSTM) and a DNN. The results match well with experimental data, but are limited to promoter and enhancer loci by design, disallowing predictions of complete Hi-C contact matrices. Other researchers have tried to design similar methods without such limitations. The work by Peng from 2017 [32] is an extension of SPEID, based on a 2016 preprint [33], additionally taking into account a “middle sequence” between enhancer- and promoter sequences, CTCF motif counts within the sequences and genomic distance between two sequence snippets. However, the network lacks generalization, i. e. the results are only good in training regions [32, figs. 4, 5]. A conceptually similar method to the one by Peng [32], but with a different neural network design has been presented by Schreiber et al., also in 2017, named Rambutan [34]. It accepts DNA-sequence, DNase-seq data and distance between two genomic loci as inputs and then uses a combination of CNNs and a DNN to predict whether the given two loci interact or not. Unfortunately, it is difficult to decide whether the results of Schreiber et al. are useful for the task at hand, since the evaluation is done only by statistical means and no actual Hi-C matrices have been published. The original paper [34] also contains a known error and seems not to have appeared in a peer-reviewed journal in improved form yet. A probably more promising method working on DNA sequence, Akita, has been published by Fudenberg et al. in 2020 [35]. It is based on two rather involved convolutional neural networks. While the first one, “trunk”, processes one-dimensional, one-hot encoded DNA sequence input through convolutional filters, the second one, “head”, converts one-dimensional representations to 2D, further processes the data with convolutional filters and enforces symmetry. Although Fudenberg et al. initially seemed to focus on determining the influence of DNA modifications on spatial structure [36], predicting complete Hi-C matrices is an integral part of their work, and a large number of images of Hi-C matrices from the test set has been published alongside the article. The predicted matrices often hardly look like experimental Hi-C matrices, but mostly still indicate highly interacting regions quite well.

A further method by Schwessinger et al. [37] also makes use of DNA sequence and additional epigenetic data for its predictions, but is conceptually different from the ones presented so far.

Here, ChIP-seq tracks are initially used to train a CNN on the relationship between sequence and the corresponding chromatin factors. The weights of this first network are then used to seed another convolutional neural network, which is responsible for predicting DNA-DNA interactions from DNA sequence. In this case, the results were blurry, but generally in good accordance with experimental Hi-C data.

Yet another machine-learning concept based on sequence data, Samarth, has been proposed by Nikumbh and Pfeifer in 2017 [38]. Here, a support vector machine is trained on 5C data, using a specific representation for DNA sequence called oligomer distance histograms. The results showed acceptable consensus with experimental Hi-C data and allowed some interesting conclusions about the importance of certain k-mers for DNA folding. However, the applicability for the task at hand is hard to assess, because none of the predicted matrices used for statistical evaluation has been published alongside the paper.

2.2 Image synthesis techniques from computer vision

With the advent of deep learning, both the number of opportunities and the demand for using machine learning techniques in image synthesis tasks have risen, as recently summarized by Tsirikoglou et al. [19]. Since Hi-C contact matrices can be seen as square grayscale images, such techniques can also be relevant for this thesis.

Probably one of the first applications of computer vision methods for Hi-C matrices was presented by Liu and Wang in 2019 [39]. Here, established image super-resolution networks – mostly deep convolutional neural networks – have been modified to enhance low-resolution Hi-C matrices.

Another technique from computer vision that has been transferred to Hi-C matrices are conditional generative adversarial networks (GANs), invented by Goodfellow, Mirza and colleagues in 2014 [40, 41]. Again, several works have employed this comparatively new and involved method to increase the resolution of given Hi-C matrices, including the ones by Liu and colleagues [42], Hong et al. [43] and Dimmick et al. [44]. In general, all three works feature the typical GAN setup with two competitive networks – a generator, which is trained to produce realistic high-resolution Hi-C matrices from its low-resolution inputs, and a discriminator, which tries to distinguish real Hi-C matrices from generated ones and thus serves as a “critique”, an additional loss function, for the generator. While the convolutional building blocks for the discriminators and the residual building blocks for the generators are conceptually similar in all cases, the three works differ in the number of building blocks used and the activation functions applied within the blocks. Furthermore, Dimmick et al. and Hong et al. include additional loss functions beyond standard generator- and adversarial losses. This includes perceptual losses derived from other (pre-trained) neural networks to obtain “visually good” Hi-C matrices and total variation loss to suppress noise while maintaining edges. The method by Dimmick et al. outperformed the others for most test cases, but it is also the most elaborated.

2.3 Discussion of existing work

Independent of chosen techniques, several of the methods shown above only allow predictions restricted to certain loci (e.g. promoters and enhancers) or yield binary predictions in the sense

2 Related work

of “interaction” or “no interaction” between certain loci [26, 27, 29, 31]. These methods are thus not directly suitable for the task at hand, but would require further development.

The chromatin-modeling based approaches [20, 21, 22, 23] allow indirect derivation of Hi-C matrices by performing sufficient simulation passes and estimating contacts from the resulting model ensembles. Depending on the chromatin model choice – which seems not straightforward [45, 46] – and the number of constraints involved, the required computations can be expensive. However, the results still seem slightly inferior compared to other methods mentioned in section 2.1, maybe because not all constraints for chromatin modeling are known or cannot be considered in the models yet.

Three of the DNA-sequence based methods mentioned above also allow direct prediction of Hi-C matrices [34, 35, 37]. At first look, it is surprising that learning spatial structure from DNA-sequence actually works, because there seems no obvious correlation between sequence and structure – instead, 3D conformation can vary considerably for different cell lines of the same organism, which all share the same DNA. On the other hand, the chosen artificial networks might be able to figure out binding sites of relevant proteins from DNA sequence, which *do* have a correlation with spatial structure. This is especially true for the method by Schwessinger [37], where the network is seeded by training on exactly such binding sites. While the methods by Schreiber and Schwessinger use secondary inputs and therefore can – at least partially – adapt to cell lines sharing the same DNA, the method by Fudenberg focuses on DNA and is thus expected to produce the same outputs for all cell lines of a given organism. All three methods require comparatively deep networks which are expensive to train. The sequence-based method by Nikumbh et al. [38] is using a support vector machine, which is generally easier to train, but the adaptability to different cell lines is expected to remain problematic due to the chosen concept.

The random-forest-based method by Zhang et al. [28] is directly targeted at the goal of this thesis, since it directly predicts Hi-C matrices, is not limited to certain loci and can adapt to different cell lines using corresponding ChIP-seq data. However, this approach has extensively been investigated in two previous study projects at the university of Freiburg, and the results could not be reproduced for unknown reasons [13, 47].

The dense neural network approach by Farrè et al. [30] is also compatible with the goals of this thesis. The published results are visually and statistically convincing, and both the presented network and the training process offer opportunities for amendments, which will be explored in section 3.1.

Since all of the image synthesis methods presented above in section 2.2 require existing Hi-C matrices for training *and* prediction, none of them is directly suitable for the task at hand. However, some of the concepts can still be used to develop novel approaches, which will be discussed in section 3.2.

3 Advancing predictions of Hi-C interaction matrices

In the following subsections, two conceptually different approaches towards the goal of the thesis, predicting Hi-C matrices from ChIP-seq data, will be explored. While the first approach is a dense neural network based on work by Farré et al. [30], the second is a novel method based on conditional generative adversarial networks.

3.1 Dense Neural Network approach

In their 2008 paper [30], Pau Farré, Alexandre Heurtau, Olivier Cuvier and Eldon Embery propose a combination of a 1D convolutional filter with a three-layer dense neural network which already fulfills most goals of this thesis with some exceptions regarding data formats and preprocessing. This thesis tries to build on the success of their method by extending the comparatively simple neural network in various ways, modifying the binsizes of the Hi-C matrices and changing the learning process. As a start, the basic network has been rebuilt and used on well-known data from human cell lines GM12878 and K562, cf. section 4.1.

3.1.1 Basic network setup

The basic network setup taken over from Farré et al. [30] is shown in simplified form in fig. 4, see section 4.4.1 for technical details.

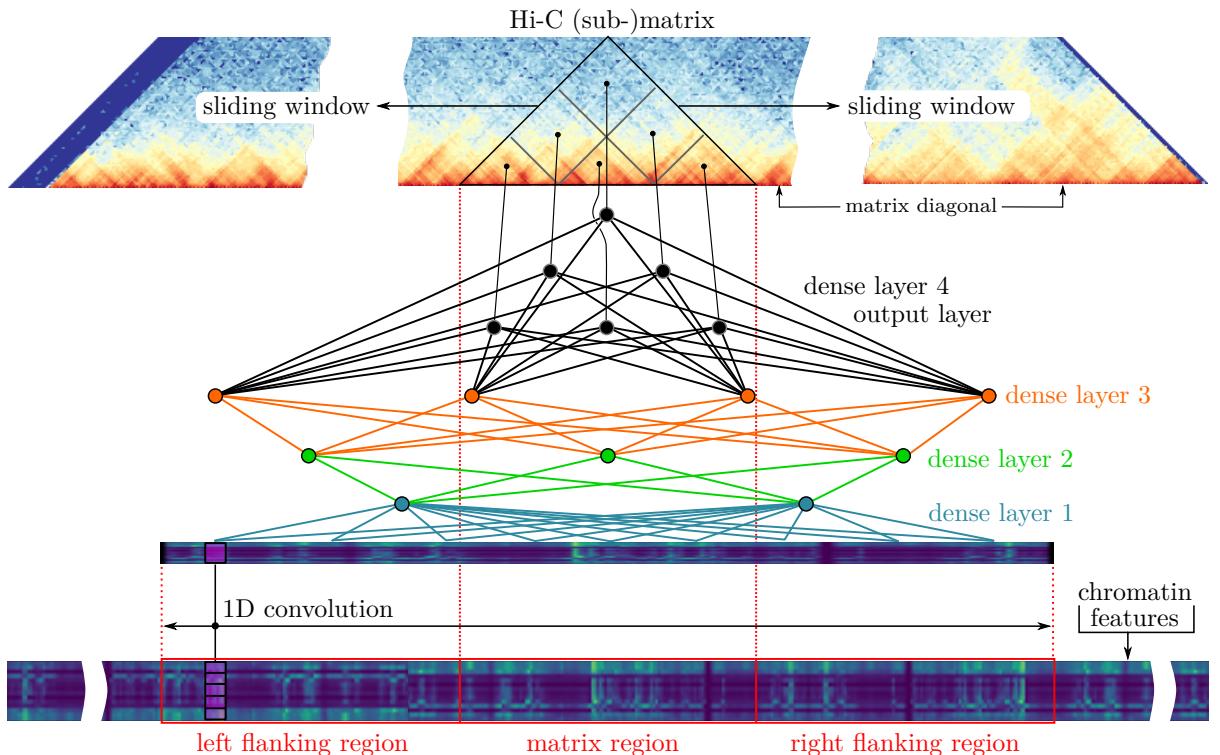


Figure 4: Principle of basic dense neural network

Since the network implements a supervised learning technique, it requires two kinds of inputs for training – ChIP-seq data of the chosen chromatin factors and target Hi-C matrices for each training chromosome. However, it is generally infeasible to learn Hi-C matrices for complete chromosomes at once with machine learning approaches, because the latter heavily depend on availability of training data. The target matrices are thus taken as submatrices of size $w \times w$ with fixed windowsize w , centered at the diagonal of the original Hi-C matrices, fig. 4 top. The ChIP-seq data of n chromatin features is taken as $3w \times n$ subarray of the original array, whereby the middle w bins are aligned with the position of the submatrix, the first w bins correspond to the left flanking region and the last w bins correspond to the right flanking region of the current submatrix region, fig. 4 bottom. Training samples are then obtained from the input data by sliding the input windows along the diagonal of the target Hi-C matrix. For the technical details of the sample generation process, see section 4.1.3.

Within the network, a 1D convolutional filter compresses the $3w \times n$ input arrays to 1D vectors of size $3w \times 1$, and four dense layers further process the compressed input, fig. 4 middle. The number of neurons in the last dense layer corresponds to the number of bins in the upper triangular part of the target submatrix, i.e. it consists of $(w \cdot (w + 1))/2$ neurons, fig. 4 top, exploiting the symmetry of Hi-C matrices. For implementation details, please refer to section 4.4.1.

Training of the network is performed by minimizing the mean squared error of the predicted matrix versus the target Hi-C matrix using stochastic gradient descent, cf. section 4.4.1.

Farré et al. propose a windowsize of $w = 80$ at $b_{feat} = b_{mat} = 10$ kbp. However, larger binsizes of $b_{feat} = b_{mat} = 25$ kbp were found beneficial for most of the data used throughout this master thesis. Additionally, larger binsizes allow for a higher coverage of the target matrix at the same windowsize, because the windowsize is specified in bins, and obviously $10w < 25w$. Results for both binsize 10 and 25 kbp are shown in section 5.1.1.

The network layout shown above is quite simple, and immediately offers some opportunities for expansion, partially already proposed in the original paper [30]. These will be explored below.

3.1.2 Modifying the convolutional part of the network

One starting point for modifying the neural network is its convolutional part.

With only a single 1D convolutional filter in one layer, the network might have difficulties capturing complex relationships between Hi-C interaction counts and more than one of the chromatin features. For this reason, an extended “longer” network was created, comprising three 1D convolutional filter layers with 16, 8 and 4 filters, respectively, replacing the single 1D-convolution in fig. 4, lower left, cf. section 4.4.2. This is still a comparatively low number of layers and filters, but the choice seemed justified in order to avoid overfitting to the low-dimensional input. The results are shown in section 5.1.2, especially figures 27 and 28.

Next, a “wider” network was created, featuring the same setup as the basic network except the width of the filter kernel, which was set to 4 instead of 1. The idea here was to allow the network to capture correlations between Hi-C interaction counts and chromatin features which span more than one bin. The actual number has been kept low, since at binsize $b = 25$ kbp, 4 bins already correspond to 100 kbp. However, the results were not as expected, cf. section 5.1.2, especially figures 25 and 26. Of course, increasing filter width and using more filters can also be

combined, hopefully allowing the “wider-longer” network to capture both correlations spanning more than one bin and more than one chromatin feature. The results for this combined approach are shown in section 5.1.2, especially figures 29 and 26.

Another approach to potentially improve the predictions that goes somewhat into the direction of the “wider” network has been proposed, but not implemented by Farré et al. in their paper [30]. ChIP-seq experiments can usually be binned at smaller binsizes than Hi-C data due to the nature of the process. This can be exploited to capture finer details in the ChIP-seq data without a need for higher (training-)matrix resolutions. To this end, the initial network can be generalized by binning the ChIP-seq data at k times the bin size of the matrices, whereby $k \in \mathbb{N}^{\geq 1}$, cf. section 4.1.5 for the technical details. This yields an input data size of $k \cdot 3w \times n$, which is then again compressed to a $3w \times 1$ vector by a 1D convolutional filter with kernel size n and strides k . For practical reasons, $k = 5$ was chosen for the thesis at hand, and the results for binsizes $b_{mat} = 25$ kbp, $b_{feat} = 5$ kbp are shown in section 5.1.2, especially figures 31 and 32.

3.1.3 Using a combination of MSE-, TV- and perceptual loss

In image regression tasks, optimizing for mean squared error is known to produce blurry images, because it is computed independently for each image pixel, ignoring spatial proximity [48, 49]. And indeed, both the predictions from the initial and the extended network according to sections 3.1.1 and 3.1.2 suffered from blurriness. To improve on this, investigations with modified loss functions were made.

It has been shown that loss functions based on the (multiscale-)structural similarity index (SSIM) [50], can outperform mean squared error (L2) and mean absolute error (L1) in image regression tasks. While Zhao et al. used a combination of L1- and multiscale SSIM loss [51], Lu proposed a custom level-weighted SSIM loss [49]. The results were better than with L1- or L2 loss alone, but sometimes not much – depending on the machine learning model in use.

Another type of loss function used to produce sharp images is the so-called perceptual- or perception loss. The idea here is not to compute L1- or L2 loss directly from the output of the network to be trained, but instead use a pre-trained loss network to determine structures in “predicted” and “real” images and then compute e.g. L1- or L2 loss on these structures, fig. 5.

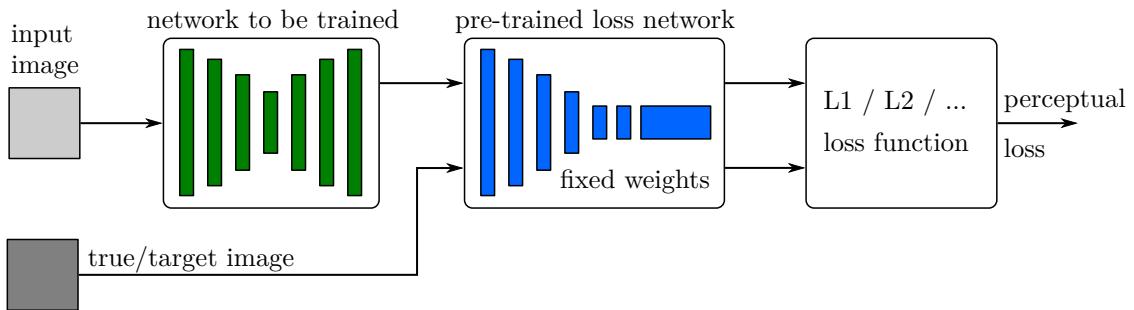


Figure 5: perceptual loss

The optimization of the target network’s trainable weights can be performed as usual, for example with gradient descent and backpropagation, simply keeping the weights of the loss networks constant. Often, complex image classification networks like VGG-16 [52] are taken as loss network, e.g. in the well-known style-transfer network by Johnson et al. [53], because these are known to be good at detecting relevant structures in images.

To check whether the given learning task benefits from using a perceptual loss, a custom combined loss function was generated, consisting of mean squared error L_{MSE} between true- and predicted matrices, perceptual loss L_{VGG} based on VGG-16 and total variation loss L_{TV} to reduce noise in the output while preserving edges [54]. This choice was inspired by the custom loss function used by Hong et al. in their successful Hi-C super-resolution network DeepHiC [43], which is otherwise not similar to the network used here. In short, the combined loss function L is shown in eq. (1). Here, the λ are individual loss weights, see section 4.4.3 for details.

$$L_{combined} = \lambda_{MSE} L_{MSE} + \lambda_{VGG} L_{VGG} + \lambda_{TV} L_{TV} \quad (1)$$

Unfortunately, there is no straightforward way for determining the optimal parameters λ , and an exhaustive parameter search was infeasible due to the computation time requirements of about 4:30 min per epoch. Therefore, only few runs were conducted with different sets of parameters, and the results for $\lambda_{MSE} = 0.8999$, $\lambda_{VGG} = 0.1$, $\lambda_{TV} = 0.0001$, which should not be considered optimal, are shown in section 5.1.3.

3.1.4 Using a TAD-based loss function

Looking at the results obtained from the networks so far, see XXX, it seemed that highly interacting regions, especially topologically interacting domains (TADs), were not well predicted and either absent in the matrix plots or blurred. Assuming availability of a TAD scoring function $tad(z_{pred})$, where z_{pred} is a predicted submatrix, this might be improved by directly optimizing a loss function as shown in eq. (2).

$$L_{combined} = \lambda_{MSE} L_{MSE} + \lambda_{TAD} (tad(z_{true}) - tad(z_{pred}))^2 \quad (2)$$

However, this approach suffers from several restrictions. First and foremost, there seems no consensus on the exact definition of TADs, and no less than 22 algorithms for TAD detection existed as of 2018 [55, 56]. Additionally, many of these algorithms have several tuning parameters, are notoriously parameter-dependent and may not even yield any results if parametrized in an unfavorable way [56]. A further restriction results from the context – since the loss function needs to be optimized, one needs to be able to compute gradients of it with respect to the network’s weights. Due to their complexity, this is generally very difficult to implement in a computationally efficient way for all known TAD calling algorithms.

To overcome the limitations, a novel loss function based on TAD scores [57] was developed. Figure 6 exemplarily shows its basic idea for a 16×16 submatrix with windowsize 4. First, the mean is taken from diamond-shaped (or rhombus-shaped) matrix cutouts along the diagonal, fig. 6 (A, C), and stored in a score vector, fig. 6 (D / E). The size of the diamonds, 2 in the figure, is configurable, but a reasonable balance with the submatrix size, i.e. the size of the sliding window w , and the expected size of TADs in the matrix must be maintained. Next, loss is

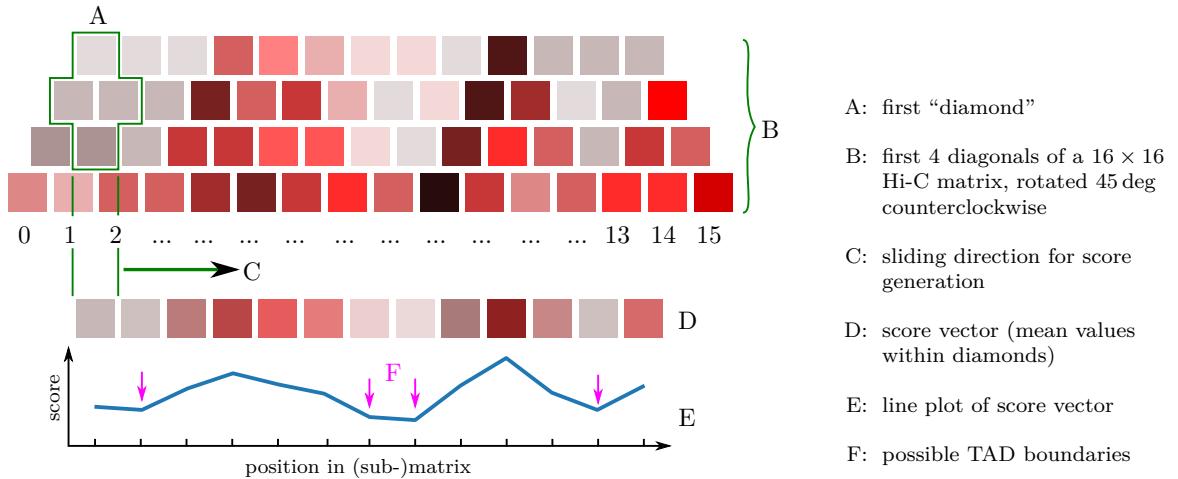


Figure 6: score vector generation for TAD-based loss

computed by taking the mean squared error between the score vectors of the “real” submatrices and the “predicted” submatrices, eq. (2).

The idea behind the score-based approach is visualized in fig. 6 (E). Local minima, i. e. dents in the line plot of the score values, fig. 6 (F), often correspond with highly interacting regions in the matrix, since the mean of diamonds from *inside* TADs is normally significantly higher than the mean of diamonds *outside* TADs. Indeed, some TAD calling algorithms like TopDom [58] and hicFindTADs [59, W12f.] do compute insulation scores – usually for more than one diamond size – and then use diverse techniques to detect meaningful local minima in the score values. However, finding meaningful local minima in the given context is still computationally involved, so it was left to the network to make sense out of the score vectors. This way, the loss function was well defined, efficiently computable and tensorflow standard functionality could be used to compute gradients with respect to weights.

For the thesis at hand, windowsize $w = 80$ and diamond size $ds = 12$ were used at binsizes of 25 kbp. For technical details please refer to section 4.4.4.

3.1.5 Modifying binsize and windowsize

In the results presented so far, larger structures were often absent. In order to improve on this, two approaches were tried.

First, predictions were made at binsizes $b_{feat} = b_{mat} = 50$ kbp, keeping the windowsize at 80 bins, which then corresponded to 4 Mbp. The idea here was to capture predominantly larger structures further away from the diagonal and then investigate various methods to combine predictions at smaller and larger binsizes. However, such a merging process was never actually developed, since the predictions at 50 kbp alone were not good enough, section 5.1.5.

Unfortunately, doubling the (matrix-)binsizes from 25 to 50 kbp directly leads to a reduction in the number of available training samples by a factor of about two, if the windowsizes are kept constant at 80 bins, see table 3. This might also be one of the causes why predictions at

50 kbp proved useless. For this reason, a second approach was made – using training samples at $b_{feat} = b_{mat} = 25$ kbp, $w = 80$ and $b_{feat} = b_{mat} = 50$ kbp, $w = 80$ at the same time. This is easily possible, since the neural network topology only depends on the windowsize *in bins* and the relation $k = b_{mat}/b_{feat}$ between the binsizes of features and matrices. This approach obviously increases the number of training samples, with the idea of allowing the network itself to figure out how to combine predictions at smaller and larger binsizes.

- use trapezoids, i.e. capped larger submatrices and flankingsize smaller than windowsize
- rationale: larger windowsize without increasing training time too much

3.1.6 DNA sequence as an additional network input branch

- use DNA as an additional input
- rationale a): allow the network to figure out true binding sites in conjunction with cs data
- rationale b): given the success of pure DNA based methods, allow the network to find yet unknown sequence structure correlations
- probably not the most important subsection, leave it out in case of time problems

3.2 Hi-cGAN approach

Because none of the results from the dense neural network approach presented in section 5.1 were overly convincing, a second, widely unrelated approach was made.

Since their invention by Goodfellow, Mirza and colleagues [40, 41], Generative Adversarial Networks have become increasingly popular for image processing tasks of many kinds, and especially for image synthesis [60]. Among their strengths is image synthesis from textual descriptions [61, 62, 63, 64] – and from an abstract point of view, this task is not very different from the goal of the thesis at hand. Considering the chromatin features on the input side as a “description” of how the target Hi-C (sub-)matrices should look like, formulating the goal of the thesis as a cGAN-problem should be possible. In the following sections, such an approach will be explored.

3.2.1 General setup of the cGAN approach

Although numerous variants exist, conditional GANs generally comprise at least two neural networks – a generator $G(x, z)$, which tries to generate realistic outputs from its inputs x and random noise z , and a discriminator $D(x, y)$, which tries to discern true inputs y , e.g. experimentally derived Hi-C matrices, from generated inputs $G(x, z)$. The optimal weights for the networks can then be found by searching an equilibrium in a two-player minimax game: The generator tries to fool the discriminator, while the discriminator tries to detect the generator’s fakes, see equations 3 and 4 [48].

$$L_{cGAN}(G, D) = \mathbb{E}_{x,y}[\log D(x, y)] + \mathbb{E}_{x,z}[\log(1 - D(x, G(x, z)))] \quad (3)$$

$$G^* = \arg \min_G \max_D L_{cGAN}(G, D) \quad (4)$$

Many different layouts and training processes for the Generator and Discriminator networks have been proposed. For the thesis at hand, the well-known “pix2pix” proposal by Isola et al. from 2017 was followed [48], amended by a convolutional embedding network for the chromatin features, which serve as the conditional input x here. The overall setup is shown in fig. 7 and will be explained in more detail below. The generator architecture is based the well known U-Net

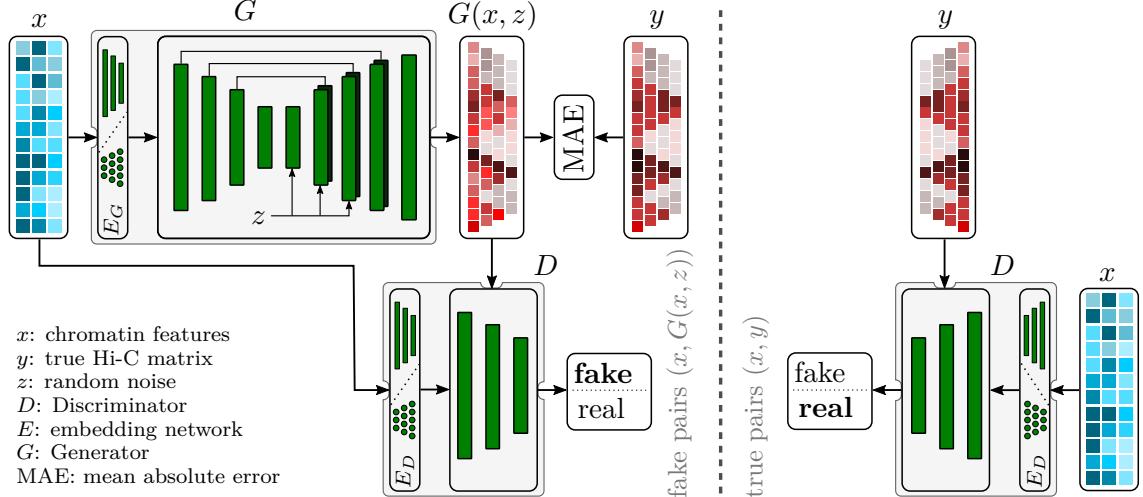


Figure 7: cGAN approach

architecture due to Ronneberger, Fischer and Brox [65], while the discriminator is implemented as a patchGAN-discriminator developed by Isola et al. especially for pix2pix [48]. This type of discriminator splits the images into patches and tries to decide which of them are real and which are fake, using convolutions. Note that pix2pix does not explicitly add noise z , but by design only relies on dropout layers for that purpose.

For the thesis at hand, few modifications were made to the actual pix2pix network. Since Hi-C matrices are symmetric by definition, symmetry of the outputs was enforced by adding additional layers to the network at the appropriate places, see section 4.5.1 for details. Furthermore, some layers in the generator and discriminator were made optional to allow processing smaller images of sizes 64×64 and 128×128 aside the 256×256 -images of the original implementation, again refer to section 4.5.1 for details.

For sample generation, we relied on the same method as described for the dense neural network described above with a few minor adaptations, see section 4.1.4. However, since pix2pix operates on images, as its name already implies, a method to add the essentially one-dimensional chromatin feature data as conditional input images into the network was needed. Unfortunately, this task seems to be without examples in literature, so two different approaches were tried, which will be discussed below in sections 3.2.2 and 3.2.2. Both approaches are distantly related to the text-encoders used by text-to-image synthesis networks [66, 67], but much less sophisticated. However, a complex encoding is not needed here, since – contrary to image captions – the chromatin features are already in a format that can be processed directly by neural networks.

Many ways for training a cGAN exist, and achieving a stable, converging training process can be tricky. For the thesis at hand, we therefore relied on the extended loss function proposed by

Isola et al. [48], amended by a TV loss function L_{TV} for noise reduction, eq. (5).

$$L_{total} = \lambda_{cGAN} L_{cGAN}(G, D) + \lambda_{MAE} L_{MAE}(G) + \lambda_{TV} L_{TV}(G) \quad (5)$$

Here, L_{MAE} is the mean absolute error (L1 error) between the generated output $G(x, z)$ and the true Hi-C submatrices y (as grayscale images), and L_{TV} is a total variation loss as in eq. (1) and eq. (9). The original idea behind using mean absolute error and PatchGAN in combination is that optimizing for MAE will minimize low-frequency errors, while optimizing the PatchGAN-loss with its small patches helps reducing high-frequency errors [48]. The TV loss has been added for edge-aware noise removal, in line with other works in the field [43], cf. section 3.1.3.

Finally, the loss function was optimized with the Adam optimizer, alternately training discriminator and generator, see section 4.5.1 for details.

Compared to other cGAN architectures, pix2pix is rather simple, but well studied for different applications like label-to-image transfer, sketch-to-image transfer, grayscale-to-color transformations or image infill tasks [48]. Since the problem at hand has likely not been tackled by a tailor-made GAN yet, choosing a “general-purpose” cGAN as a starting point seemed reasonable. Another advantage towards pix2pix, and U-Net architectures in general, is that they can achieve good performance even with comparatively few training samples [48, 65].

3.2.2 Using a DNN for feature embedding

The embedding network required for processing the conditional inputs has one obvious mandatory property: it must be capable of embedding the input of shape $(3w, n)$ into a 2D grayscale image of shape $(w, w, 1)$. Naturally, this can be achieved by using the dense neural network explored above and reshaping its “upper triangular” output vector back into a symmetric “image” tensor with the required shape.

The rationale here was to first use the DNN to get a coarse estimate of the predicted matrix, and then the cGAN to refine the results, somewhat similar to the two-stage approach by Zhang et al. [62], albeit much less sophisticated. Naturally, this approach also allows to pre-train the dense network as shown above and then use transfer learning to provide better starting values for the embedding network, potentially improving both stability and convergence speed of the cGAN network.

A disadvantage of the dense network approach is that the number of neurons in its output layer quadratically depends on the windowsize w . Taken together with the three fully connected layers underneath, this leads to a superlinear increase in the number of parameters along w , further aggravated by the fact that the cGAN requires w to be powers of two, cf. section 4.5.1 and 4.5.2 (table 4, p. 34). The DNN embedding has thus only been explored for $w = 64$, both with and without pretraining. The results are to be found in section 5.2.1.

3.2.3 Using a CNN for feature embedding

Since the results from the DNN embedding were not convincing, another approach based on convolutional layers was created. Here, the idea was to have a trainable embedding that keeps the localization information in the chromatin feature data, has less parameters than a dense

embedding and can efficiently be trained along with the rest of the network, allowing for a standalone solution.

Since no example for such an embedding was found in literature, a first try was made with a simple 8-layer convolutional network. The complete setup is shown in section 4.5.3 (fig. 18, p. 42). Compared to the dense approach, the number of parameters in the CNN embedding is widely independent of the windowsize and stays between 4.24 million and 4.29 million for the tested windowsizes 64, 128 and 256, cf. table 4 (p. 34).

3.2.4 Using DNN and CNN embedding for generator and discriminator

4 Methods

In the following sections, the methodical details for the tasks within the thesis will be discussed in more detail. First, input data and data preprocessing will be discussed, followed by an explanation of the quality metrics used to assess the predictions of Hi-C matrices made throughout the thesis. Next, sections 4.4 and 4.5 will give details with regard to the two different approaches made in this thesis to advance the state of the art in predicting Hi-C matrices. Finally, the hardware used for the computations within this thesis will be outlined in section 4.7.

4.1 Input data

For the thesis at hand, data from human cell lines GM12878, K562, HMEC, HUVEC and NHEK was used. The exact data sources and data preprocessing for the Hi-C matrices and ChIP-seq data will be outlined in the following subsections 4.1.1 and 4.1.2.

4.1.1 Hi-C matrices

Hi-C data due to Rao et al. [11] was downloaded in .hic format from Gene Expression Omnibus under accession key GSE63525. Here, the quality-filtered “combined_30” matrices were taken, which contain only high-quality reads from both replicates.

Next, matrices at 5 kbp binsize were extracted and converted to cooler format using `hic2cool` and subsequently coarsened to resolutions of 10, 25, 50 and 100 kbp using `cooler coarsen`, see listing 1.

Contrary to the work from Farré et al. [30], which is using a distance-based normalization, and many others in the field which are using ICE- or KR-normalization, these matrices have not been normalized for the thesis at hand because no benefit of doing so was found during the study project [13].

4.1.2 ChIP-seq data

For this thesis, ChIP-seq data for 13 chromatin features and DNaseI-seq data was used, cf. table 2. Here, the data was downloaded in .bam format either via the ENCODE project [16, 17] or directly from the file server of the University of California (UCSC). In all cases, bam-files for replicate 1 and 2 were downloaded in their most recent versions (if applicable); the UCSC-(wgEncode...) or GEO- (GSM...) identifiers are also given in table 2. For convenience, the pdf version of this document also provides download links for concrete files in section 7.1.

After downloading, the bam files were converted to bigwig format, which was found more convenient to handle, and the replicates were merged into one bigwig file by taking the mean, as in the study project [13]. Pseudocode for the full conversion process is given in listing 2.

The choice of chromatin features is widely in line with the work by Zhang et al. [28]; it contains structural proteins like CTCF and Cohesin subcomponents RAD21 and SMC3 as well as active/passive markers.

feature name	cell line	
	GM12878	K562
CTCF	GSM733752	GSM733719
DNaseI	wgEncodeEH000534	wgEncodeEH000530
H3k27ac	GSM733771	GSM733656
H3k27me3	GSM733758	GSM733658
H3k36me3	GSM733679	GSM733714
H3k4me1	GSM733772	GSM733692
H3k4me2	GSM733769	GSM733651
H3k4me3	GSM733708	GSM733680
H3k79me2	GSM733736	GSM733653
H3k9ac	GSM733677	GSM733778
H3k9me3	GSM733664	GSM733776
H4k20me1	GSM733642	GSM733675
Rad21	wgEncodeEH000749	wgEncodeEH000649
Smc3	wgEncodeEH001833	wgEncodeEH001845

Table 2: chromatin features used for the thesis

4.1.3 Sample generation process for the dense neural network

This thesis follows the sliding window approach proposed by Farré et al. [30].

First, all chromatin features were binned to binsize b_{feat} by splitting each chromosome of size cs into $l_{feat} = \left\lceil \frac{cs}{b_{feat}} \right\rceil$ non-overlapping bins of size b_{feat} and taking the mean feature value within the genomic region belonging to each bin. All n chromatin factors were processed in this way and then stacked into a $l_{feat} \times n$ array. Here, the number of chromatin features was constant for all investigations within this thesis, $n = 14$ (cf. section 4.1.2).

Separate Hi-C matrices for each chromosome were derived from the cooler format as $(l_{mat} \times l_{mat})$ -matrices, $l_{mat} = \left\lceil \frac{cs}{b_{mat}} \right\rceil$ being the number of bins in the given chromosome. Initially, $b_{feat} = b_{mat}$ was used, which leads to $l_{feat} = l_{mat}$, because cs is a constant for a given chromosome.

A sliding window approach was now applied to generate training samples for the neural networks G described below. Here, subarrays of size $(3w_{mat} \times n)$ were cut out from the feature array such that the i -th training sample corresponded to the subarray containing the columns $i, i + 1, i + 2, \dots, i + 3w_{mat}$ of the full array. Sliding the window along the array with stepsize one obviously yields $N = l - 3w_{mat} + 1$ training samples. The corresponding Hi-C matrices were then cut out along the diagonal of the original matrix as submatrices with corner indices $[j, j], [j, j + w_{mat}], [j + w_{mat}, j + w_{mat}], [j + w_{mat}, j]$ in clockwise order, where $j = i + w_{mat}$. The idea here is that the first $0, 1, \dots, w_{mat}$ columns of each feature sample form the left flanking region of the training matrix, the next $w_{mat} + 1, w_{mat} + 2, \dots, 2w_{mat}$ correspond to the matrix' region and the last $2w_{mat} + 1, 2w_{mat} + 2, \dots, 3w_{mat}$ columns form the right flanking region. Since Hi-C matrices are symmetric by definition, only the upper triangular part of the submatrices was used, flattened into a $w \cdot (w + 1)/2$ vector.

Figure 8 exemplarily shows the sample generation process for a (16×16) -matrix with $w_{mat} = 4$ and $n = 3$ chromatin features. In this case, five training samples would be generated – the one

encircled in green and four more to the right, as the window is sliding from left to right until the right flanking region hits the feature array boundary.

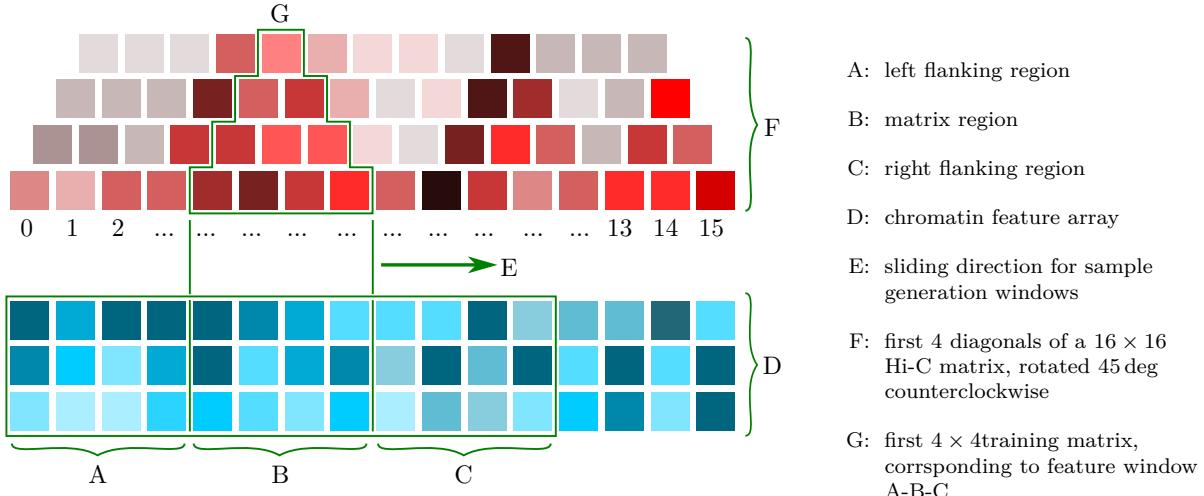


Figure 8: Sample generation process

The sample generation process for predicting (unknown) matrices was the same as for training, except that no matrix window was generated. Due to the sliding window approach, the output of the network is a set of overlapping submatrices along the main diagonal of the actual target Hi-C matrix. To generate the final submatrix, all submatrices were added up in a position-aware fashion and finally all values were divided by the number of overlaps for their respective position. Figure 9 exemplarily shows the prediction process for $N = 5$ samples with windowsize $w = 4$ for a 16×16 matrix. Note that the first and last w bins in each row (matrix diagonal) always remain empty due to the flanking regions, as do all bins outside the main diagonal and the first $w - 1$ side diagonals. To improve visualisation, all predicted matrices were scaled to value range 0...1000 after re-assembly and stored in cooler format for further processing. Conveniently, cooler also supports storing only the upper triangular part of symmetric matrices, minimizing conversion effort for the data at hand.

Training samples were drawn from GM12878, chromosomes 1, 2, 4, 7, 9, 11, 13, 14, 16, 17, 18, 20 and 22 of GM12878, validation samples from GM12878, chromosomes 6, 8, 12 and 15 and test samples from K562, chromosomes 3, 5, 10, 19 and 21. This approximately implements a 60:20:20 train:validation:test split, see table 3 for details. Note that windowsize $w = 80$ is not suitable for resolutions beyond 50 kbp, because the number of training samples becomes too small to train a network with more than seven million parameters, cf. section 4.4.1.

4.1.4 Sample generation for the cGAN

The samples for the cGAN were generated the same way as the samples for the dense neural network described in section 4.1.3, with two exceptions. First, Hi-C (sub-)matrices were not entered as vectors corresponding to their upper triangular part, but were instead taken as $w \times w \times 1$ grayscale images with value range [0...1] in 32-bit floating point format. Second, for the cGAN approach the input matrices need not only be square, but their sizes also needed to be powers

chrom.	length/bp	samples at w = 80 and binsize...					
		5k	10k	25k	50k	250k	500k
1	249250621	49612	24687	9732	4747	759	260
2	243199373	48401	24081	9489	4625	734	248
4	191154276	37992	18877	7408	3585	526	144
7	159138663	31589	15675	6127	2944	398	80
9	141213431	28004	13883	5410	2586	326	44
11	135006516	26763	13262	5162	2462	302	32
13	115169878	22795	11278	4368	2065	222	
14	107349540	21231	10496	4055	1908	191	
16	90354753	17832	8797	3376	1569	123	
17	81195210	16001	7881	3009	1385	86	
18	78077248	15377	7569	2885	1323	74	
20	63025520	12367	6064	2283	1022	14	
22	51304566	10022	4892	1814	788		
\sum train samples		337986	167442	65118	31009	3755	808
6	171115067	33985	16873	6606	3184	446	104
8	146364022	29034	14398	5616	2689	347	54
12	133851895	26532	13147	5116	2439	297	29
15	102531392	20268	10015	3863	1812	172	
\sum valid. samples		109819	54433	21201	10124	1262	187
3	198022430	39366	19564	7682	3722	554	158
5	180915260	35945	17853	6998	3380	485	123
10	135534747	26868	13315	5183	2472	304	33
19	59128983	11587	5674	2127	944		
21	48129895	9387	4574	1687	724		
\sum test samples		123153	60980	23677	11242	1343	314
\sum total samples		570958	282855	109996	52375	6360	1309

Table 3: training, validation and test samples for sliding window approach

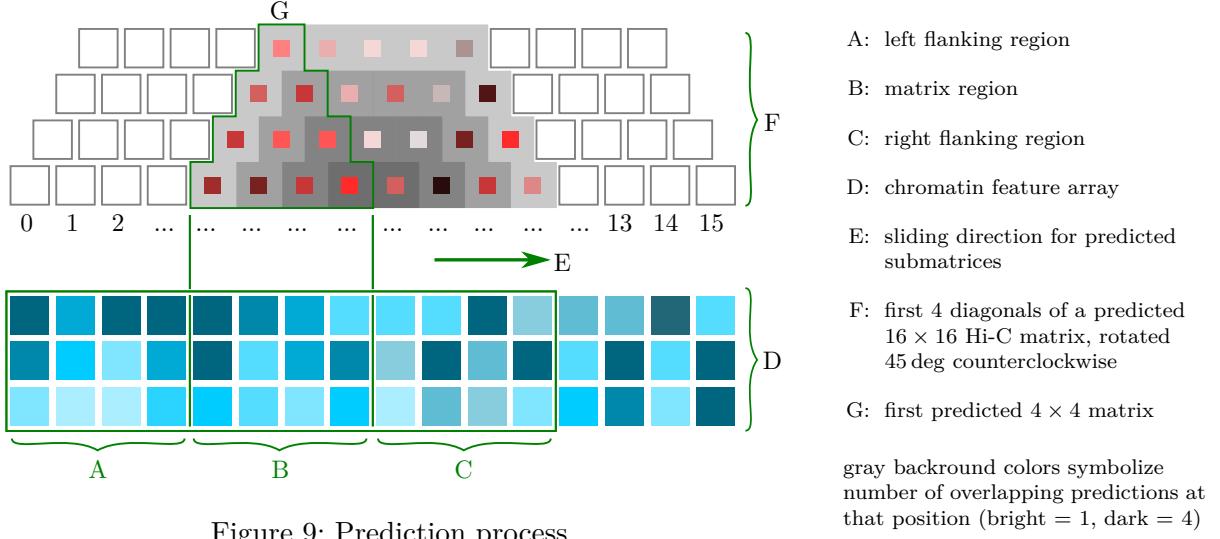


Figure 9: Prediction process

of two. This ensured the required shapes for the connected up- and downsampling operations in the generator, which essentially are 2D-convolutions and transposed 2D-convolutions with strides two. Within the thesis, windowsizes of $w = \{64, 128, 256\}$ were used, see section 5.2.

4.1.5 Generalization of feature binning

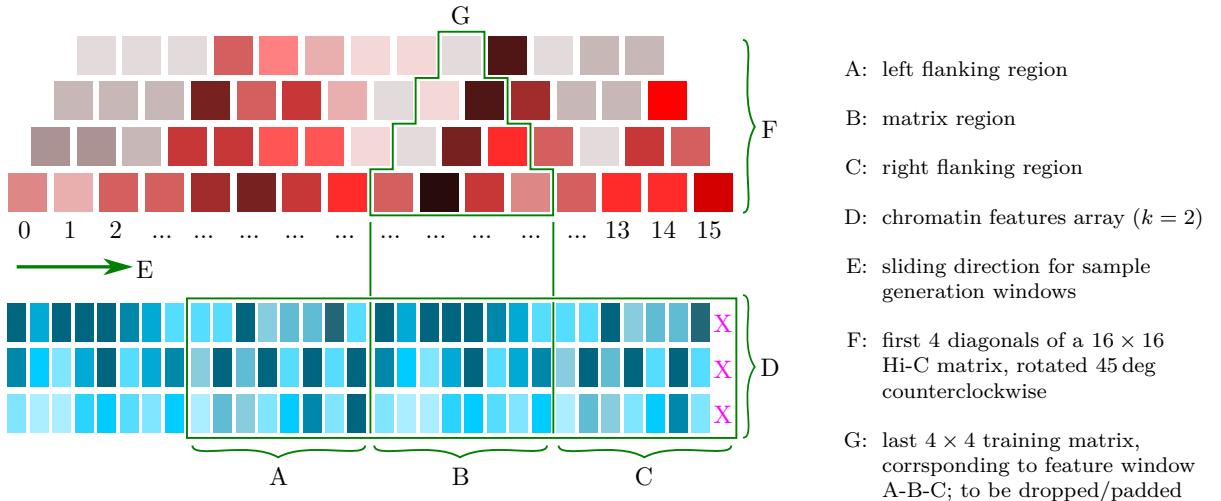
Most of the binning- and sample generation procedures described above also work for binsize relations $k = \frac{b_{mat}}{b_{feat}} \in \mathbb{N}^{>1}$.

The training matrices remain unchanged, i. e. $(l \times l)$ -arrays, from which training submatrices of size $w_{mat} \times w_{mat}$ can be extracted. With $k \in \mathbb{N}^{>1}$, one bin on the matrix diagonal corresponds to k bins of the feature array, so the feature windowsize needs to be k times the submatrix windowsize, $w_{feat} = k \cdot w_{mat}$. Since the first layer of all neural networks used in this thesis is a 1D convolution, this can be achieved by setting the filter width and strides parameters of the (first) convolutional layer to k , leaving the rest of the network unchanged. However, the number of bins along the matrix diagonal is generally not k times the number of bins in the feature array, see eq. (6).

$$l_{feat} = \left\lceil \frac{cs}{b_{feat}} \right\rceil = \left\lceil \frac{cs}{k \cdot b_{mat}} \right\rceil \neq k \cdot \left\lceil \frac{cs}{b_{mat}} \right\rceil = k \cdot l_{mat} \quad (6)$$

For the training process, this discrepancy was resolved by simply dropping the last training sample, if the feature window belonging to it had missing bins. For the prediction process, the feature array was padded with zeros on its end. This procedure ensures that no errors are introduced into the *training* process by imputing values, but keeps the size of the *predicted* matrix consistent with the training matrix binsizes.

Figure 10 shows the generalized training process with a (16×16) -training matrix and $k = 2$. If $15 \cdot b_{mat} + 1 \leq cs < 15 \cdot b_{mat} + b_{feat}$ held for the chromosome size cs in the example, then the number of bins on the matrix diagonal would be $l_{mat} = 16$, while the number of chromatin

Figure 10: Generalized sample generation process f. $k = 2$

feature bins would be $l_{feat} = 31 \neq 2 \cdot l_{mat}$. In this case, the 5th sample would be dropped for training, while a column with zero bins – symbolized by pink crosses in fig. 10 – would be added to the feature array for prediction so that the resulting matrix would still have the desired size of 16×16 .

4.2 Quality metrics for predicted Hi-C matrices

Assessing the quality of synthetically generated images is a long-standing problem, which has not yet been solved, see e.g. [60, p. 19]. Within this thesis, distance-stratified Pearson correlations were computed between predicted and real matrices to measure the quality of the predictions. While the suitability of suchlike correlations as a quality metric for Hi-C matrices seems debatable in general [68], distance stratified Pearson correlation is quite common in the field of Hi-C matrices and thus useful at least for comparisons with other approaches.

To compute the correlations, the w bins at the left and right boundaries of the investigated matrices were first removed, because these never contain valid values due to the chosen sample generation approach, cf. section 4.1.3, fig. 9 and 4.1.4. Next, the remaining values were grouped into vectors according to their genomic distance d , up to the maximum distance w , and then Pearson correlations ρ were computed separately for each vector as usual, eq. (7). Here, $true|_d$ and $pred|_d$ are the true and predicted values restricted to bin distance d , while $\sigma(true|_d)$ and $\sigma(pred|_d)$ are the respective standard deviations and $cov(\cdot, \cdot)$ is the covariance.

$$\rho(true|_d, pred|_d) = \frac{cov(true|_d, pred|_d)}{\sigma(true|_d) \sigma(pred|_d)} \quad (7)$$

Since a numerically stable and efficient implementation of the Pearson correlation is non-trivial, the corresponding function from the pandas library **XXX** was used for actual calculations. An obvious disadvantage of distance-stratified Pearson correlations is that they are undefined if either the predicted matrices or – not observed in practice – the true matrices have the same value, and thus zero standard deviation for a certain distance.

In addition to the correlations themselves, the area under the correlation curve (AUC) was computed for all curves in each plot to obtain a single-valued, albeit very abstract quality metric. In line with Zhang et al. [28], trapez integration method was used for numerical area computations and the values were normalized to the interval [-1, 1] by dividing through the maximum bin distance in each dataset to allow for comparisons across distances. Furthermore, all Pearson correlation plots show the correlation between the “true” GM12878 matrix and the corresponding target matrix as a baseline, i.e. the Pearson correlation that is obtained when simply using the corresponding GM12878 matrix and chromosome as a “prediction” for a given target matrix and chromosome.

In computer graphics, other similarity metrics like the structural similarity index (SSIM), peak signal-to-noise ratio (PSNR) or, especially for images generated by GANs, Fréchet inception distance (FID) seem more common than Pearson correlation. However, their suitability for plots of Hi-C matrices seems not to have been investigated so far. Unlike Pearson correlation, SSIM and PSNR both depend on absolute values in the matrices to be compared, so that for example $PSNR(\mathbf{X}) \neq PSNR(k \cdot \mathbf{X})$ for $k \in \mathbb{R}^{>1}$, which seems not optimal for a use case where structures are more interesting than actual values. Computing FID would require reshaping our outputs into images of shape (299, 299, 3) to meet Inception v3 specifications [69]. This is a non-trivial task for the given matrix plots, where the longer edge – defined by *chromsize/binsize* – is usually much longer and the shorter edge – defined by the windowsize w – is usually shorter than 299 bins, or pixels, respectively. While computing the FID for individual (w, w) -submatrices would be possible, generalizing such results to the whole matrix seems without example in literature so far.

Since relying on one metric alone seemed problematic, selected areas of the test set were plotted against the true Hi-C matrices in these areas, see section 4.3 below.

4.3 Matrix plots

To allow for a visual comparison, selected areas of the predicted matrices were plotted against their true counterparts using pygenometracks [70]. Since it was impossible to plot the full test set every time due to the amount of figures needed, three exemplary regions were selected, chromosome 21, 19 and 5, 30 to 40 Mbp, respectively. These regions were chosen because they feature both small and large TADs as well as nested TADs and subregions with very little chromatin feature signal values.

In general, most matrix plots additionally feature the sign of the first eigenvector in a purple track, named “binarized PCA” (values +1 or -1) and the summarized value of all 14 chromatin features in a green track, named “feature sum”, see e.g. fig. 22. Feature sum was computed by binning all chromatin features at 25 kbp and summing up the values across all features for each bin. Eigenvectors were computed using `hicPCA` [59] and then the sign for each 25 kbp bin was extracted using a simple python script. Finally, eigenvectors and feature sum were written out in bedgraph format, which pygenometracks can plot natively.

All tracks were plotted with an original resolution of 200 dpi and a diagram width of 150 mm. For the interaction counts in the Hi-C matrix plots, “log1p” transformation was applied and the true matrices were inverted, i.e. reflected at the diagonal.

4.4 Dense neural network approach

In the following sections, the setup for the Dense Neural network inspired by Farré et al. [30] and its variations will be discussed in detail.

4.4.1 Basic setup

The basic setup for the dense neural network approach closely follows the proposal by Farré et al. [30], so a windowsize of $w = 80$ and $b_{feat} = b_{mat} = 10\text{ kbp}$ was initially used. With these parameters, the network has a total of 7,486,436 trainable weights in five trainable layers; one convolutional layer and four densely connected layers, fig. 11. For brevity, the sigmoid activation after the 1D convolution and the ReLU activations after the Dense layers are not shown. The dense layers all use a “L2” kernel regularizer and the dropout layers following all but the last dense layer have a dropout probability of 10%.

The training goal for the neural network G is to find weights ω^* for its five trainable layers such that the mean squared error L_2 between the predicted submatrices $M_s = G_s(\omega)$ and the training submatrices T_s becomes minimal for all N training samples $s \in (1, 2, \dots, N)$. Here, M_s is given by the activations of the last dense layer, which are to be interpreted as the upper triangular part of a symmetric $(w \times w)$ Hi-C matrix. Formally, one needs to optimize

$$\omega^* = \arg \min_{\omega} L_2(\omega) = \arg \min_{\omega} \frac{1}{N} \sum_{s=0}^N (M_s - T_s)^2 \quad (8)$$

For the thesis at hand, stochastic gradient descent (SGD) with learning rate 10^{-5} was used to find ω^* . Initial values ω_{init} were drawn from a Xavier initializer, a uniform distribution with limits depending on the in- and output shapes. Following [30], optimization was performed on minibatches of 30 samples, assembled randomly from the N training samples to prevent location-dependent effects and improve generalization. For training, the last batch was dropped, if $N/30 \notin \mathbb{N}$.

The network and its learning algorithm were implemented in python using tensorflow deep learning framework, partially with keras frontend [71, 72], see repository [XXX](#).

4.4.2 Modifying kernel size, number of filter layers and filters

For the “wider” variant, the kernel size of the of the 1D convolutional layer was increased to $4k$ with strides k , where $k = b_{mat}/b_{feat}$ is the relation beetwen matrix- and feature binsize. Mirror-type padding was used to maintain the output dimensions of the basic network, which now had 7 486 478 trainable weights for $k = 1$.

For the “longer” variant three 1D-convolutional layers with 4, 8 and 16 filters were used in place of the basic network’s single convolutional layer, cf. fig. 12. This replacement was also made for the “wider-longer”-variant, additionally increasing the respective kernel sizes to $4k$ (with strides k), 4 and 4, cf. fig. 12 (right). In both cases, the dropout rate was increased to 20%. The “longer” variant had 9 142 665 trainable weights and the “wider-longer” had 9 143 313 for $k = 1$.

For the variant with generalized binning, features were binned at $b_{\text{feat}} = 5 \text{ kbp}$ while keeping the matrix binsize $b_{\text{mat}} = 25 \text{ kbp}$, so the factor for the relation between the two binsizes was $k = 25/5 = 5$. This yields input feature arrays of size $3w \cdot k \times n = 3 \cdot 80 \cdot 5 \times 14 = 1200 \times 14$. Replacing the first convolutional layer of the basic network by a 1D convolutional filter with kernel size $k = 5$ and strides $k = 5$ without padding, this input was again compressed to a $3w \times 1 = 240 \times 1$ tensor and fed into the flatten layer, cf fig. 11. The rest of the network remained the same so that the number of trainable parameters increased only slightly to 7 486 492.

4.4.3 Combination of mean squared error, perception loss and TV loss

For computing the combined MSE, perception- and TV-loss, input features were first passed through the network as normal, and mean squared error was computed on the predicted “upper triangular vectors” vs. the real vectors. Next, both the output- and target vectors were converted to symmetric grayscale images by embedding them into $w \times w \times 1$ tensors, where w is again the windowsize.

For a pixel-image y , total variation can be defined as the sum of the absolute differences for neighboring pixel-values, eq. (9) [54]

$$\sum_{i,j} \sqrt{|y_{i+1,j} - y_{i,j}|^2 + |y_{i,j+1} - y_{i,j}|^2} \quad (9)$$

For efficiency, the tensorflow implementation from `tf.image.total_variation` was used, taking the sum across batches as loss value, as recommended in the tensorflow documentation [73].

For perception loss, the predicted images and the true images were first fed through a pre-trained VGG-16 network with fixed weights, truncated after the third convolution layer in the fourth block (“block4_conv3”), the last layer used by the influential work of Johnson et al. [53]. The loss was then computed as mean squared error between the “predicted” and “true” output activations of the truncated VGG-16 network.

Let $M_s = G_s(\omega)$ again be the output of the neural network G described above, and T_s the true matrices for training samples s in vector form, and let M'_s and T'_s be their grayscale image counterparts as described above. Furthermore, let $tv(x)$ be the total variation of image x and $vgg(x)$ the output of the perception loss network for image x . The goal of the modified network was now to find weights w^* such that

$$\omega^* = \arg \min_{\omega} (\lambda_{MSE} \frac{1}{N} \sum_{s=0}^N (M_s - T_s)^2 + \lambda_{TV} \sum_{s=0}^N tv(M'_s) + \lambda_{VGG} \frac{1}{N} \sum_{s=0}^N (vgg(M'_s) - vgg(T'_s))^2) \quad (10)$$

Weight initialization for network G and minibatching was done as described in section 4.4.1. The weights for the VGG16 network were taken from the pre-trained keras implementation and can here be considered as constants. In addition, the usage of VGG16 imposes the restriction $w \geq 32$ on the windowsize w , which is not problematic, since again $w = 80$ was chosen for all experiments.

The network $G(\omega)$ could in principle be any of the variants described above in section 4.4.2, but for the thesis at hand, only the initial network from section 4.4.1 was used.

4.4.4 Combination of mean squared error and TAD-score-based loss

Formally, the the following optimization task can be defined by means of a combination between MSE and TAD-score based loss:

$$\omega^* = \arg \min_{\omega} (\lambda_{MSE} \frac{1}{N} \sum_{s=0}^N (M_s - T_s)^2 + \lambda_{score} \frac{1}{N} \sum_{s=0}^N (score(M'_s, ds) - score(T'_s, ds))^2) \quad (11)$$

where M_s is again the Hi-C submatrix predicted by the network $G(\omega)$ for sample s and T_s is the corresponding true Hi-C submatrix.

To compute the TAD-score-based loss $score(\cdot, \cdot)$, the predictions and true Hi-C matrices M and T in vector form (upper triangular part) were first converted back to complete, symmetric Hi-C matrices M' , T' . Next, in a custom network layer, all diamonds with size ds inside the submatrices of size w were cut out using tensor slicing and the values inside the diamonds were reduced to their respective mean. This yields score vectors – more exactly, tensors with shape $(w - 2ds, 1)$. After computing the latter for both predicted- and real Hi-C submatrices, the mean squared error between them was computed as usual and weighted with a user-selected loss weight λ_{score} , see eq. (11).

While it would also have been possible, and probably faster, to slice the outputs of the networks directly in vector form, this is rather unintuitive and was therefore not implemented for the thesis.

4.5 Hi-cGAN approach

4.5.1 Modified pix2pix network

Several implementations of the original pix2pix network are publicly available, usually for the original image size of 256×256 . For the thesis at hand, implementation concepts from two tutorials [74, 75] were combined and the code was adapted to the given requirements.

Within the generator, two of the down- and upsampling layers inside the U-Net portion were made optional to allow processing smaller images of sizes 64×64 and 128×128 , see fig. 13. Note that the generator (still) only supports square images with edge lengths that are powers of two and at least $2^6 = 64$. Furthermore, symmetry of the output was enforced by adding its transpose and multiplying by 0.5, cf. [35]. The down- and upsampling layers shown in fig. 13 are custom blocks detailed in fig. 15 and 16. All 2D-convolutions and 2D-deconvolutions had kernel size $(4, 4)$ and were initialized with values drawn from a normal distribution with mean $\mu = 0$ and standard deviation $\sigma = 0.02$. Finally, the activation of the output layer was changed from tanh to sigmoid, because better results were observed in conjunction with the given Hi-C matrices, probably because these contain no negative values by definition.

Compared to the original pix2pix setup, one downsampling layer was omitted in the discriminator for windowsize $w = \{256, 128\}$ and another one for $w = 64$. This made the discriminator patches larger, especially for the smaller windowsizes, cf. fig. 14. Symmetry was enforced after all convolutions in the same way as in the generator. Kernel sizes and initializations for all 2D

convolutions also were the same as for the generator, and leaky ReLU-activations were used with parameter $\alpha = 0.2$, as in all up- and downsampling layers.

Both the discriminator and the generator feature their own, trainable embedding network to convert the conditional input, i.e. the chromatin feature data of shape $(3w, n)$, into grayscale images of shape $(w, w, 1)$. These networks will be discussed below in section 4.5.2 and section 4.5.3.

The loss function was implemented as shown in eq. (5) with parameters $\lambda_{cGAN} = 10^{-5}$, $\lambda_{MAE} = 1.0$, $\lambda_{TV} = 10^{-12}$. Optimization was performed on minibatches of size 32, 4 and 2 for windowsizes 64, 128 and 256, respectively, using Adam optimizers with learning rate $2 \cdot 10^{-5}$ and $\beta_1 = 0.5$ for both generator and discriminator. The choice of batchsizes was partially dictated by memory limitations of the available GPUs, cf. section 4.7.

- a) the number of trainable parameters

4.5.2 Using a DNN for 1D-2D embedding

In order to use the DNN described in section 4.4.1 as an embedding network for the cGAN, only small amendments were required to adjust the input shapes, i.e. to provide symmetric Hi-C matrices as grayscale images instead of the upper-triangular-vector representation native to the DNN, see fig. 17. The triu-reshape layer is a custom tensorflow network layer which generates an output tensor of appropriate shape (w, w) and sets its upper triangular part to the values given by the input vector. Symmetrization was then performed by adding this tensor to its transpose and dividing the values on the diagonal by two, because the diagonal is contained both in the upper triangular part of the matrix and its transpose. Finally, the required third axis was added to get the shape of a grayscale image. The number of trainable parameters for the DNN embedding is shown in table 4. Note that all trainable parameters stem from the DNN here; the reshaping layers do not have any trainable parameters.

windowsize	trainable weights	
	CNN	DNN
64	4243968	5502796
128	4260416	16034732
256	4293312	57877612

Table 4: trainable weights for embedding networks

For the thesis, the DNN-embedding was used in two ways. First, it was trained together with the rest of the cGAN with weight initialization as described in section 4.4.1 and 4.5.1. Second, the DNN was pre-trained as described in section 4.4.1 and the learned weights were transferred to the cGAN once before the start of the training process, which was then continued as described in section 4.5.1. The results of the pre-training are visualized in section 7.4.2 (fig. 61); the state after 250 epochs was the one transferred to the cGAN.

DNN embeddings were only used with windowsize $w = 64$ due to the large number of parameters to be trained at windowsizes 128, 256 and higher.

4.5.3 Using a CNN for 1D-2D embedding

The convolutional embedding network consists of 8 convolutional blocks and a final 1D convolution layer, as shown in fig. 18. Each of the convolution blocks start with a 1D convolution with kernel size 4, strides 1, padding “same” and “L2” kernel regularization with parameter $l = 0.02$, followed by batch normalization and leaky ReLU activation with parameter $\alpha = 0.2$. The last 1D convolution consists of w filters with kernel size 4, strides 3 and padding “same”, followed by sigmoid activation; this last convolution layer was not using kernel regularization. All kernel weights of the 1D convolutions in the embedding network were initialized by a Xavier initializer.

For the three windowsizes $w = \{64, 128, 256\}$ used within this thesis, the embedding network shown in fig. 18 contained about 4.2 to 4.3 million trainable weights, see table 4 for details.

4.6 Comparsion with other approaches

To compare our results with the ones due to Zhang et al. [28], predictions for cell line K562, chromosomes 14 and 17 were downloaded from Zenodo XXX. Here, results from the “WINDOW” and “MULTICELL” approaches were selected, the random forest being trained on GM12878, chromosome 14 and 17, respectively, because these results were deemed to offer the best comparability to the cGAN and DNN approaches of this thesis. The downloaded data is in text format and was first converted into cooler format, whereby we noticed that the data offered is surprisingly sparse. For chromosome 14, only 42.4 % and for chromosome 17, only 59.9 % of all possible interacting pairs for the chosen windowsize $w = 200$ at binsize $b = 5000$ were contained in the datasets, and no interacting pairs with predicted interaction counts below 0.01 were found in the data. Instead, the value range was between 0.36 and 5.10 across all four datasets, with similar values for each dataset alone. The missing pairs were found to be distributed all across the chromosome and all distance ranges, except for distance zero, which was not contained in the datasets at all. For the conversion to cooler format, the missing values were assumed to be zero. Next, the cooler matrices were coarsened to binsize 25 kbp to allow comparisons with the cGAN and DNN data.

For the comparison between HiC-Reg and Hi-cGAN, two additional cGAN models with CNN embedding were trained on chromosome 14 and 17, respectively, using (arbitarily selected) chromosome 10 for validation. Due to the small number of training samples for in this setting, windowsize was set to 64 and batch size to 2. The rest of the training process remained the same, cf. section 4.5.1. Additionally, HiC-Reg was compared to a cGAN-CNN model with windowsize 256 and CNN embedding, trained on the typical training data set, which includes chromosomes 14 and 17, cf. sections 4.5.1, 4.1.3 and 4.1.4.

When computing the distance-stratified Pearson correlations only from the available (non-zero) predicted values in the dataset XXX, the results for chromosome 17 showed very good accordance with the data published in the paper [28, fig. 10], fig. 19, allowing the conclusion that the correlation computations should be comparable between this thesis and the paper. For the comparsion with the DNN and cGAN approach and for the transfer from GM12878, the full matrices (including zeros) where considered, yielding comparatively high correlation values and visually pleasing matrix predictions, cf. 5.3.

It seems strange that zero values have not been considered when computing the correlations for the publication. First, the paper gives no reason why the predicted data could be sparse, and second, correctly predicted zero-values strongly increase the quality of the predicted matrices and should thus be considered when computing correlations. It is also unknown how TADs could be computed on the data without assuming the missing values as zeros, and, among others, TAD detection was used as a measure of prediction quality in the paper.

4.7 Hardware

For the thesis, three virtual machines were used to train the neural networks, see table 5. All training for section 5.2 was done on machine 2, computations for sections 5.1.1, 5.1.2 and 5.1.5 were done on machine 3 without GPU and computations for sections 5.1.3 and 5.1.4 were done on machine 2.

Training the DNN with perception- or score-based losses as well as the cGAN was not reasonably possible without GPU. For the cGAN, it was found that GPU memory should not fall short of the given values (table 5) to avoid undue limitations on batchsizes and/or windowsizes. 20 GB of main memory, as in machine 2, were not enough to train the cGAN at windowsize 64 for more than 80 epochs. It could not be clarified throughout the thesis whether this was due to a memory leak in tensorflow or due to the chosen implementation.

Training samples were stored as tensorflow tfrecords (on the fly at runtime) and a custom pipeline including a shuffle buffer and prefetching was employed to balance workload between CPUs and GPU. This approach was found to be faster than generator-based approaches by a large margin.

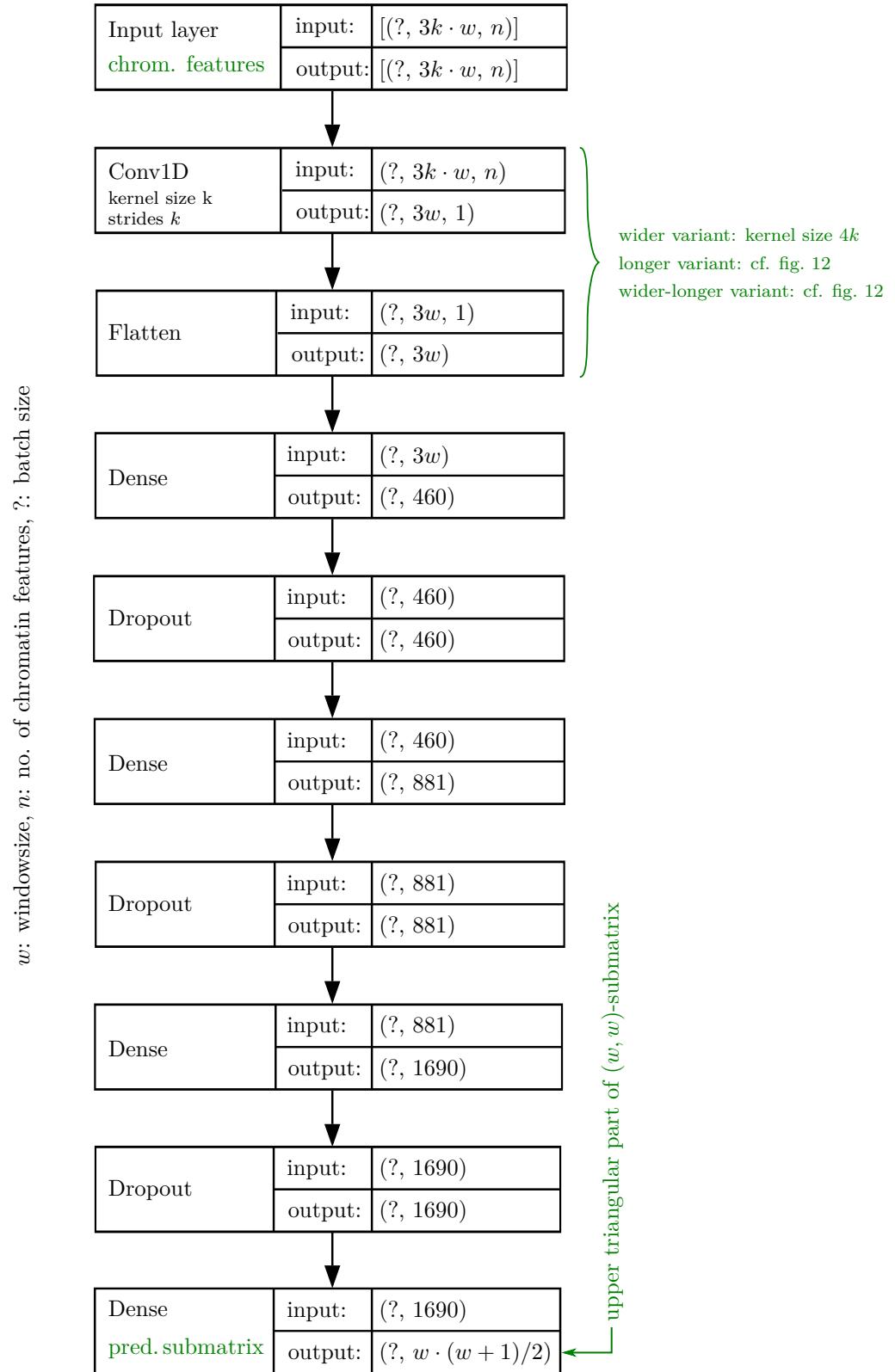


Figure 11: Basic dense neural network with generalized feature binning

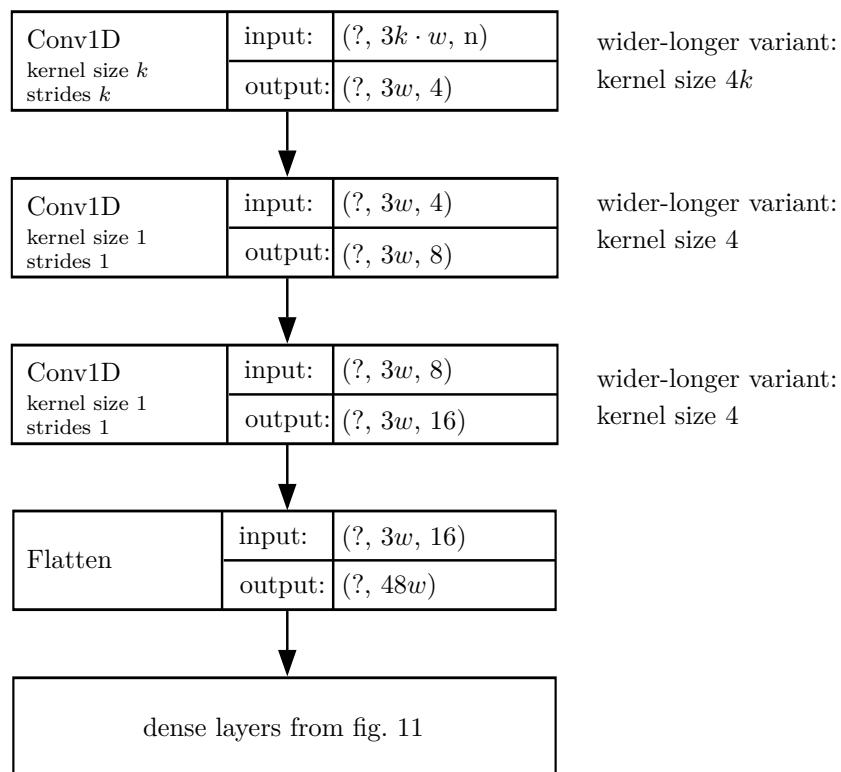


Figure 12: “longer” and “wider-longer” variants for DNN

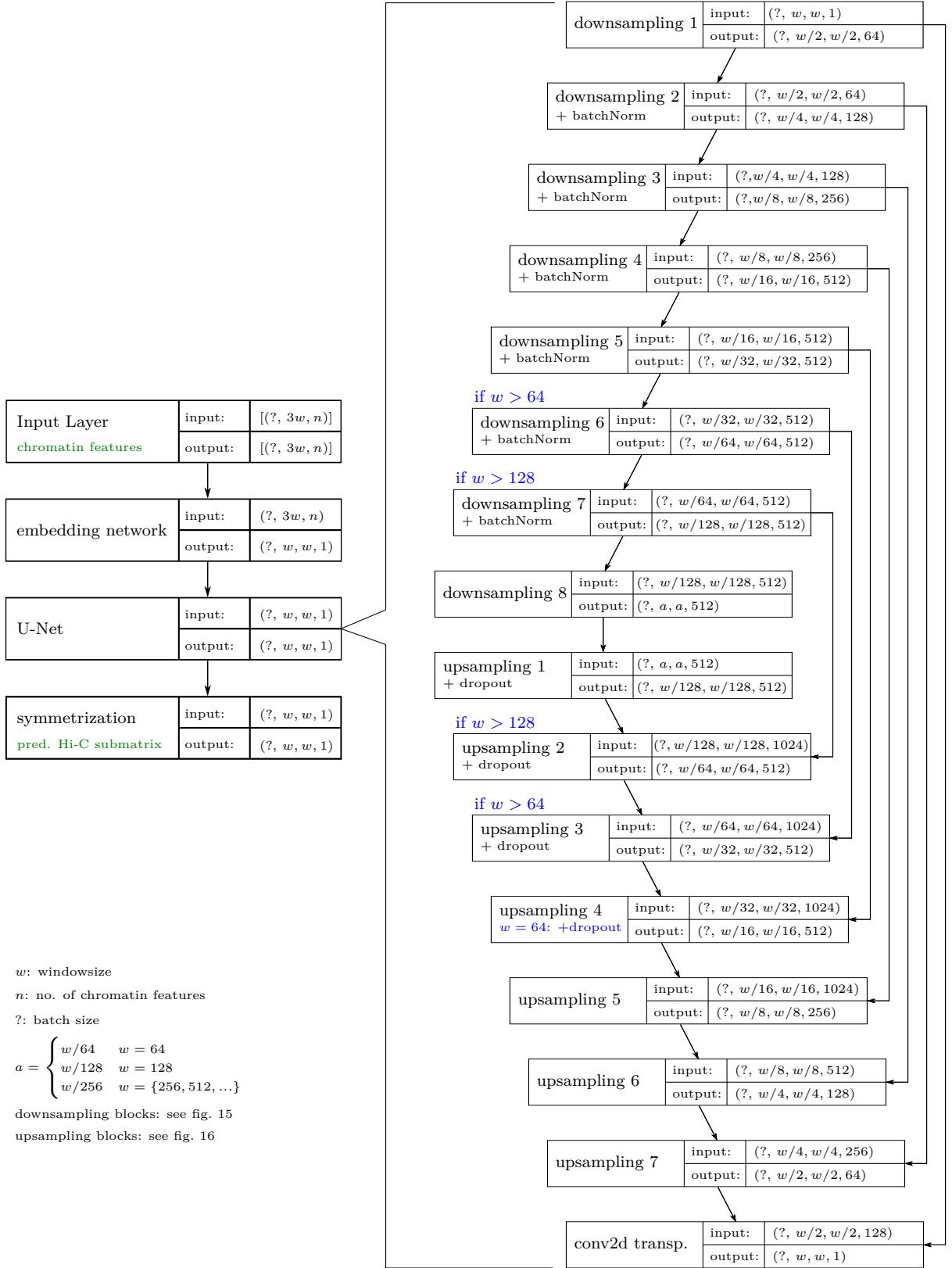


Figure 13: Adapted generator model from pix2pix

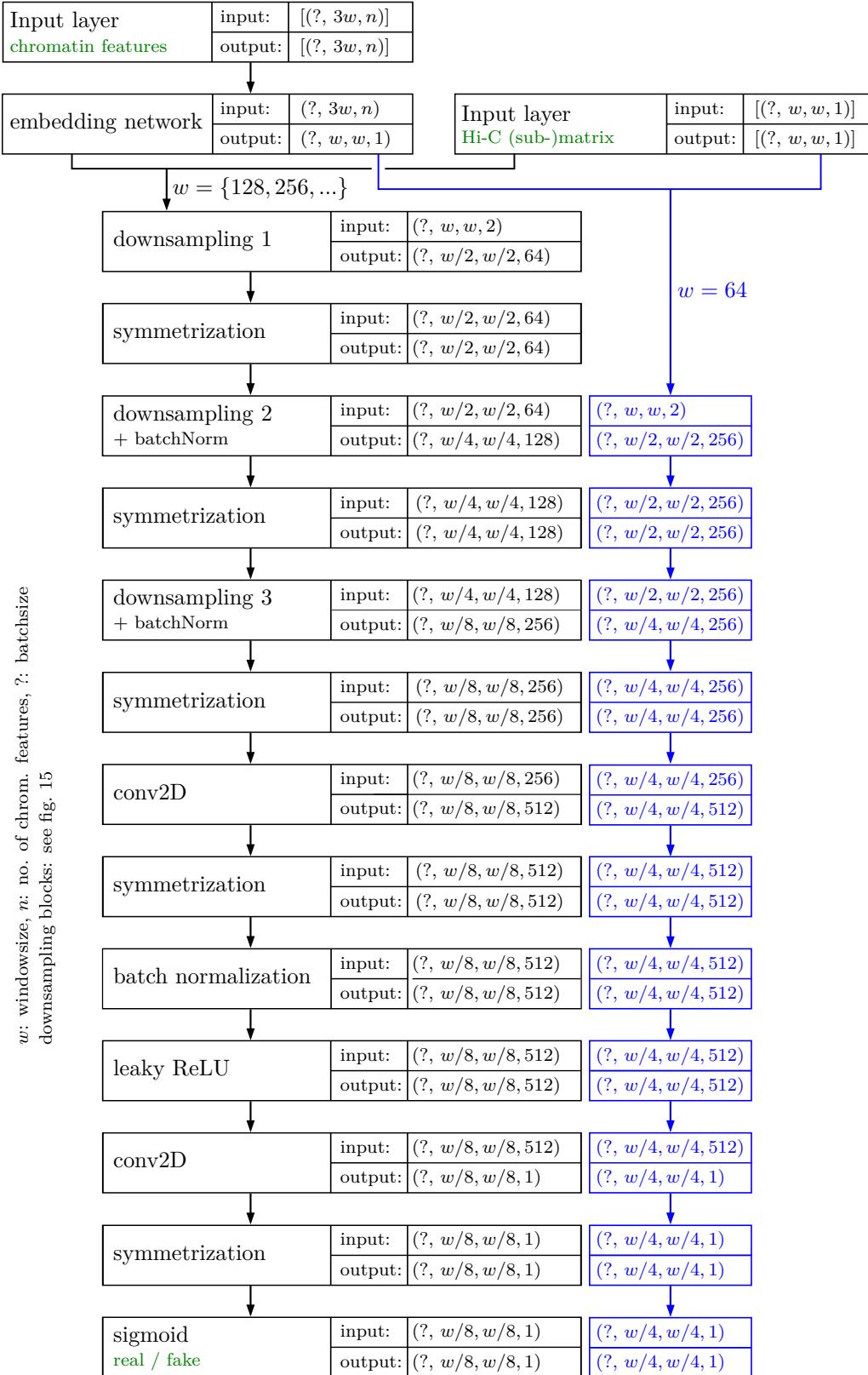


Figure 14: Adapted discriminator model from pix2pix

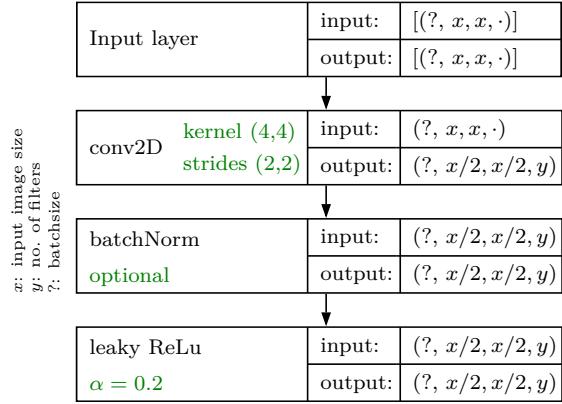


Figure 15: downsampling block

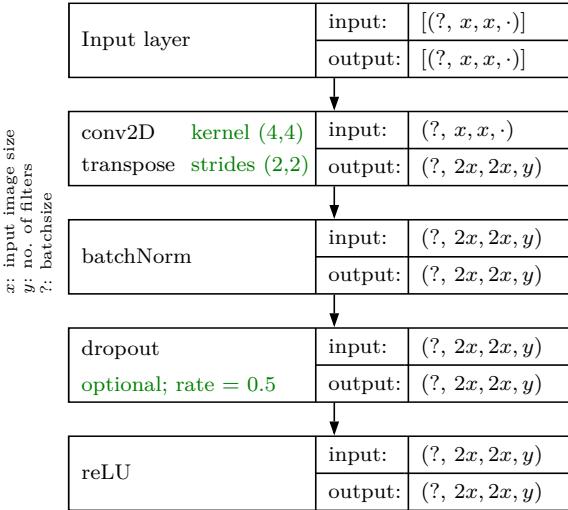


Figure 16: upsampling block

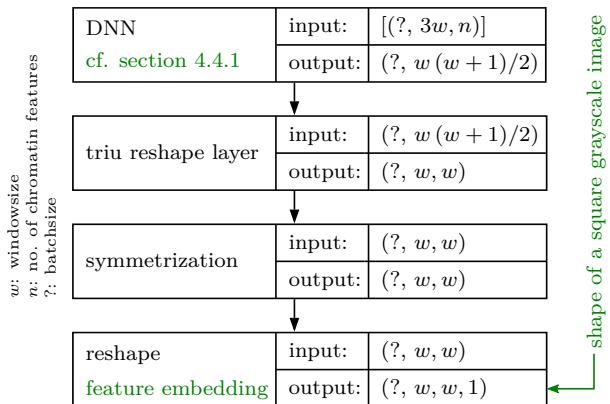


Figure 17: embedding network, DNN

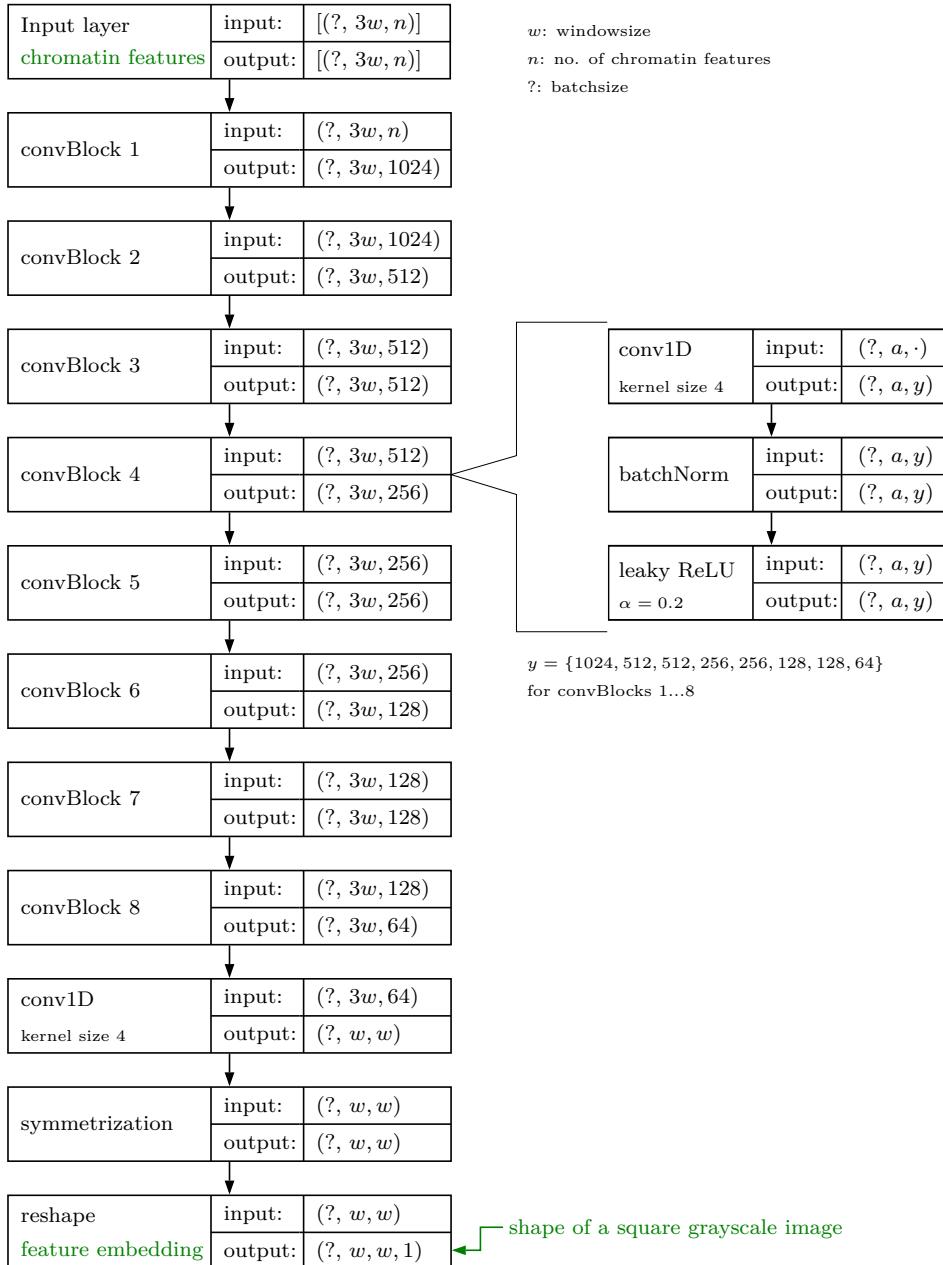


Figure 18: embedding network, CNN

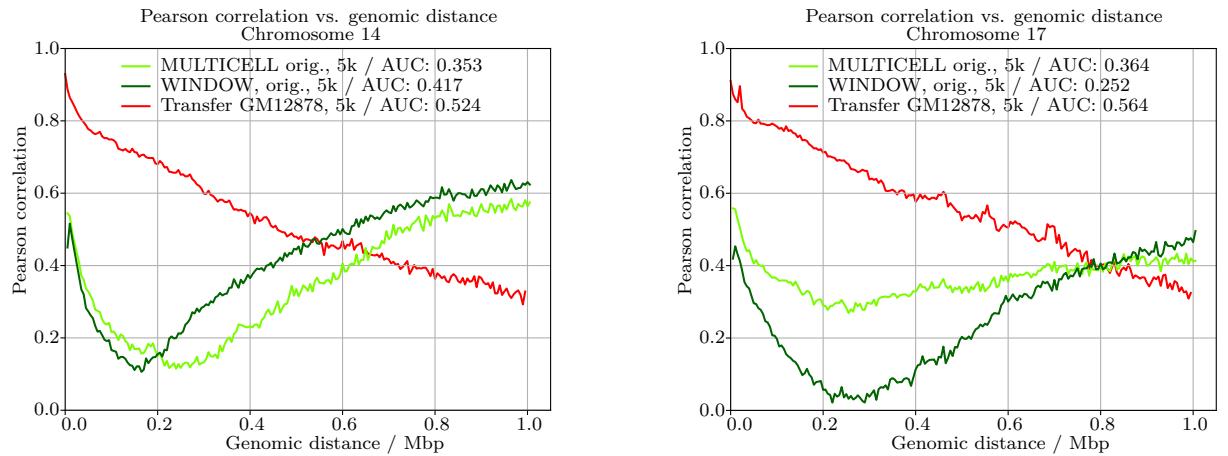


Figure 19: Pearson correlations reconstructed from [28]

5 Results

In the following two sections, the results of the DNN- and cGAN-based approaches for predicting Hi-C contact matrices will be presented. While the various modifications of the dense neural network described in section 3.1 did not really help improve the status quo, the novel conditional generative adversarial network laid out in section 3.2 showed interesting properties.

5.1 Dense Neural Network approaches

In the following subsections, the resulting predictions for modifications of the dense neural network originally conceived by Farré, Heurteau, Cuvier and Emberly [30] will be shown. This includes variations of the convolutional layer(s), custom loss functions with the intent of reducing blurriness in the predictions and tuning window- and bin size, cf. section 3.1. As a start, however, the results of the initial network without any modifications will be shown for comparison.

5.1.1 Initial results for comparison

The basic network was setup and trained as explained in section 4.4.1. Here, the validation error (MSE) reached its minimum of about 150 000 after approximately 500 epochs for 25 kbp bin size and around 400 epochs for 10 kbp bin size, fig. 20f and 21f. Beyond that, the learning curve indicated overfitting, but the resulting test matrices often still looked fairly similar, compare e.g. the matrix plots after 500 and 1000 epochs in figures 22 and 23.

Figure 20 and fig. 21 show the distance stratified Pearson correlations (cf. section 4.2) alongside area under the correlation curve (AUC) for the five test chromosomes at bin sizes 25 and 10 kbp, respectively. The red curves in each correlation plot show the correlation between the corresponding training chromosome from GM12878 and the target chromosome from K562. It is obvious that all predicted test matrices have a strictly positive Pearson correlation with respect to the experimentally derived matrices by Rao et al. [11], but are not better than simply taking data from the training cell line as prediction for the target cell line.

The predicted matrices themselves looked modest when plotted with pygenometracks. While the DNN generally produced high interaction counts in regions with many true interactions and low interaction counts in regions with few true interactions, the (TAD-)boundaries between different interacting domains were mostly not discernible, fig. 22 and 23. This finding is in line with the clearly positive, but medium-valued Pearson correlations. Exceptions with more distinct boundaries existed in all of the five test chromosomes, for example chr19, 34 to 35 kbp (fig. 22b), but were rare. Interestingly, medium-sized interacting structures, for example chr21, 31 to 32.5 kbp or between chr19, 31.2 to 32.7 kbp often seemed to be missing altogether – while structures larger than the window size, for example chr3, 34 and 36.7 kbp and 36.7 and 39.5 kbp sometimes were at least indicated.

Reducing the bin size to $b_{feat} = b_{mat} = 10$ kbp as in the paper by Farré et al. [30] led to somewhat different results. The area under the correlation curves was approximately the same for test chromosomes 3 and 5, slightly better for chromosome 10, but worse for chromosome 19 and 21, cf. fig. 20 and 21. However, the ability to predict larger structures was lost, and

the matrix plots thus did not look better than the ones for bin size 25 kbp. The comparatively bad result for test chromosome 21 might result from the low chromatin feature coverage of this particular chromosome.

No obvious correlation between comparatively “good” and “bad” predictions with open and closed states of the chromatin was observed. However, formally computing such a correlation is challenging, because no adequate objective measure for “good” and “bad” is known, especially considering the rather blurry results obtained so far. Furthermore, even if suchlike correlations existed, exploiting them for improving predictions would still be, at best, not straightforward.

5.1.2 Results for variations of the convolutional part

The results for the “wider” network, which featured a wider convolutional filter in the first network layer, cf. section 3.1.2 and 4.4.2, were generally similar to the initial results, both in terms of Pearson correlations and in terms of matrix plots, fig. 25 and 26. Given the small increase in the number of trainable parameters and overall similar network topology, this is not surprising. Overfitting was less obvious than with the initial setup and the training process looked more smooth, but the remaining validation error was slightly higher than for the initial approach, fig. 25f.

The predictions from the “longer” variant with three convolutional filter layers instead of just one were better than the initial ones in terms of Pearson correlations for test chromosomes 10, 19 and 21, but worse for test chromosomes 3 and 5, fig. 27. Interestingly, correlations for some of the larger distances could not be computed after 250 and 500 epochs, which generally means that the same values were predicted for these distances, cf. section 4.2. The reason for this behavior is not fully understood yet, but comparatively few neurons in the outermost layer are responsible for predictions at longer distances due to the chosen network setup, cf. section 4.1.3, fig. 9. Since the longer network variant has a considerably larger number of trainable parameters, it is assumed that 500 epochs might not have been enough to activate some of the outer neurons. Apart from that, the learning process for the “longer” variant in general looked more smooth and reached a lower validation error than before, fig. 27f, but the matrix plots did not show any obvious improvement over the initial ones, fig. 28.

Combining the “longer” and “wider” variants in the “wider-longer” setup with more convolutional layers and wider filter kernels also did not perform as expected. While improvements in the Pearson correlations could again be seen for 3 of 5 test chromosomes compared to the initial network, fig. 29, the observed correlations were worse than the ones from the highly similar “longer”-variant alone. Like with the similar “longer”-approach predictions at longer distances were partially missing. Compared to the other variants, the validation error was generally higher and stopped decreasing after very few epochs, fig. 29f. In terms of matrix plots, the predictions surprisingly were still quite similar to the initial ones, but seemed a bit more blurry, 30.

Predictions and metrics from the generalized DNN approach with feature bin size 5 kbp and matrix bin size 25 kbp are shown in fig. 31 and 32. Unfortunately, the results did again not improve compared to the initial predictions. While the learning curve was smooth and showed signs of slight overfitting beyond 300 epochs, fig. 31f, the matrix plots seemed worse than the initial ones. For example, the large structure at chromosome 3, 34 to 36.7 kbp, which had been detected by the previous approaches, was now completely missing.

5 Results

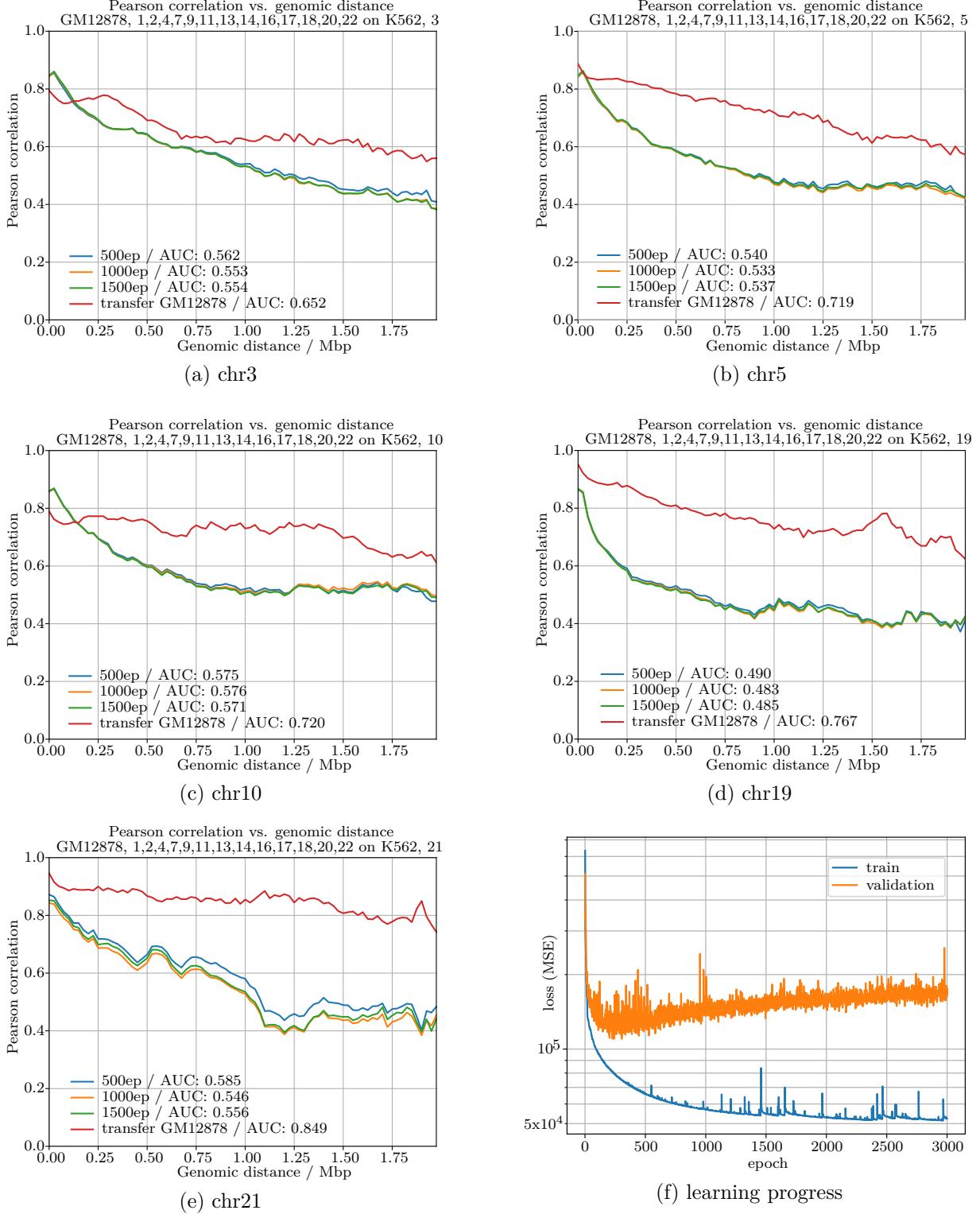


Figure 20: results / metrics, basic DNN, 25 kbp, test chromosomes

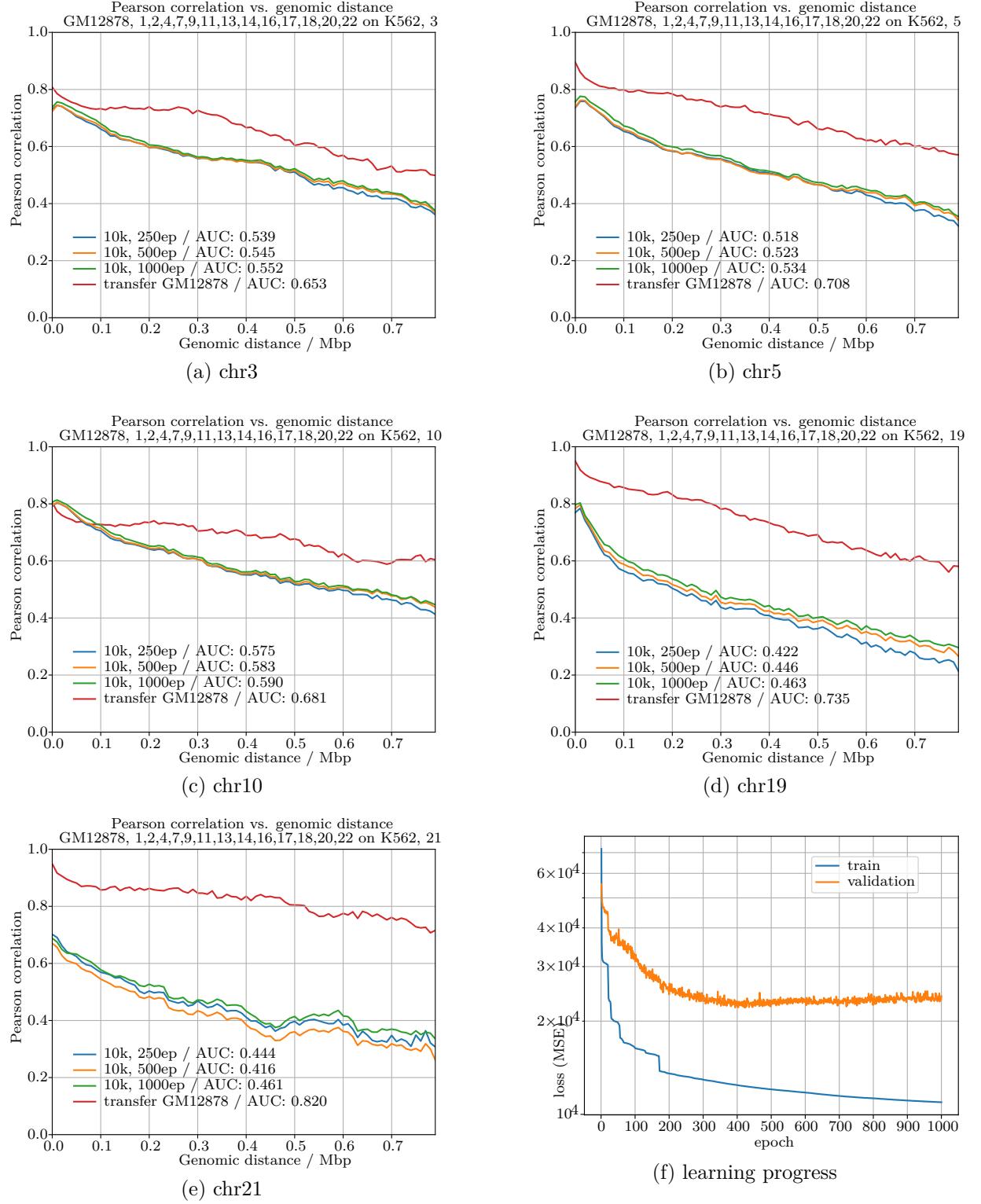


Figure 21: results / metrics, basic DNN, 10 kbp, test chromosomes

5 Results

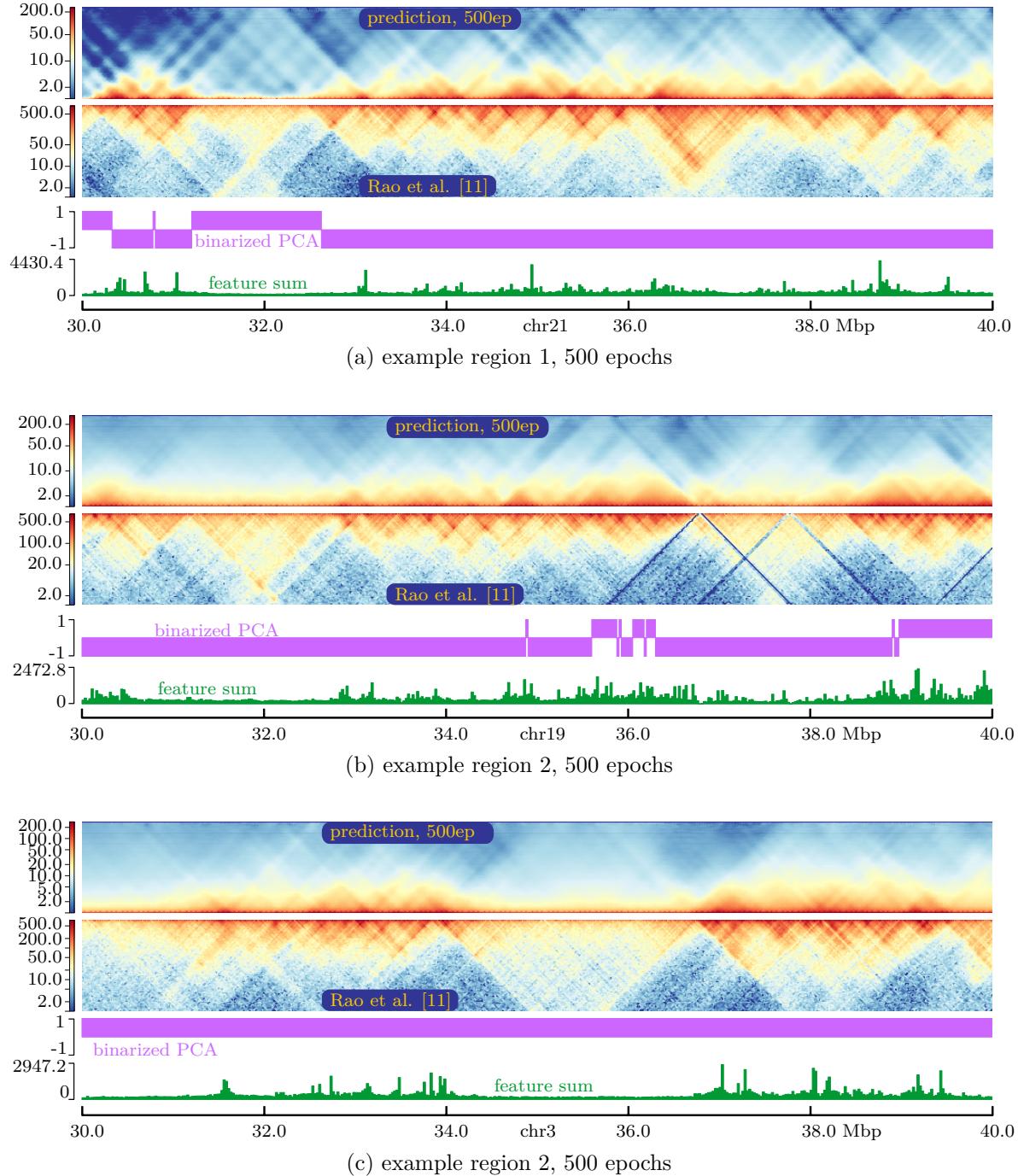


Figure 22: example predictions, basic DNN, 25 kbp, 500 epochs

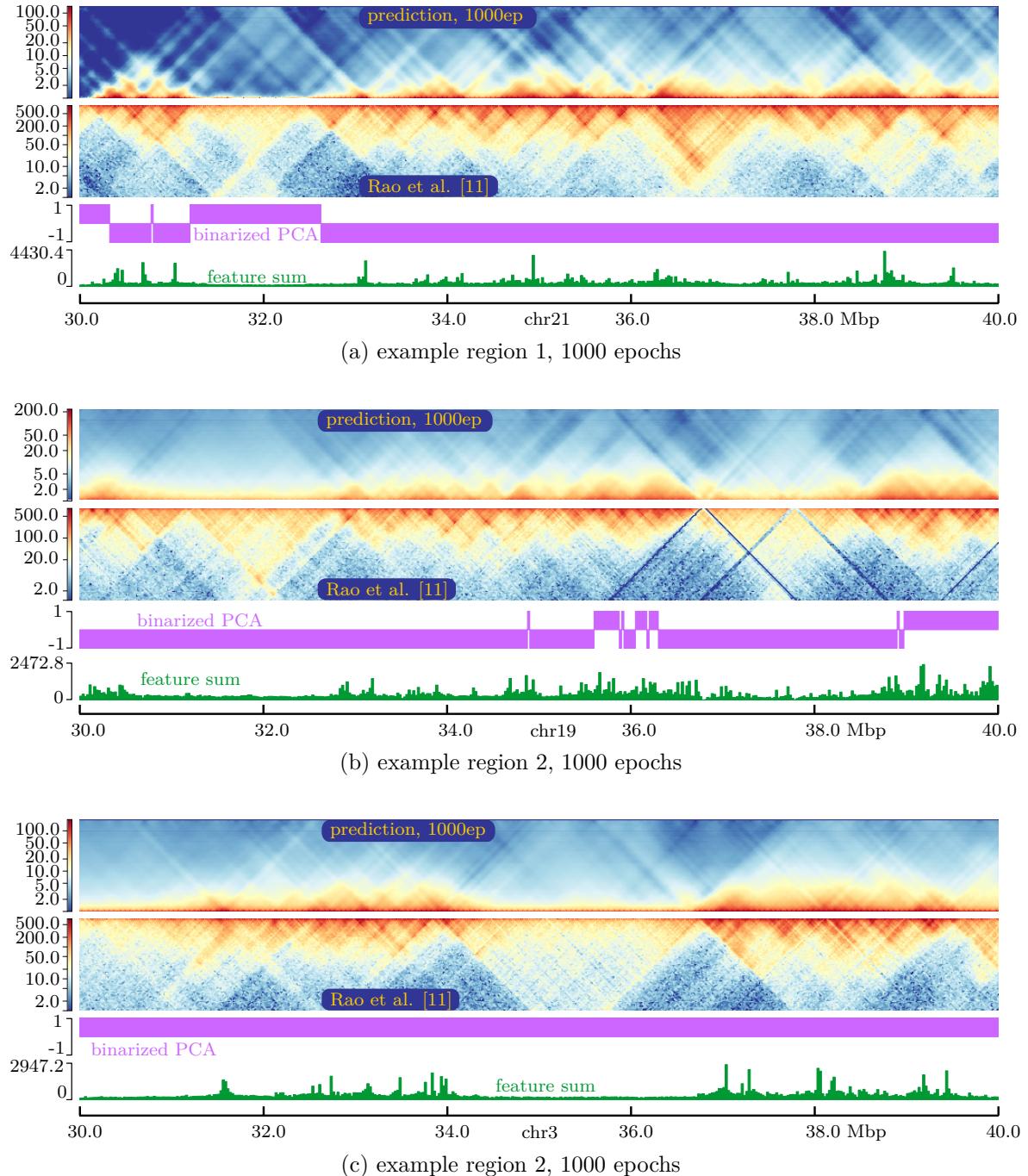


Figure 23: example predictions, basic DNN, 25 kbp, 1000 epochs

5 Results

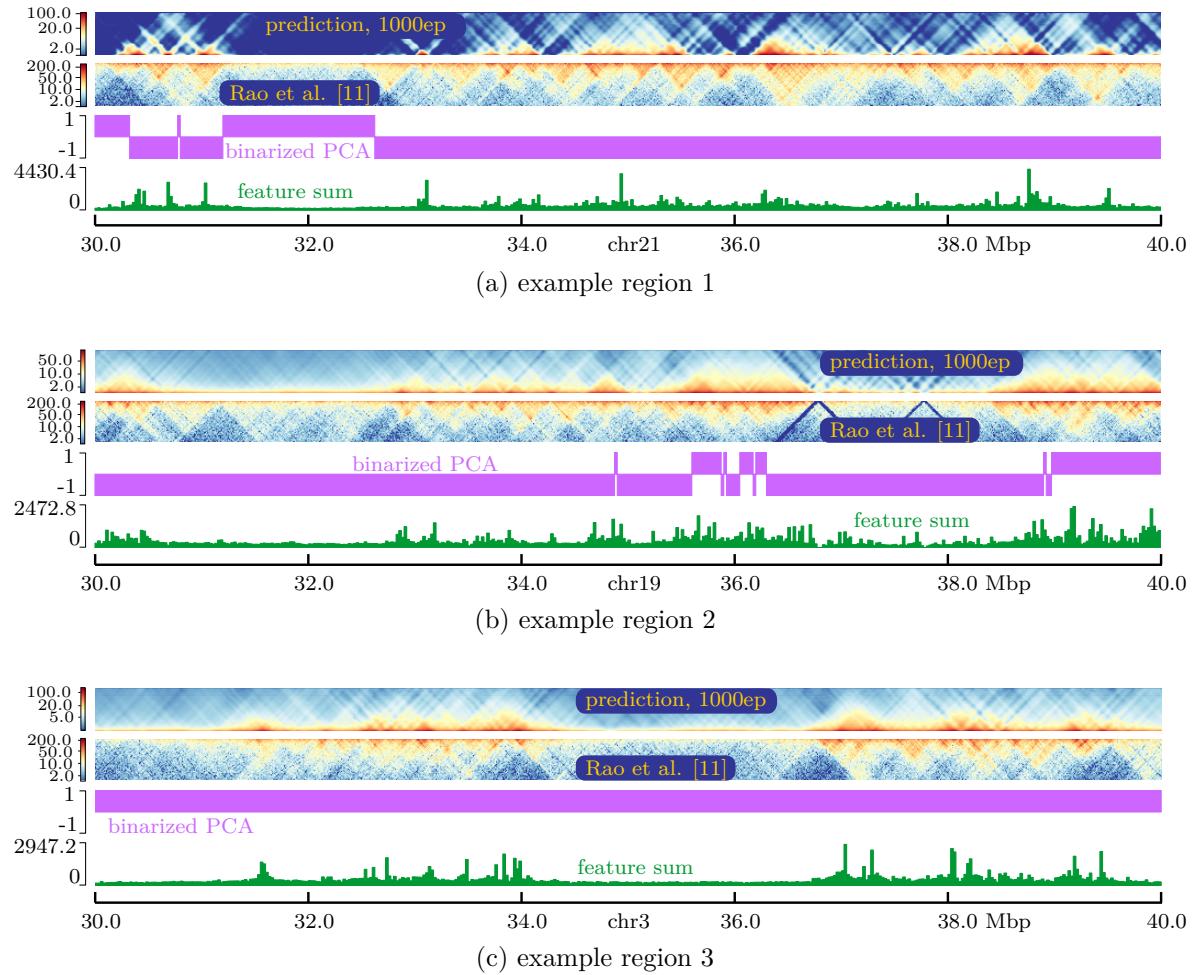


Figure 24: example predictions, basic DNN 10 kbp, 1000 epochs

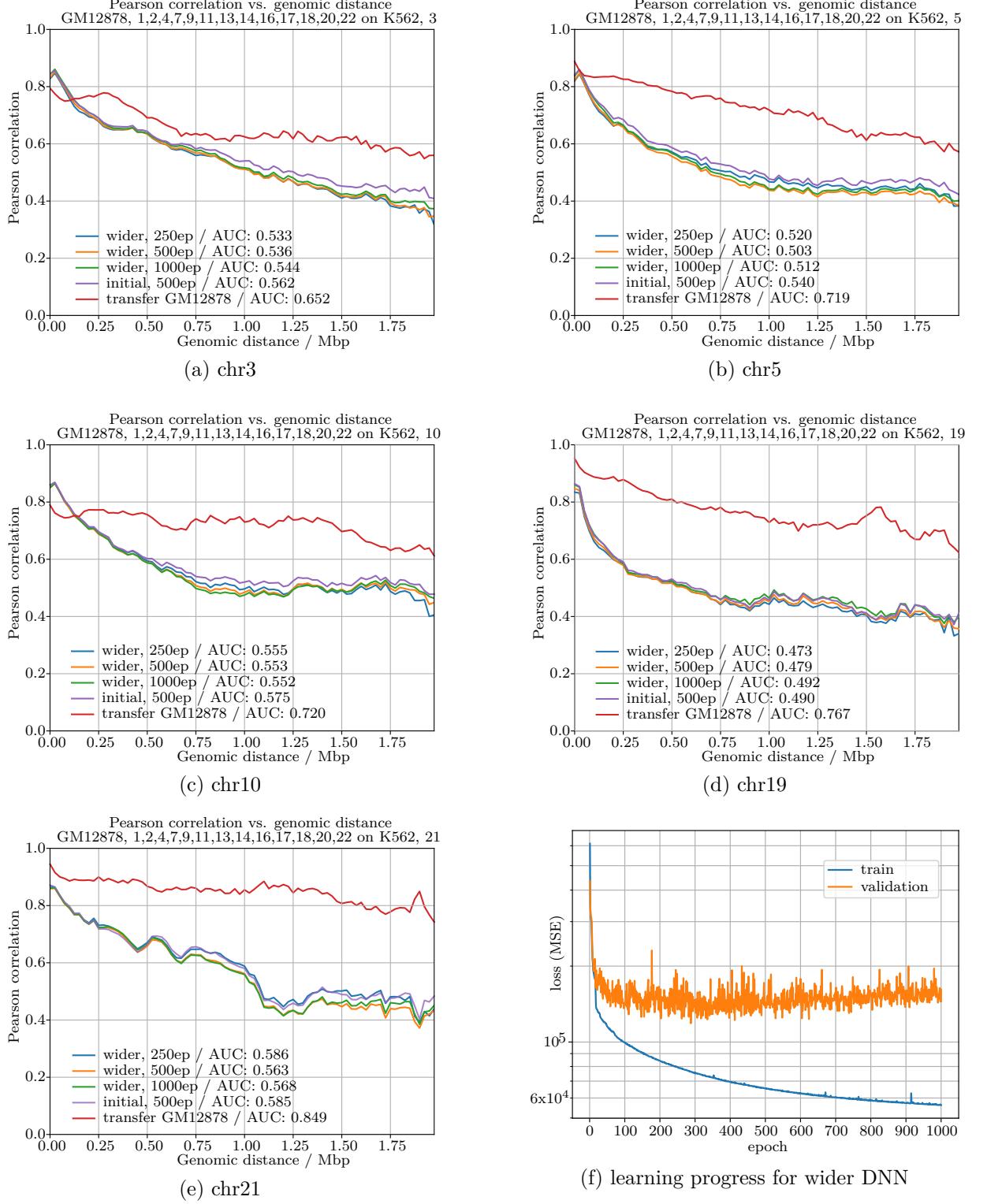


Figure 25: Pearson correlations, “wider” variant of DNN, test chromosomes

5 Results

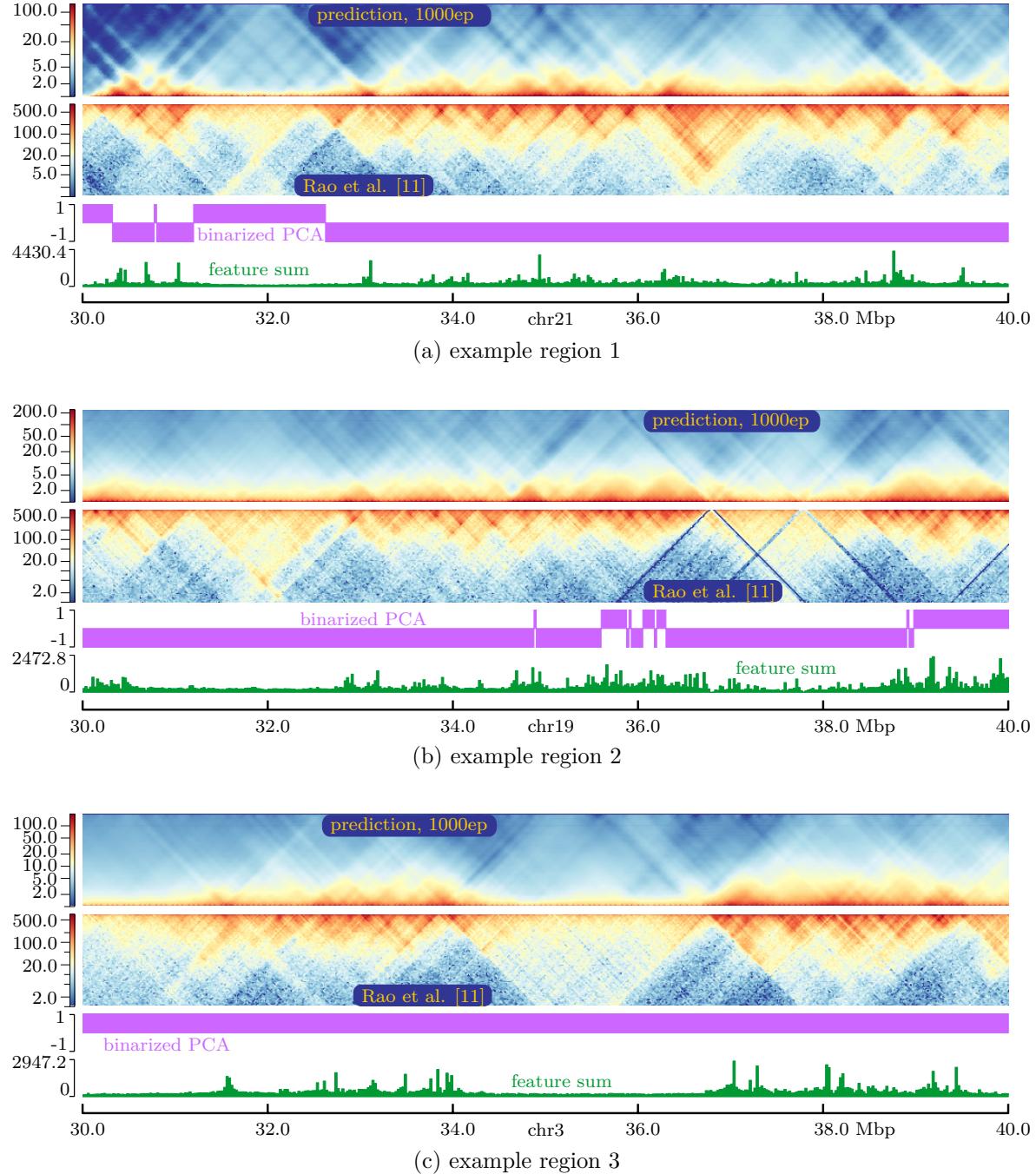


Figure 26: example predictions, “wider” variant of DNN 25 kbp, 1000 epochs

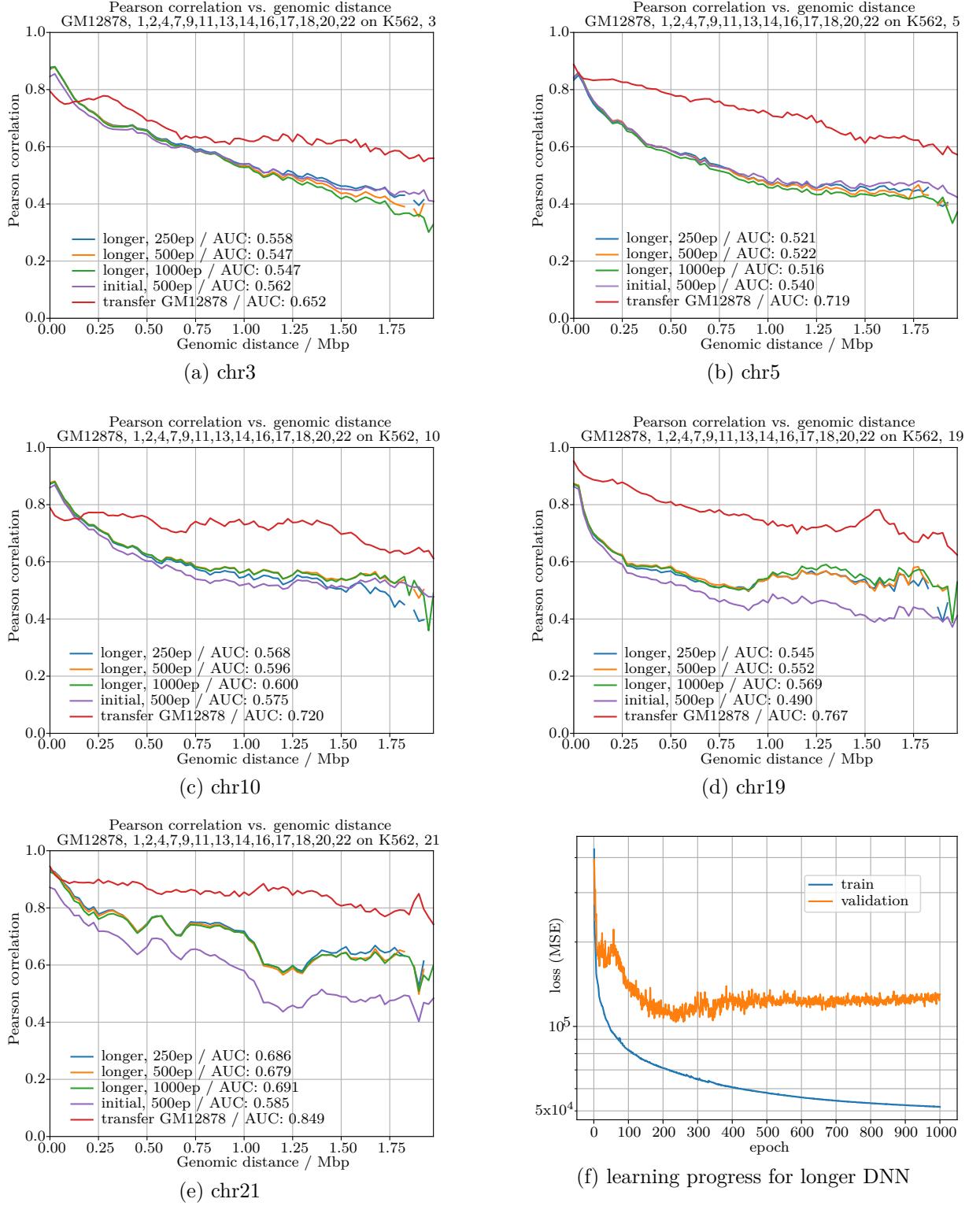


Figure 27: results / metrics, “longer” variant of DNN, test chromosomes

5 Results

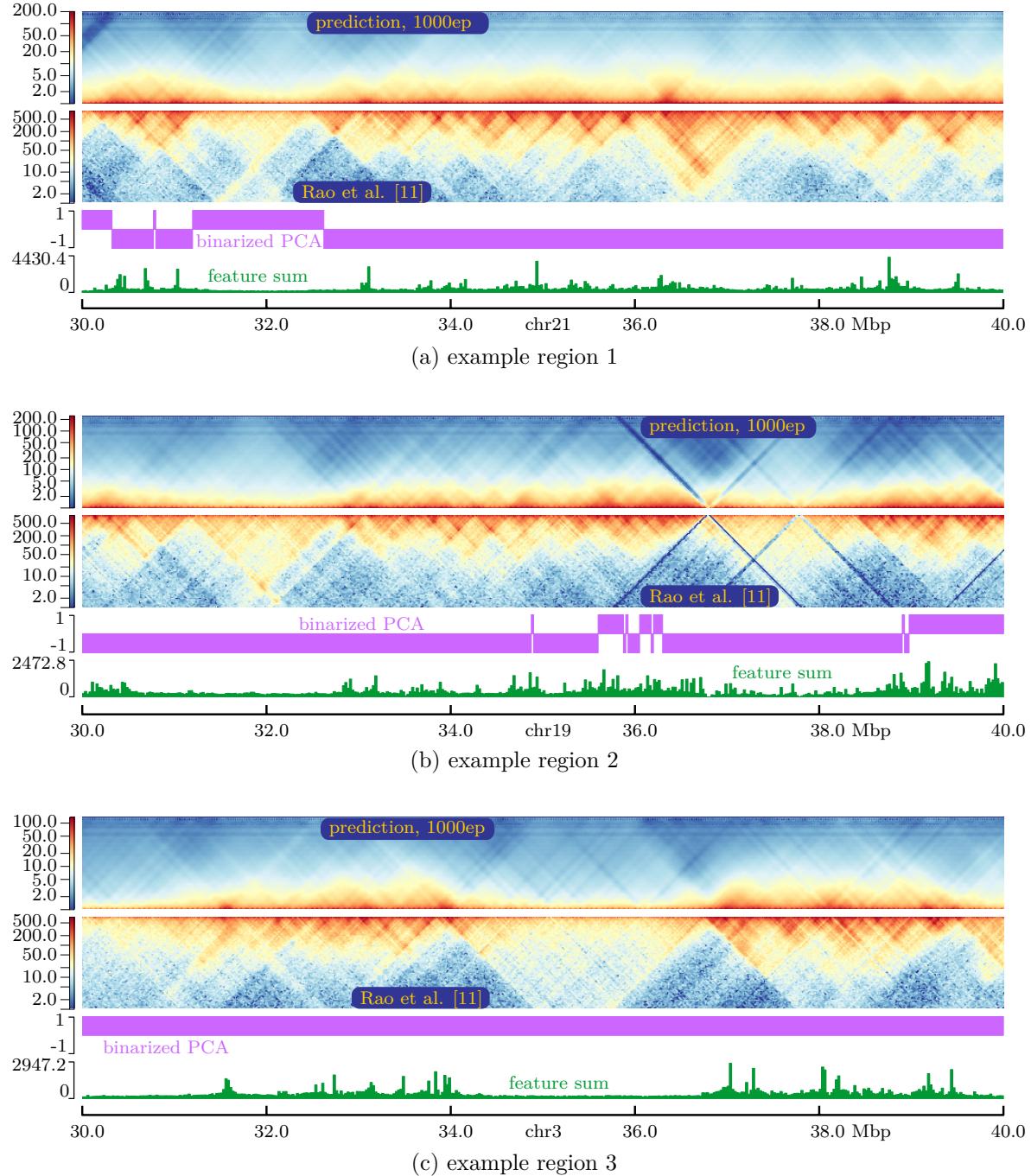


Figure 28: example predictions, “longer” variant of DNN 25 kbp, 1000 epochs

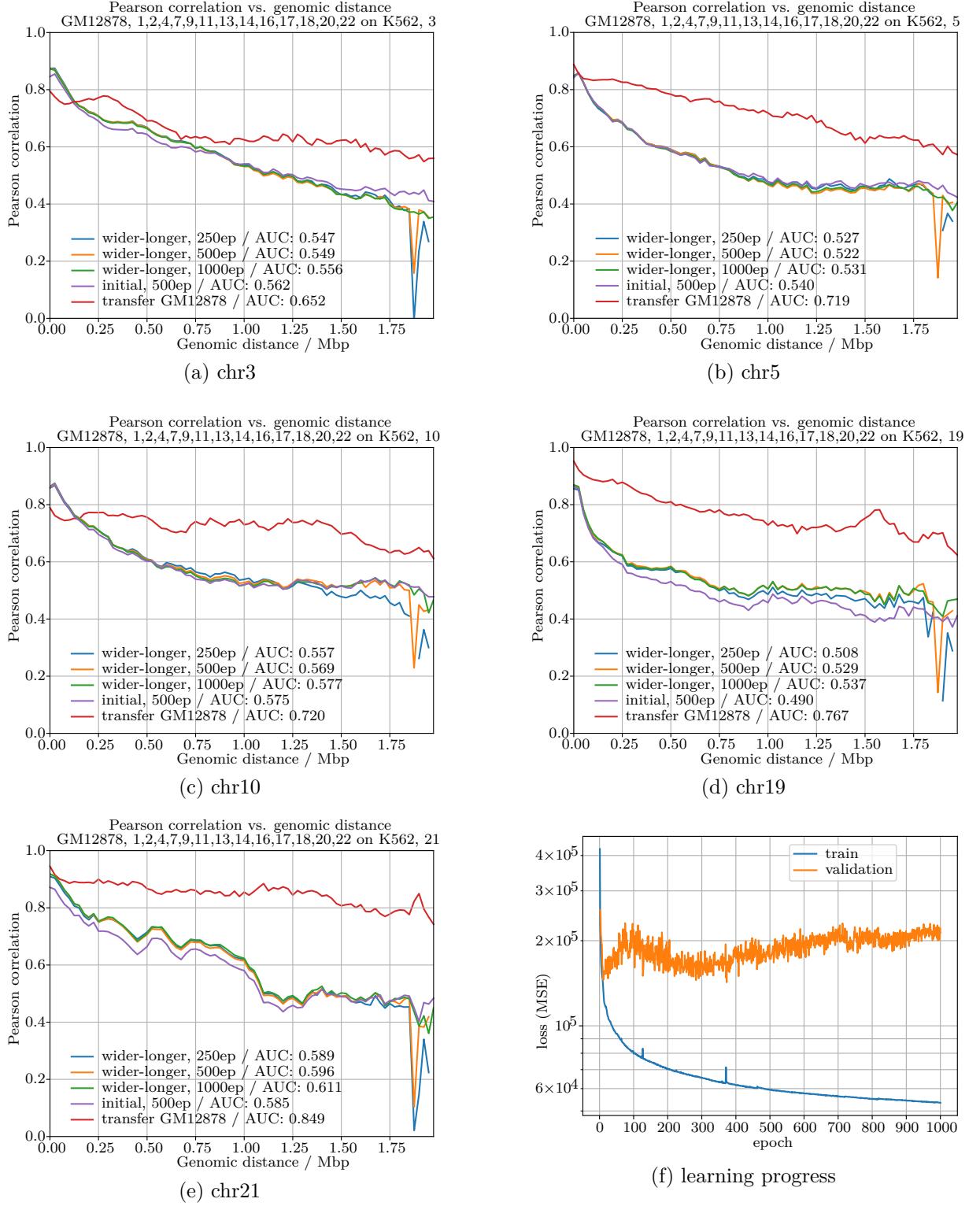


Figure 29: results / metrics, “wider-longer” variant of DNN, test chromosomes

5 Results

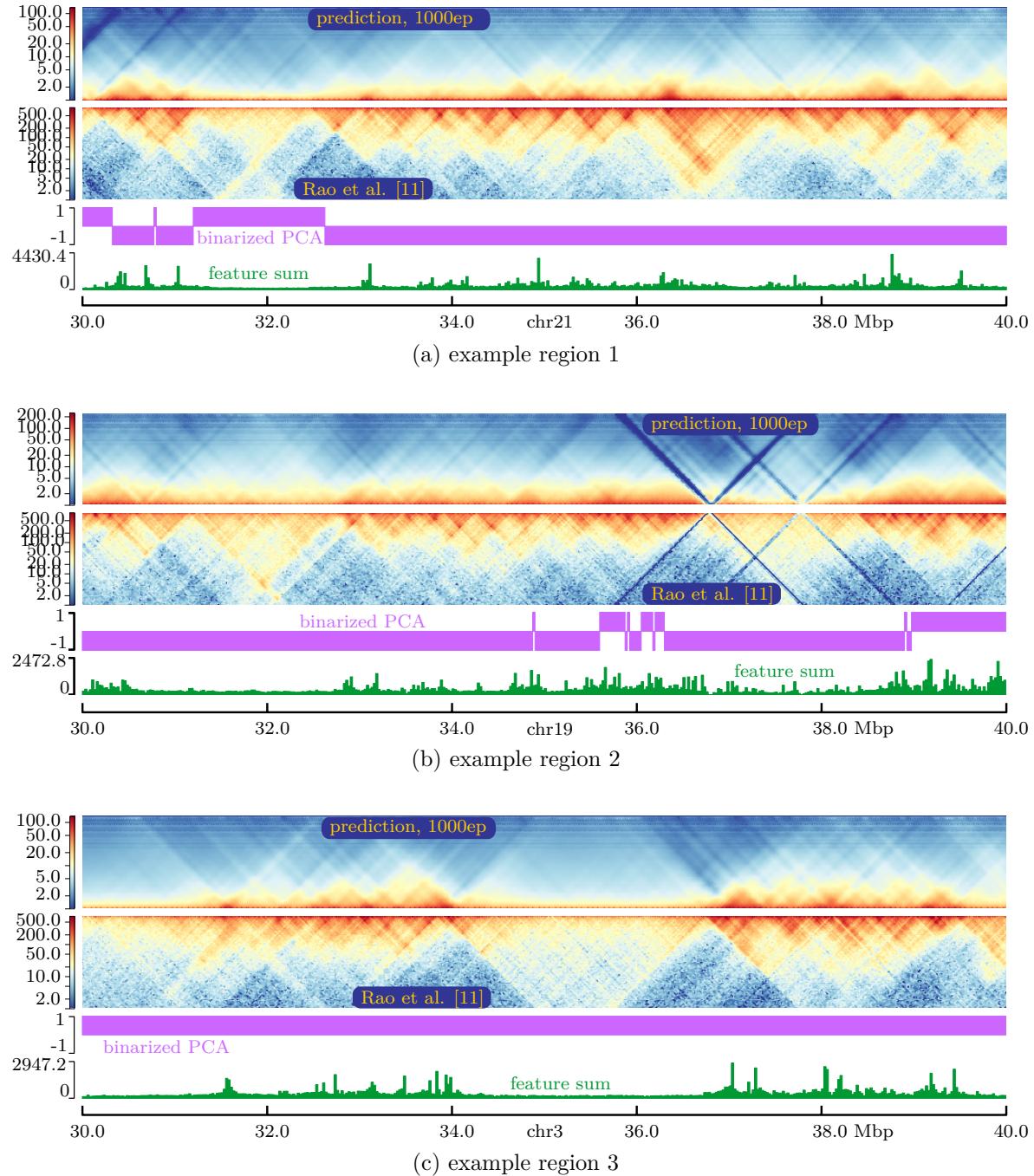


Figure 30: example predictions, “wider-longer” variant of DNN 25 kbp, 1000 epochs

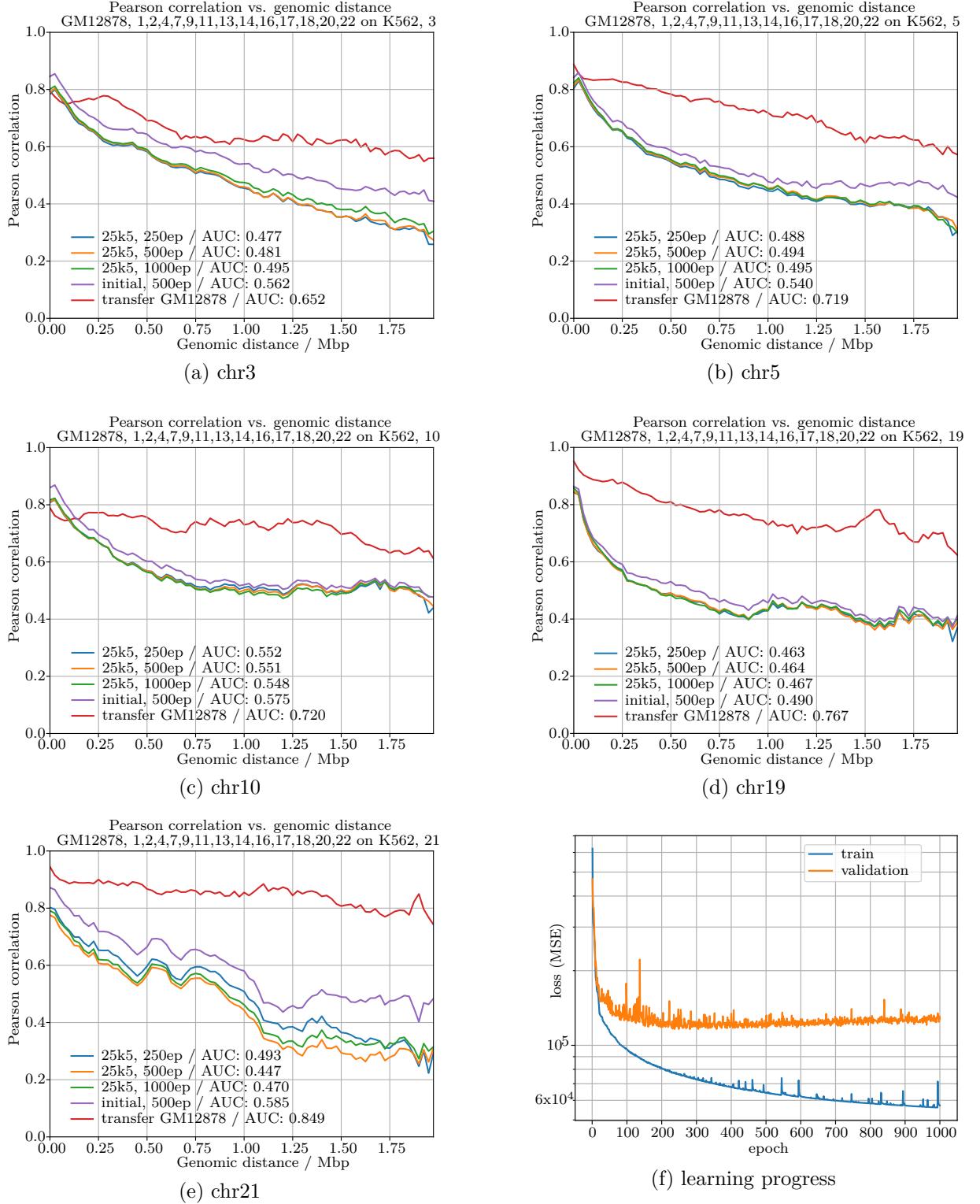


Figure 31: results / metrics, “5k – 25k” variant of DNN with $b_{feat} = 5$ kbp and $b_{mat} = 25$ kbp, test chromosomes

5 Results

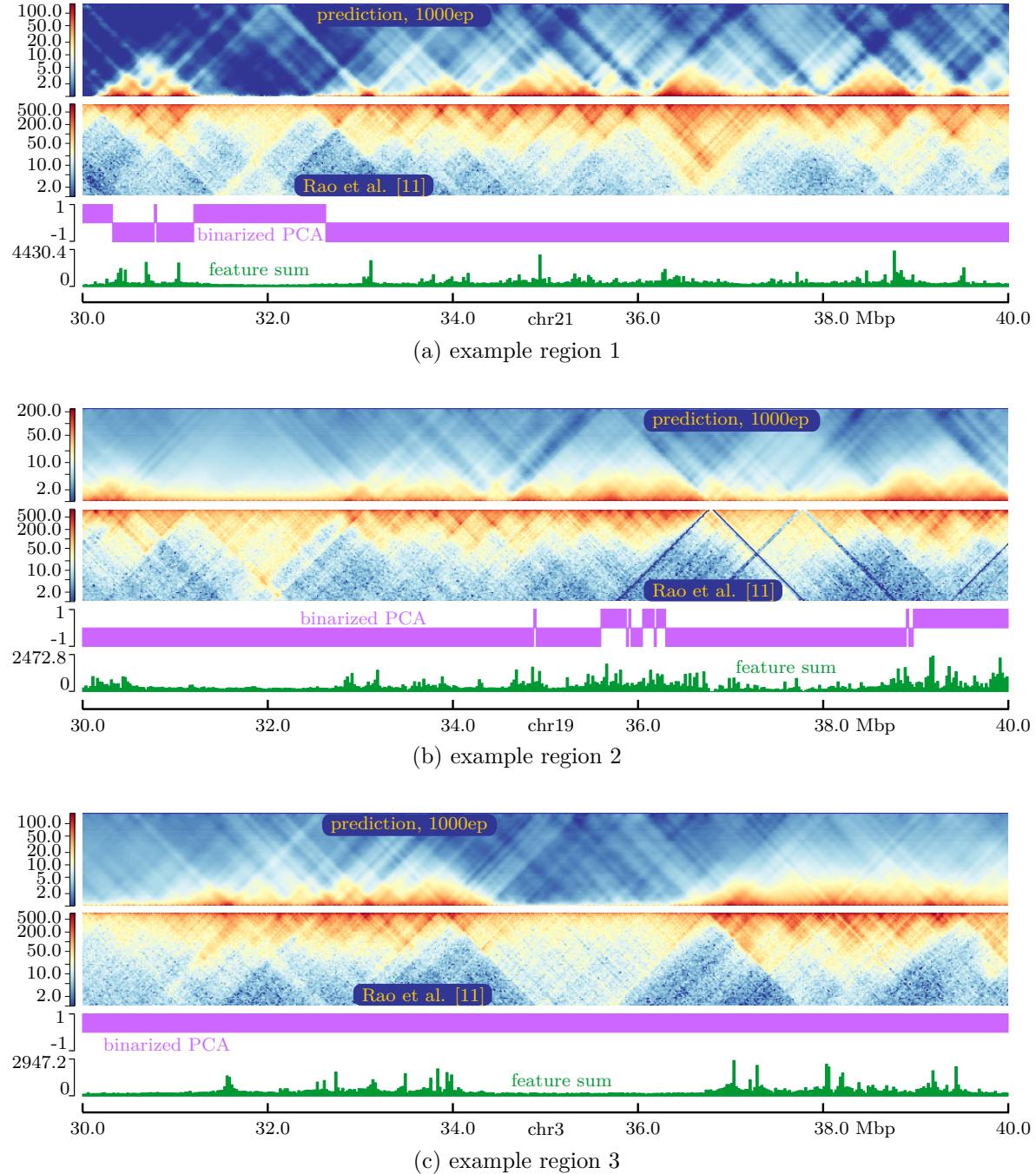


Figure 32: example predictions, “5k – 25k” variant of DNN, 1000 epochs

5.1.3 Results for combined loss function

Exchanging the mean squared error for a combined loss function consisting of MSE, TV loss and perceptual loss did not improve the results in the given setting. The results for optimizing a loss function according to eq. (10) with weighting parameters are shown in fig. 33 and 34.

For all test chromosomes, the correlations were highly similar to the initial network's, fig. 33, and the matrix plots also looked similar, chromosome 21 probably being the most different, fig. 34. The results plotted are the best ones obtained by manual tuning of the multiplicative parameters λ . Guided parameter tuning was unfortunately infeasible within the thesis at hand due to the training times required for optimizing the combined loss function. Other options which where not explored for the same reason include truncating the VGG-16 network at a different layer, using a loss function based on more than just one of the intermediate VGG-16 layers [53] or taking another loss network. However, the results obtained thus far were also not encouraging towards such investigations. While manually searching better parameters λ was not successful, it was found that the TV loss weight λ_{TV} needed to be much smaller than the two other weights. Otherwise, many true interactions off the matrix diagonals were considered as noise and optimized away early in the training process, cf. fig. 60 (p. 96).

5.1.4 Results for score-based loss function

Exchanging the MSE- loss function by a combination between score-based and MSE loss allowed for a smooth learning process and a slightly lower validation error compared to the initial approach. However, at around 7 min per epoch on a GPU, the training process also was about seven times slower than the initial approach on CPU. Unfortunately, the higher effort did not lead to obvious improvements.

The Pearson correlations for a score-based loss function with parameters $\lambda_{MSE} = 1.0$, $\lambda_{score} = 100$, $ds = 12$ are shown in figure 35. While a slight improvement was achieved for test chromosome 21, the correlations of the others remained widely unchanged. The matrix plots also looked fairly similar to the initial ones, fig. 36, chromosome 21 again being the most different compared to the initial predictions.

In the matrix plots, the true- and predicted scores have been added as a second track, replacing the PCA track. Indeed, the score curve computed from the true matrices showed local minima at putative TAD boundaries, as set forth in 3.1.4, so score computation with the chosen diamond size seemed sound. However, despite the optimization term in the loss function, the score curve of the predicted matrices compared to the true curve somewhat like the predicted matrices compared to the true ones: The predicted score was generally high, when the true score was high, and low when the true score was also low, but high peaks (local maxima) and steep valleys (local minima) in the plots were usually averaged out.

Long training times forbade a targeted parameter tuning by grid- or tree-search, so the results presented in this section should not be interpreted as the optimal ones achievable by a score-based loss function.

5 Results

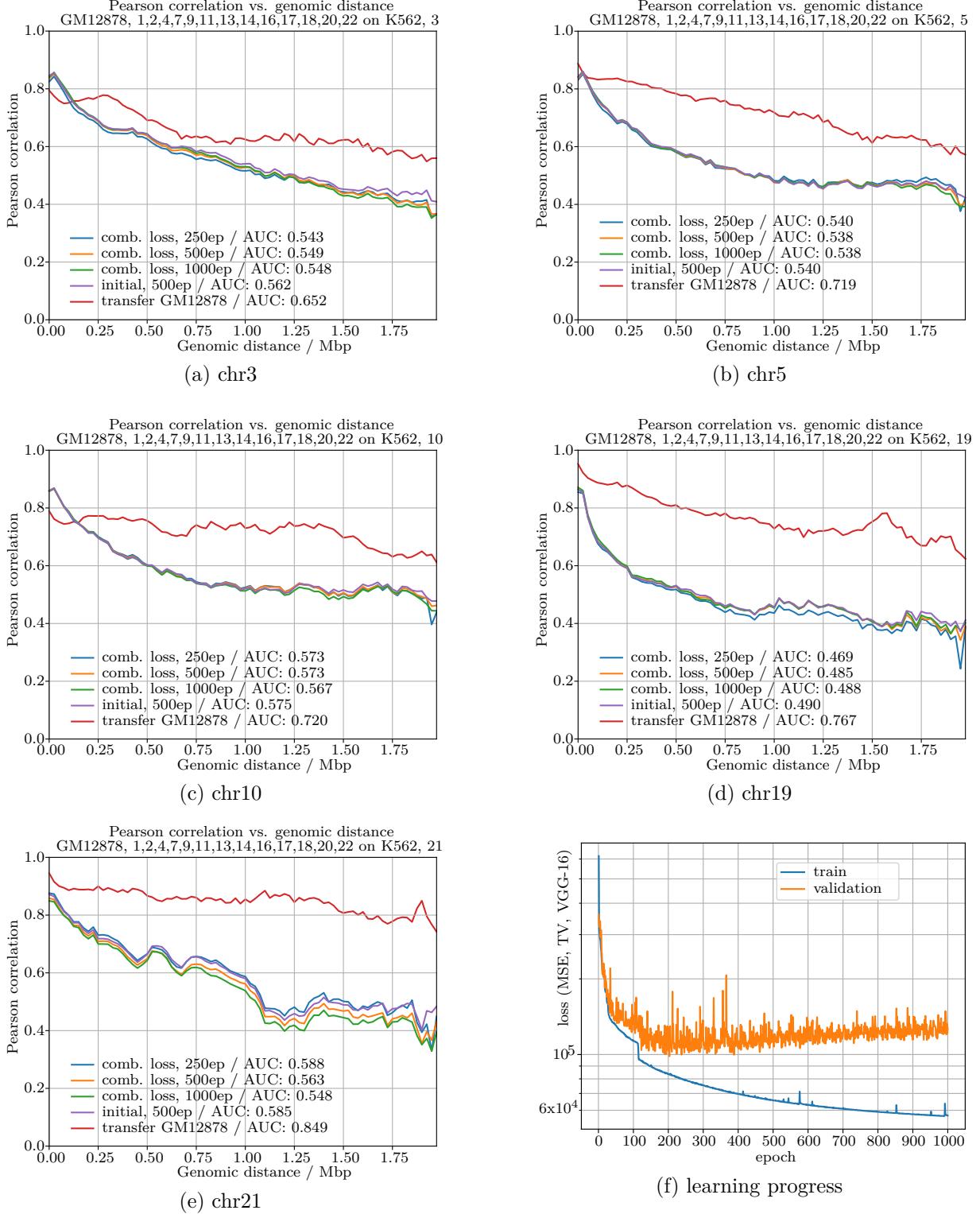


Figure 33: results / metrics, DNN with combined loss function (MSE, TV, VGG-16), test chromosomes

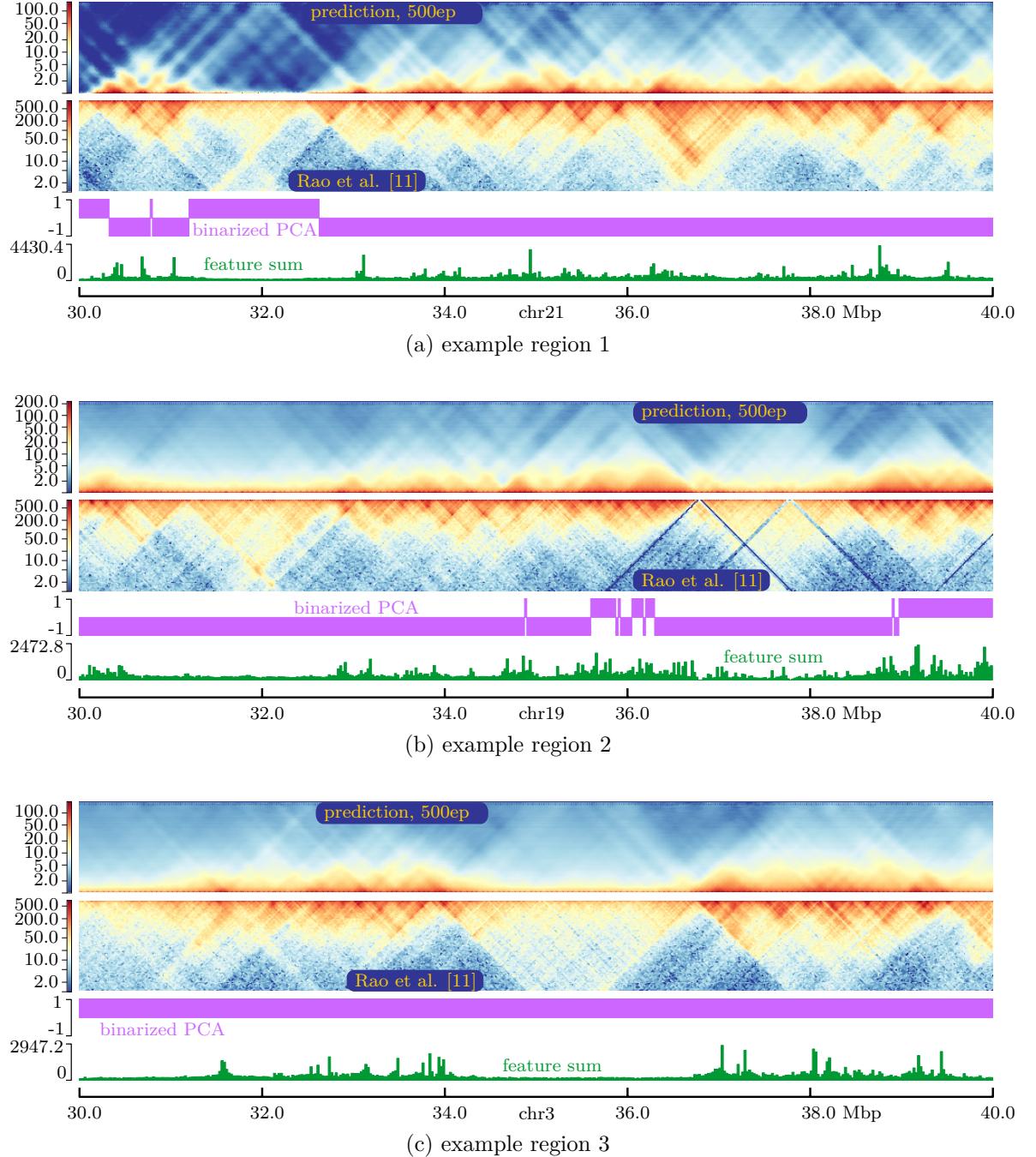


Figure 34: example predictions, DNN with combined loss function (MSE, TV, VGG-16), 500 epochs

5 Results

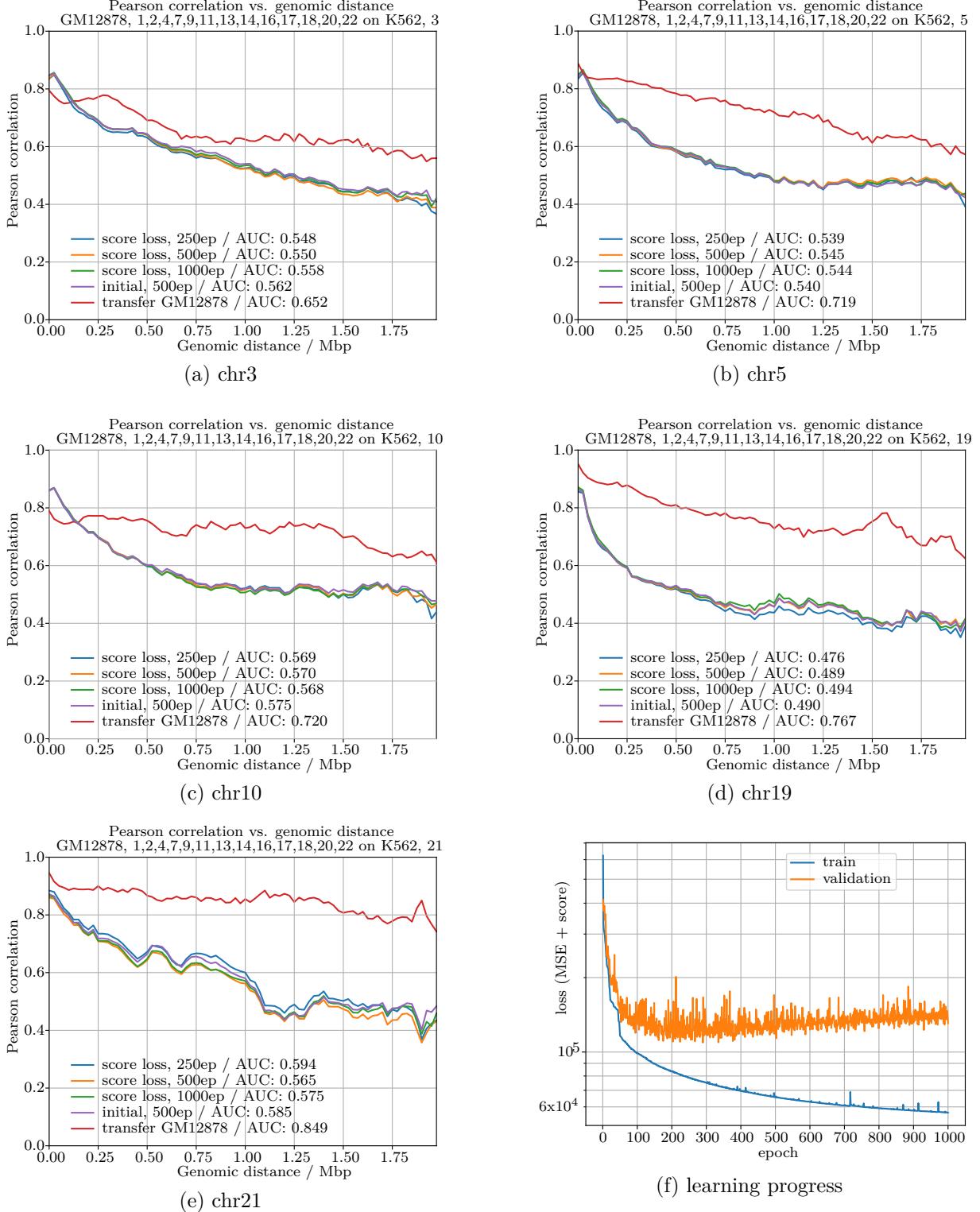


Figure 35: results / metrics, DNN with score-based loss function, test chromosomes
 $(\lambda_{MSE} = 1.0, \lambda_{score} = 100, ds = 12)$

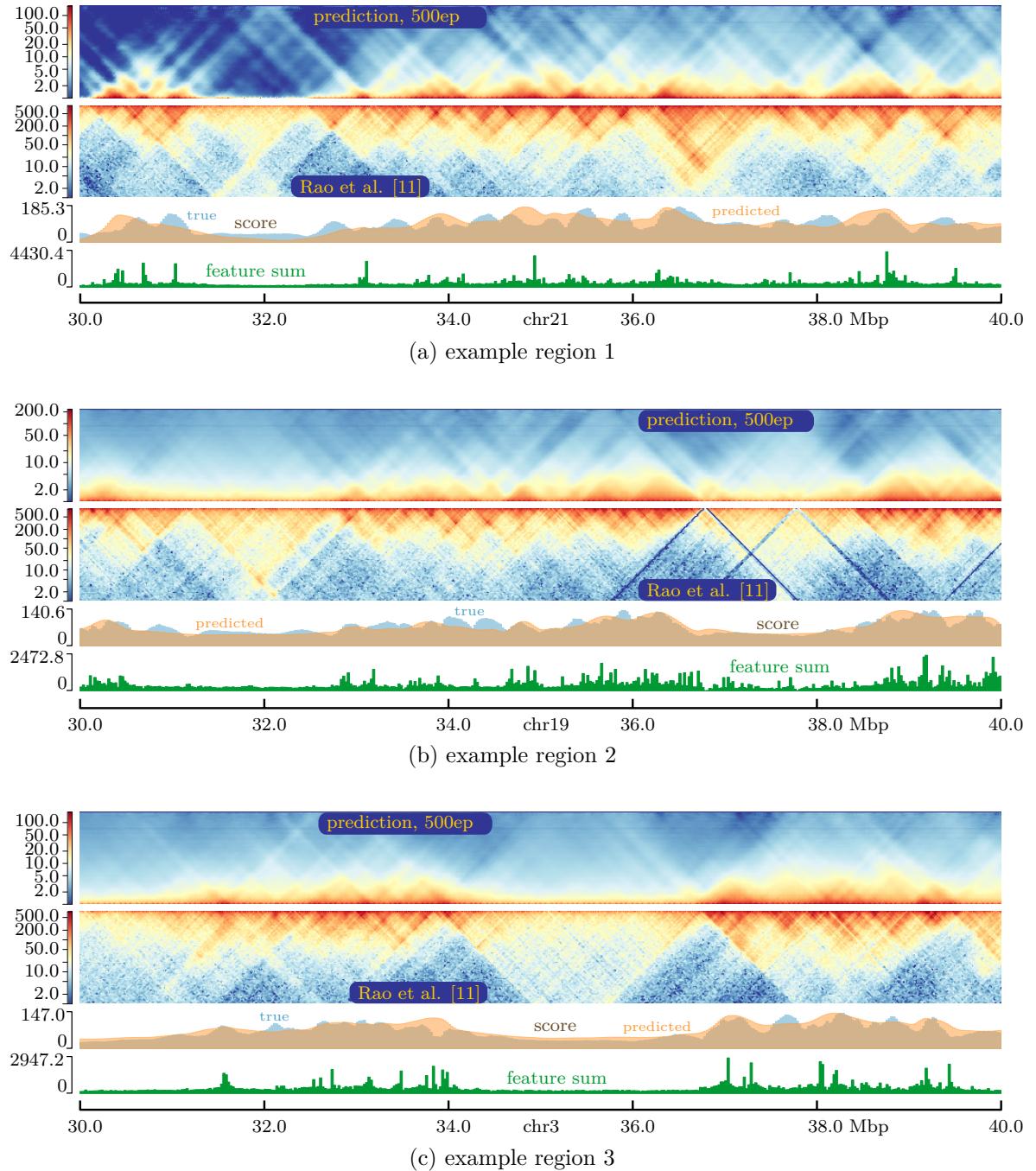


Figure 36: example predictions, DNN with score-based loss function, 500 epochs

5.1.5 Results for different binsizes and windowsizes

To assess predictions at larger bin sizes, five different approaches were compared, cf. section 3.1.5:

- a) “50k direct”:
directly training a network at bin size 50 kbp and predicting at that same bin size
- b) “initial 25k coarsened”:
coarsening the results of the initial network discussed above (section 5.1.1) by summarizing bins via `cooler coarsen`, cf. section 4.1.1
- c) “initial 25k→50k”:
using the initial network trained at 25 kbp (cf. section 5.1.1) to predict at 50 kbp
- d) “25k+50k→50k”:
predicting at 50 kbp from a network *simultaneously* trained with bin sizes 25 and 50 kbp
- e) “25k+50k→25k”:
predicting at 25 kbp from a network *simultaneously* trained with bin sizes 25 and 50 kbp

The best Pearson correlations at bin size 50 kbp were generally obtained either by coarsening the initial results to 50 kbp (method b) or by taking the network trained at 25 kbp for predicting at 50 kbp (method c), fig. 37. Compared to coarsening, the latter approach had the advantage of doubling the window size (in base pairs) and it worked better for test chromosome 21.

Looking at the corresponding matrix plots, the desired effect of making larger structures more prominent by increasing the bin size was only partially achieved, fig. 39. While all larger structures in the example cutout of test chromosome 3 indeed looked more prominent, fig. 39c, no obvious improvement was observed for the medium-sized structures in the example regions of chromosome 19 and 21, figures 39a and 39b.

Direct predictions at bin size 50 kbp (method a) were definitely not better than indirect methods derived from networks trained at 25 kbp. Both the Pearson correlations and the matrix plots were clearly better for method b) and c), figures 37, 38 and 39. It is not known why the direct predictions turned out worse. Potential reasons include the reduced number of samples (cf. table 3, p. 27) and the binning process, or a combination of both. However, first investigations showed that binning the proteins using the maximum instead of the mean across the 50 kbp-bins, cf. section 4.1.3, did not improve the results.

Notably, the training process for the direct prediction at bin size 50 kbp (method a) diverged after about 420 epochs. One possible reason for this could be too high a learning rate, which could have been avoided by decreasing the learning rate over time. However, no further investigations were made into the case, because the divergence occurred only after overfitting, fig. 37f, and was thus not seen as too problematic here. The minimum validation error was reached after about 150 epochs and thus about 100 epochs earlier than in the initial setup at 25 kbp. This is not surprising, since there are only about half as many training samples at 50 kbp compared to 25 kbp, cf. table 3 (p. 27).

Simultaneously training a network with matrix- and feature bin sizes of 25 kbp and 50 kbp (methods d, e) turned out unproblematic with regard to convergence, fig. 40f, but the Pearson correlations when predicting at both 25 kbp and 50 kbp were – often significantly – worse than the initial

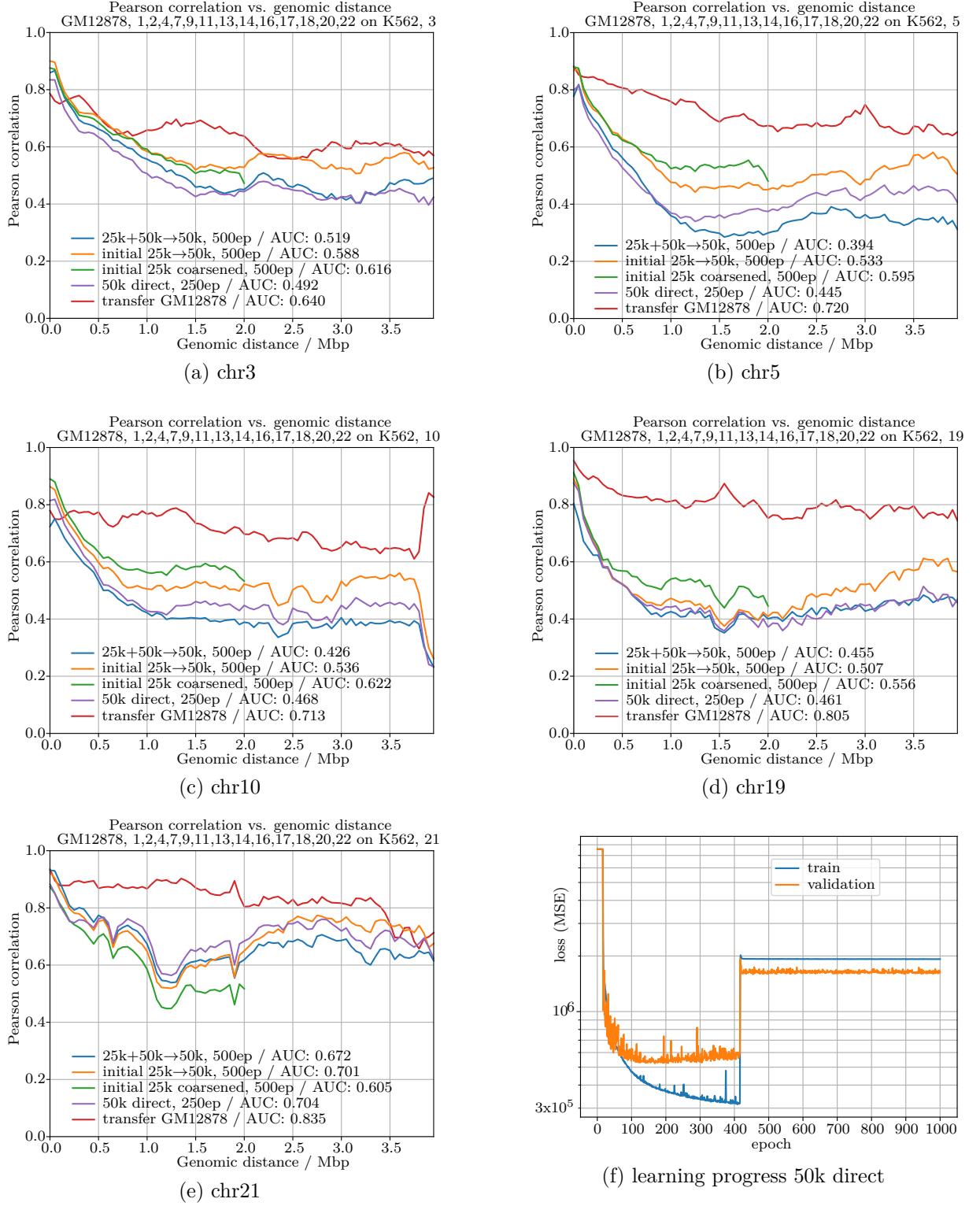


Figure 37: results / metrics, various DNNs at 50 kbp

5 Results

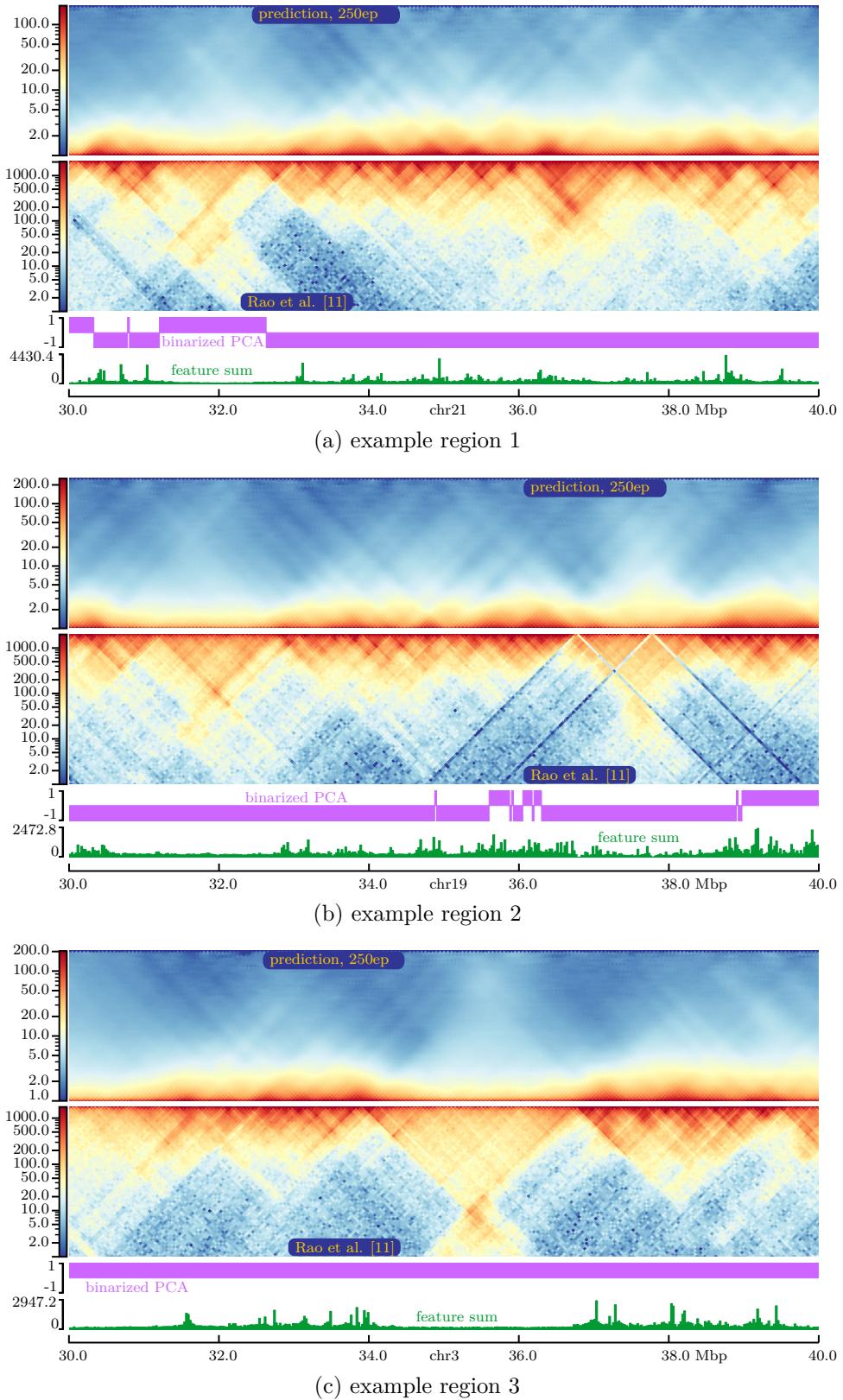


Figure 38: example predictions, DNN at 50 kbp direct, 250 epochs

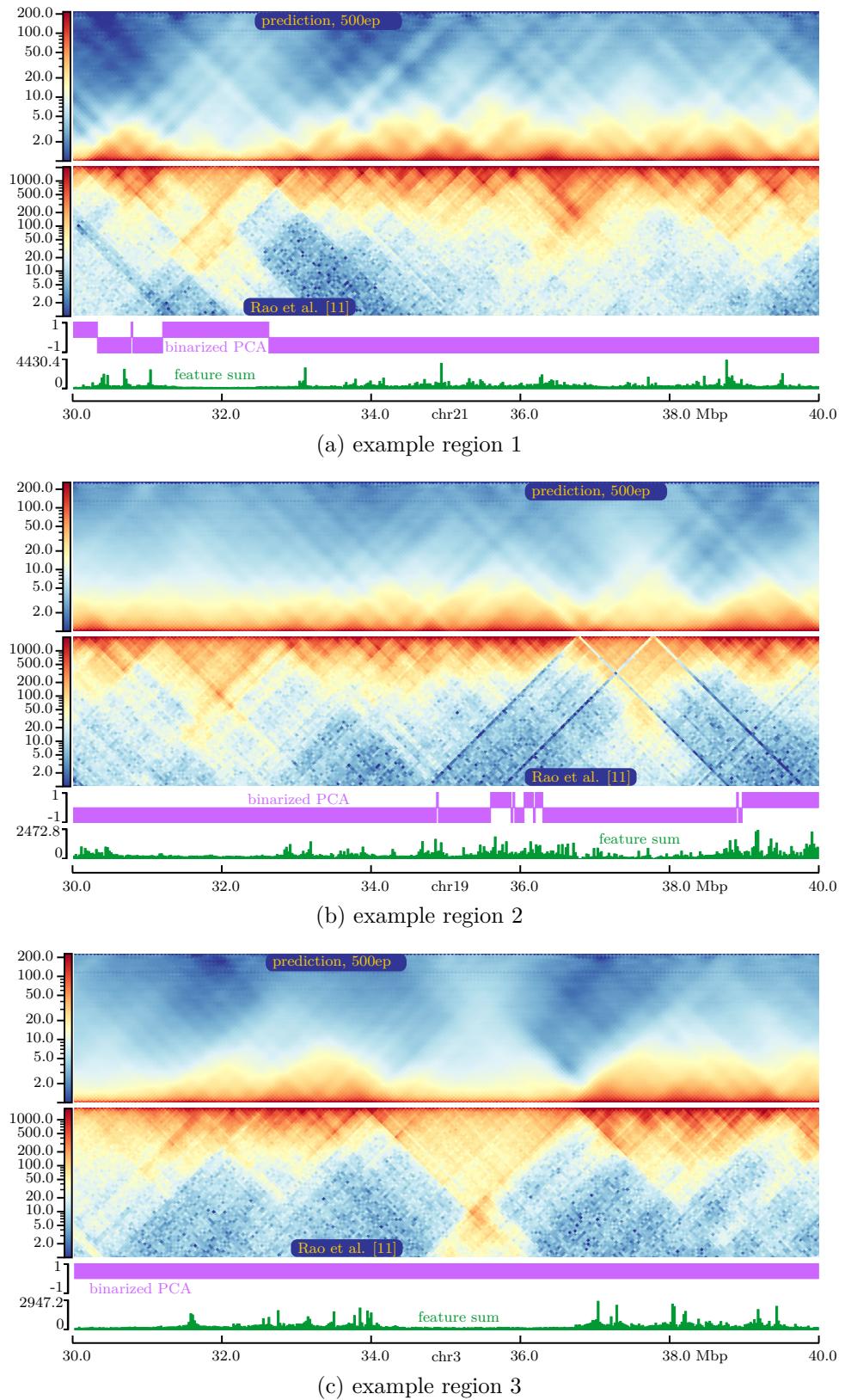


Figure 39: example predictions, DNN trained at 25 kbp predicting at 50 kbp, 500 epochs

5 Results

predictions at the respective bin size, fig. 37 (“25k+50k→50k”) and fig. 40 (“25k+50k→25k”). Looking into the matrix plots shown in fig. 41, all predictions seemed equally useless and definitely worse than the results obtained by the other approaches investigated thus far. It could not be clarified what caused the improvement in Pearson correlations for chromosome 21 compared to the initial predictions at 25 kbp, fig. 40e, but it is interesting that even predictions with such a high degree of blurriness as in fig. 41a can reach an AUC of around 0.65.

XXXget this section in shape Another finding relates to the training progress curve – such smooth courses of the training loss usually occur together with gradient-style predictions as observed in test chromosome 19. These gradients seem to be a local minimum with regard to mean squared error in which the optimizer may end up under certain circumstances. However, these types of predictions generally cause Pearson correlations close to zero, and it remained unclear why this did not happen here – the other parts of chromosome 19 did not look much better than the 10 Mbp-cutout shown in fig. 41b. **XXX**

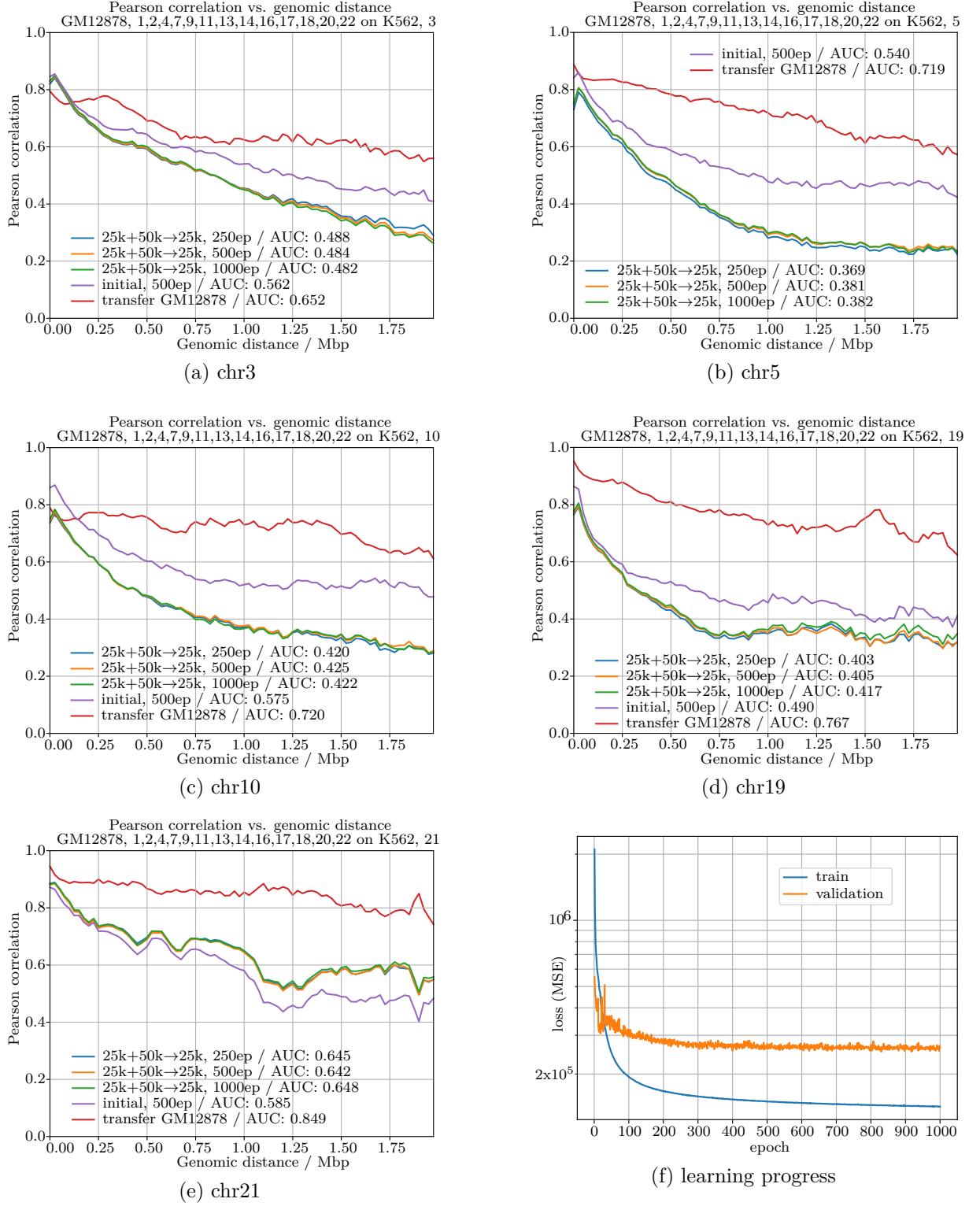


Figure 40: results / metrics, DNN trained at 25 kbp and 50 kbp simultaneously

5 Results

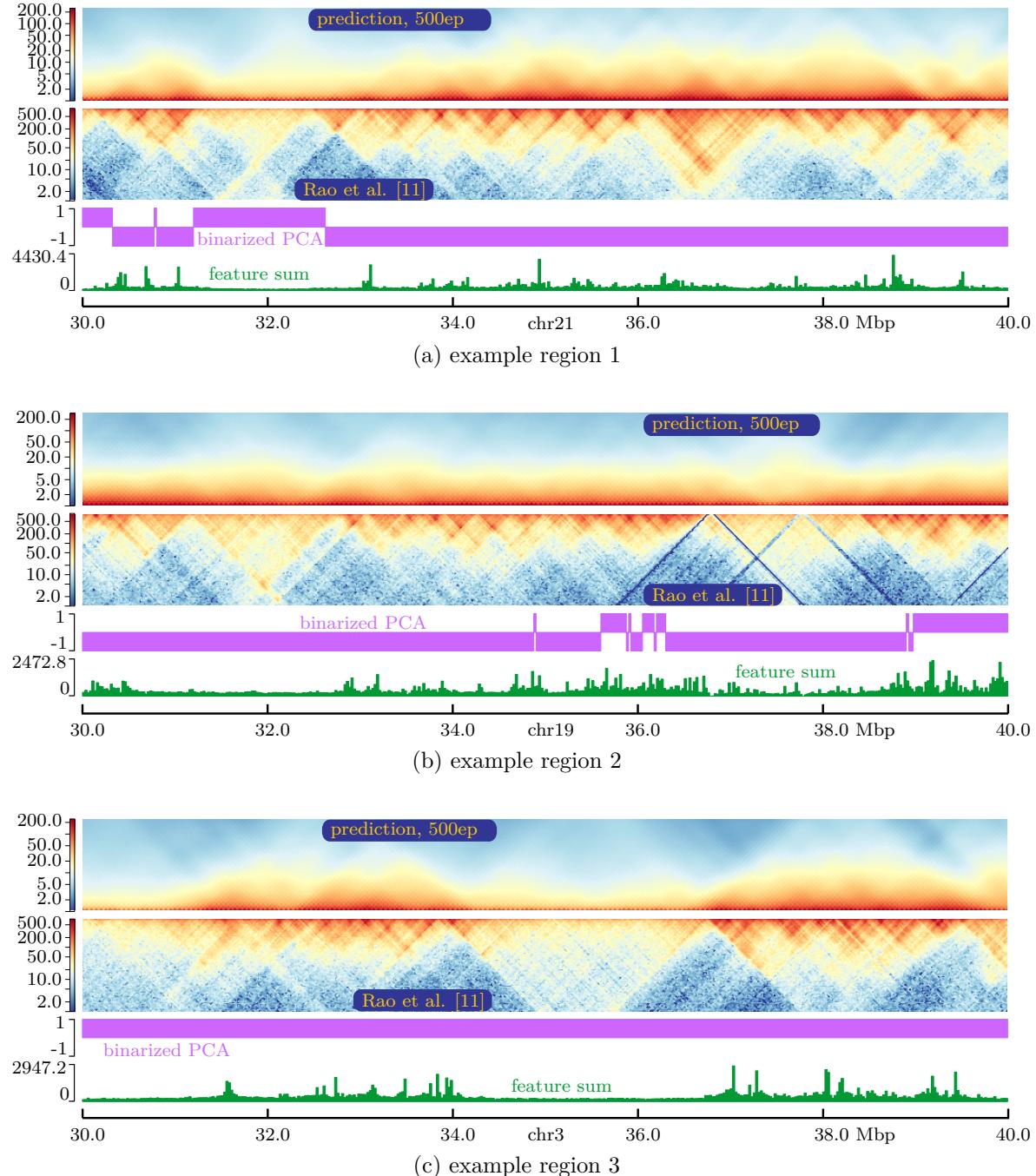


Figure 41: example predictions, DNN trained at 25 kbp and 50 kbp simultaneously, 25 kbp, 500 epochs

5.2 Hi-cGAN approaches

5.2.1 cGAN with DNN embedding

Results after pretraining see figures 44 and 45. Results without pretraining see figures 42 and 43.

5.2.2 cGAN with CNN embedding

The results from the cGAN were generally better than the best results from the DNN, and from windowsize 128, they were also close to the baseline or better. Interestingly, acceptable results were obtained already after only 25 epochs. Fast convergence is well known from pix2pix [48], but it is still surprising that this property was maintained despite the changes made to the original network.

For windowsizes 64 and 128 bins, the optimal number of epochs seemed to be around 80, while for windowsize 256, a number greater 100 epochs might have further improved the results. However, this would have come at a large computation time, since average training time was around 108 min per epoch on the given hardware.

5.2.3 cGAN with mixed DNN / CNN embedding

The results from the cGAN with mixed embedding, i.e. DNN-embedding for the generator and CNN-embedding for the discriminator are shown in fig. 54 and 55 (with pre-trained DNN-embedding network) and **XXX, XXX**(with standard weight initialization)

5 Results

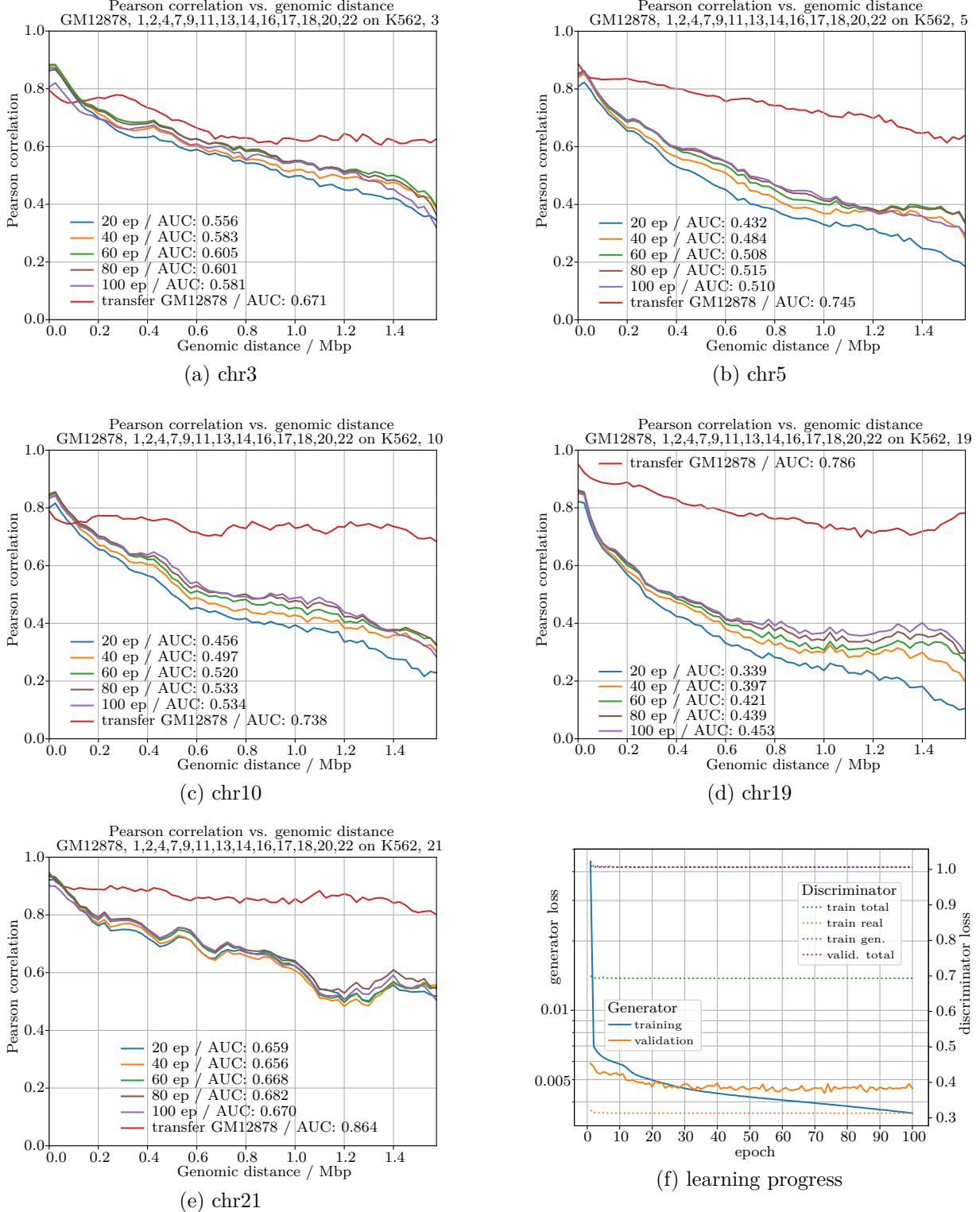


Figure 42: results / metrics cGAN, DNN embedding, no pre-training, $w = 64$, test chromosomes

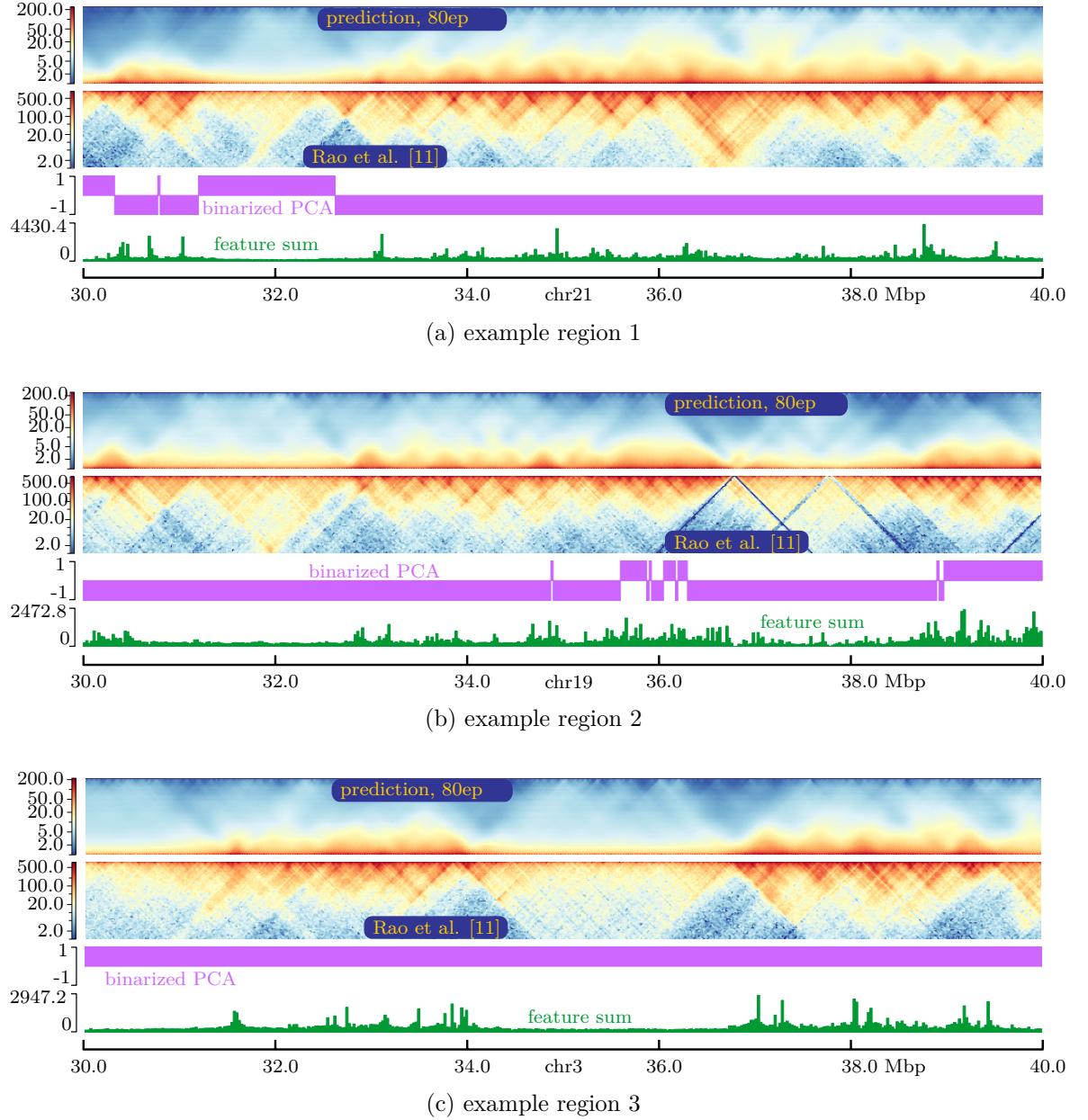


Figure 43: example predictions, cGAN, DNN embedding, no pre-training, $w = 64$, 80 epochs

5 Results

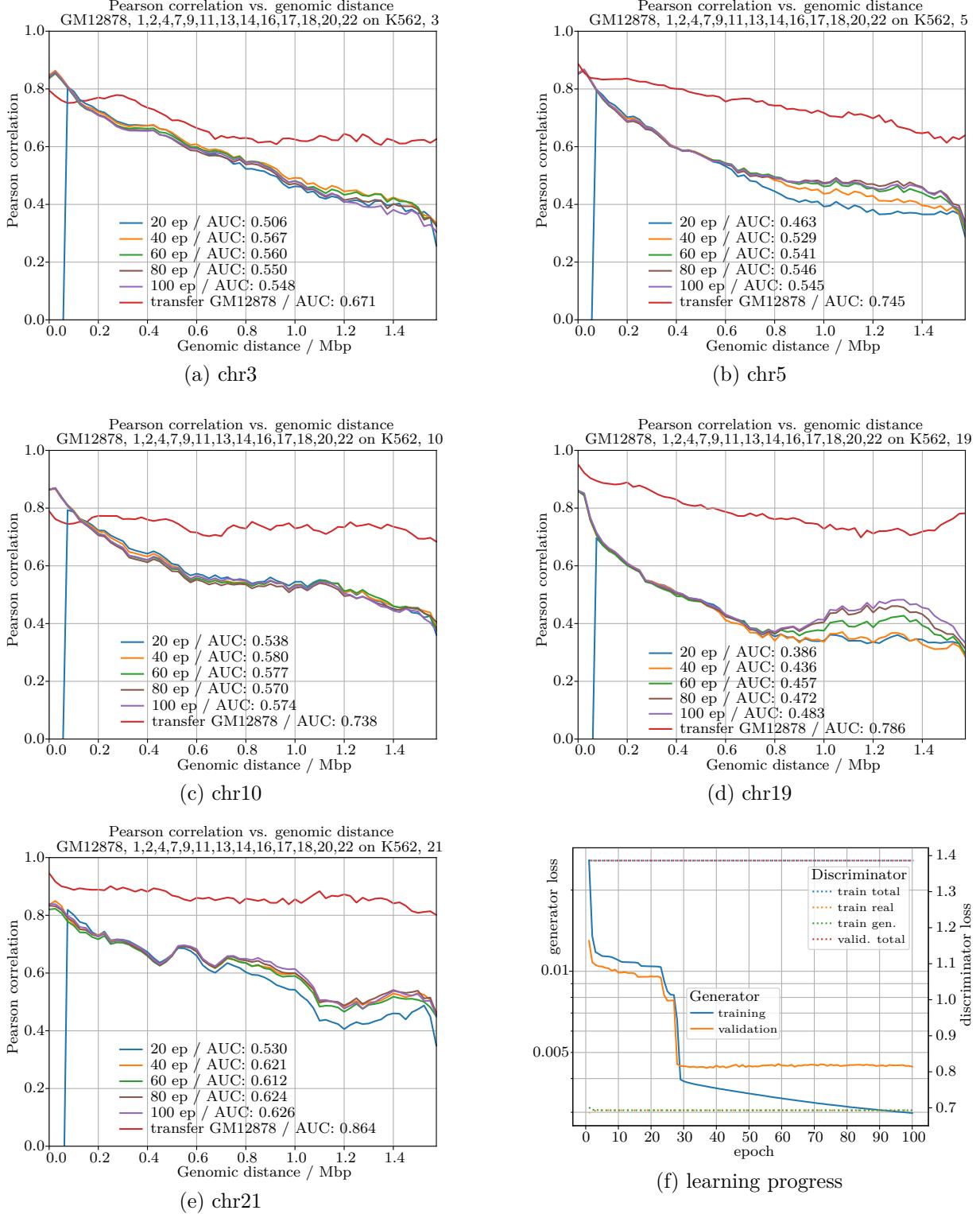


Figure 44: results / metrics, cGAN, DNN embedding, pre-trained, $w = 64$, test chromosomes

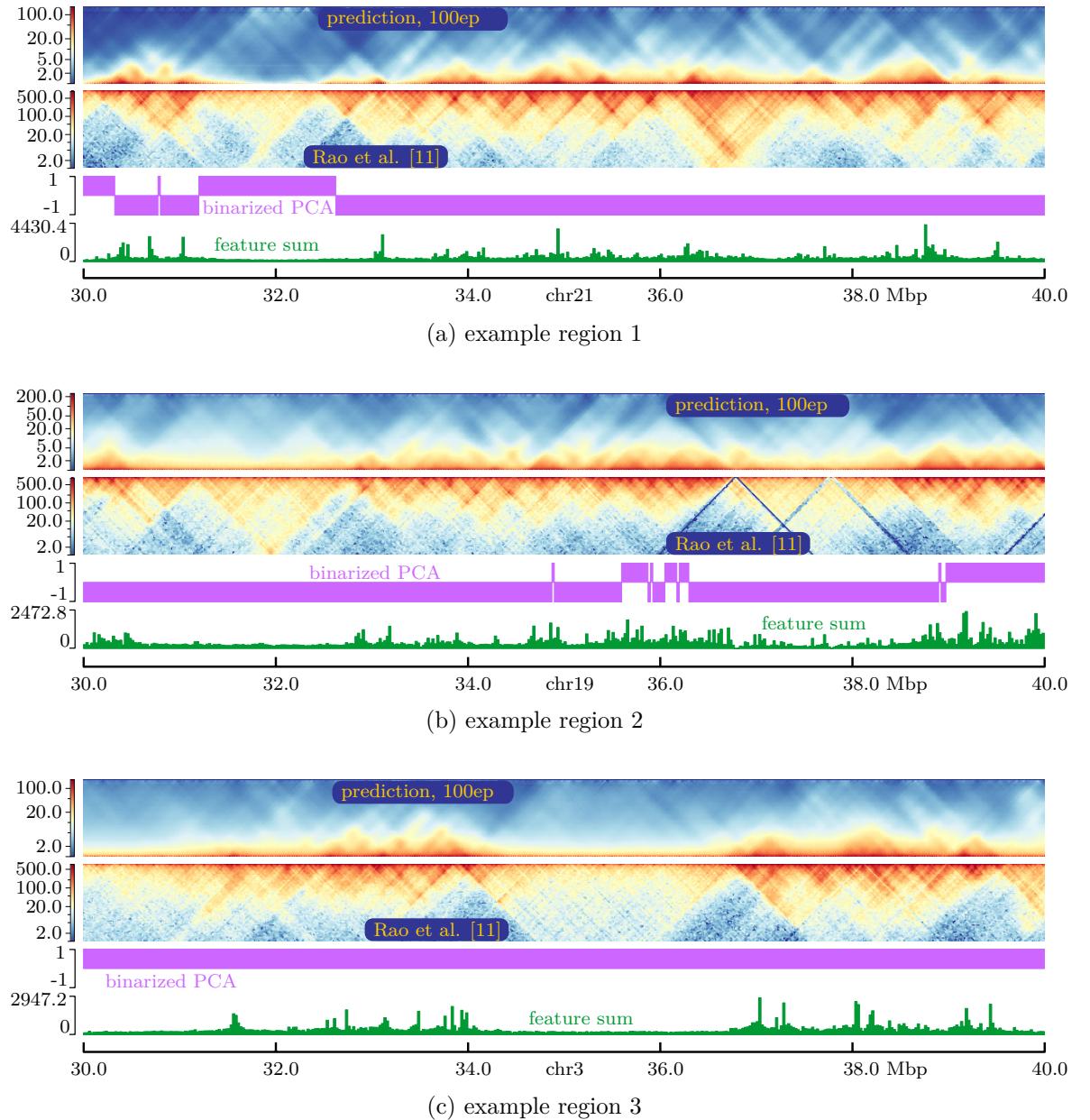


Figure 45: example predictions, cGAN, DNN embedding, pre-trained, $w = 64$, 100 epochs

5 Results

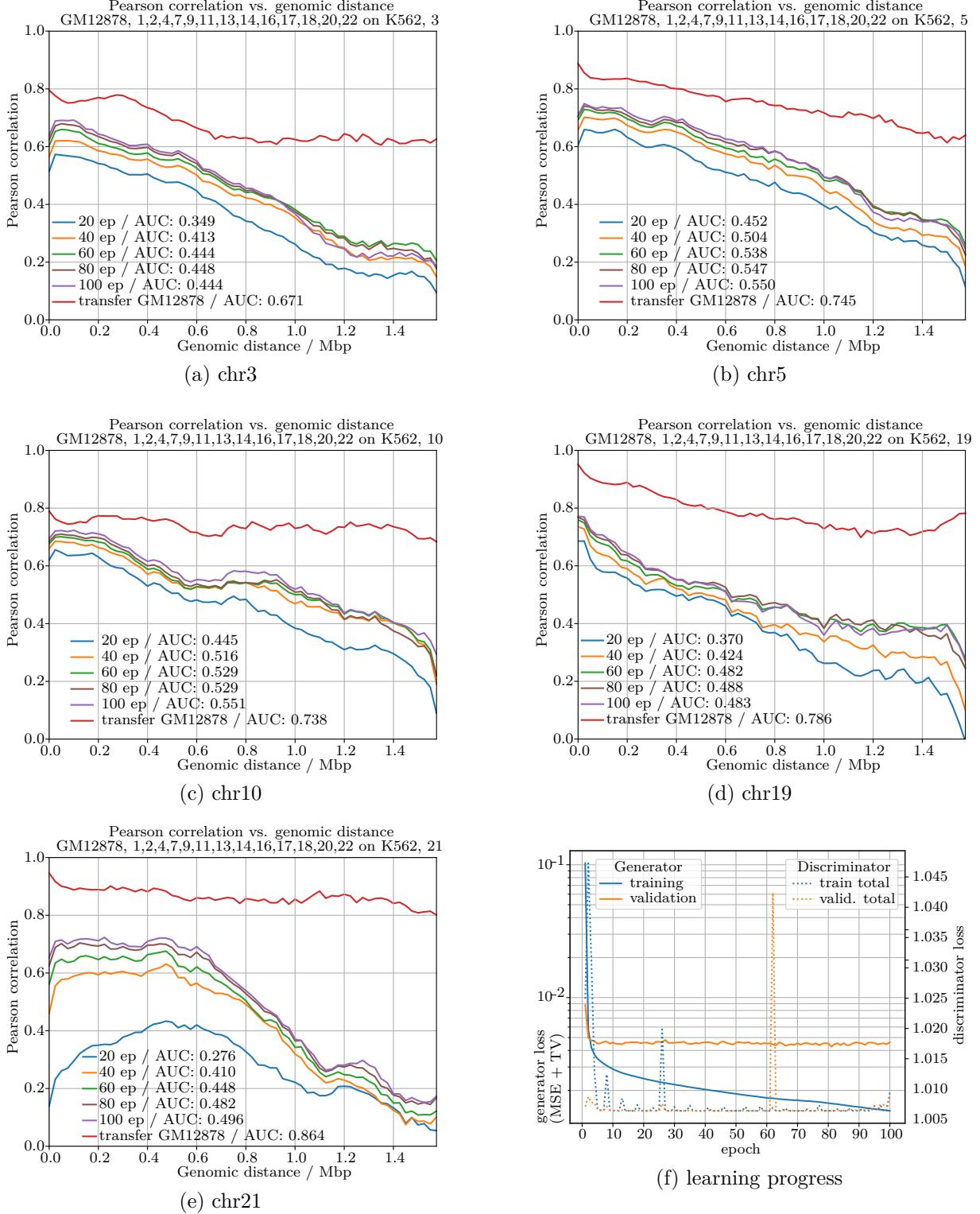
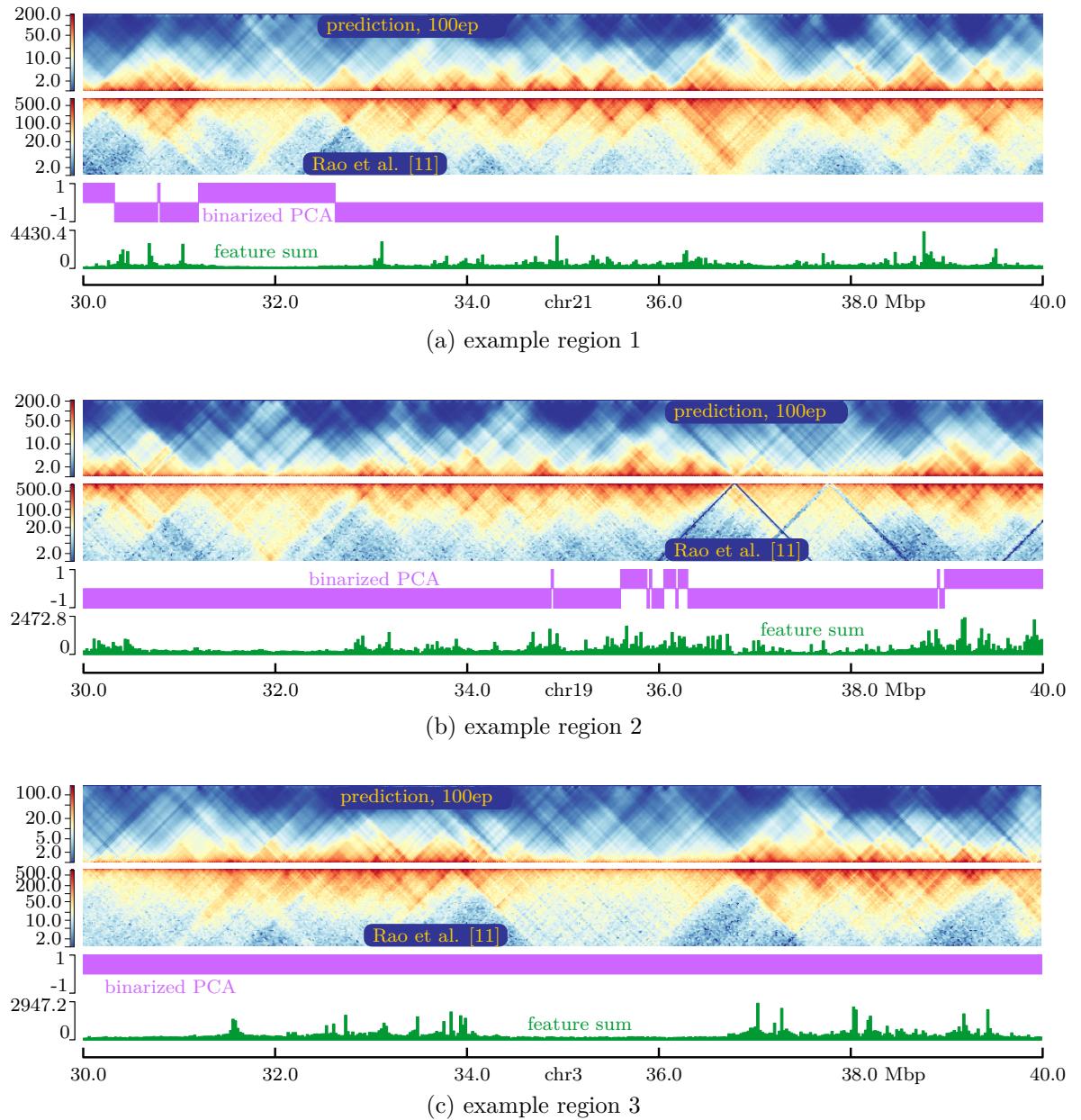


Figure 46: results / metrics cGAN, CNN embedding, $w = 64$, test chromosomes

Figure 47: example predictions, cGAN, CNN embedding, $w = 64$, 100 epochs

5 Results

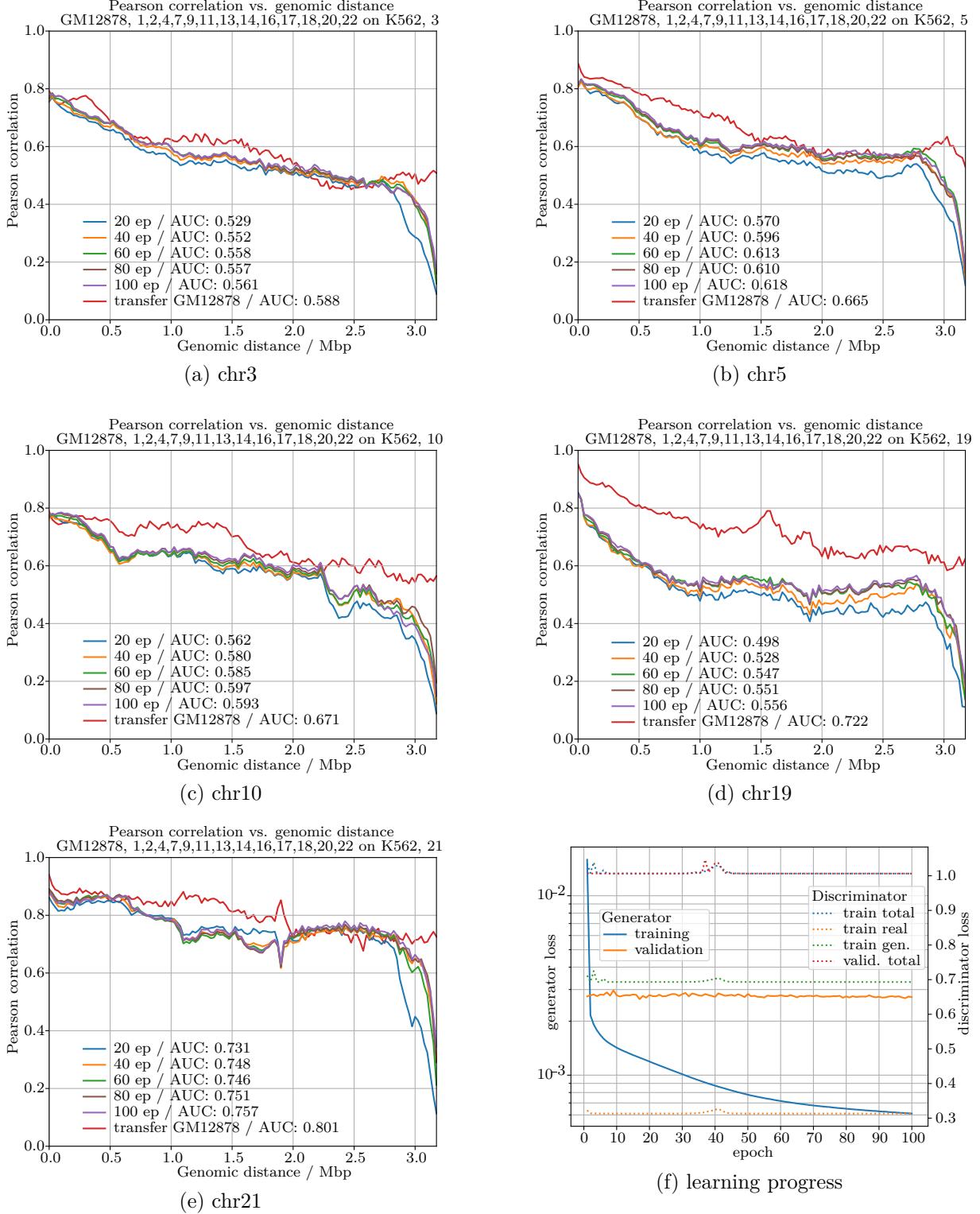
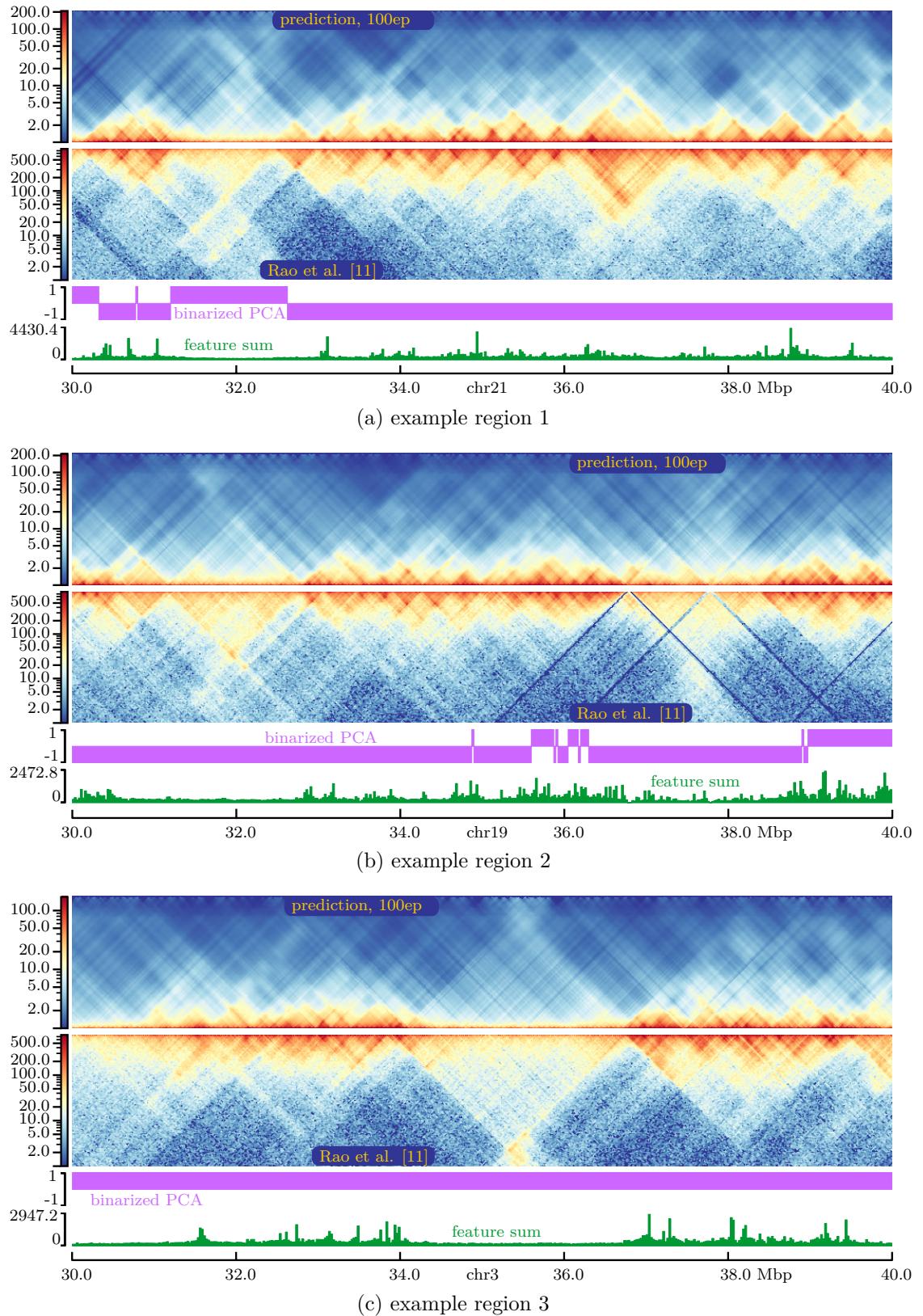


Figure 48: results / metrics cGAN, CNN embedding. $w = 128$, test chromosomes

Figure 49: example predictions, cGAN, CNN embedding, $w = 128$, 100 epochs

5 Results

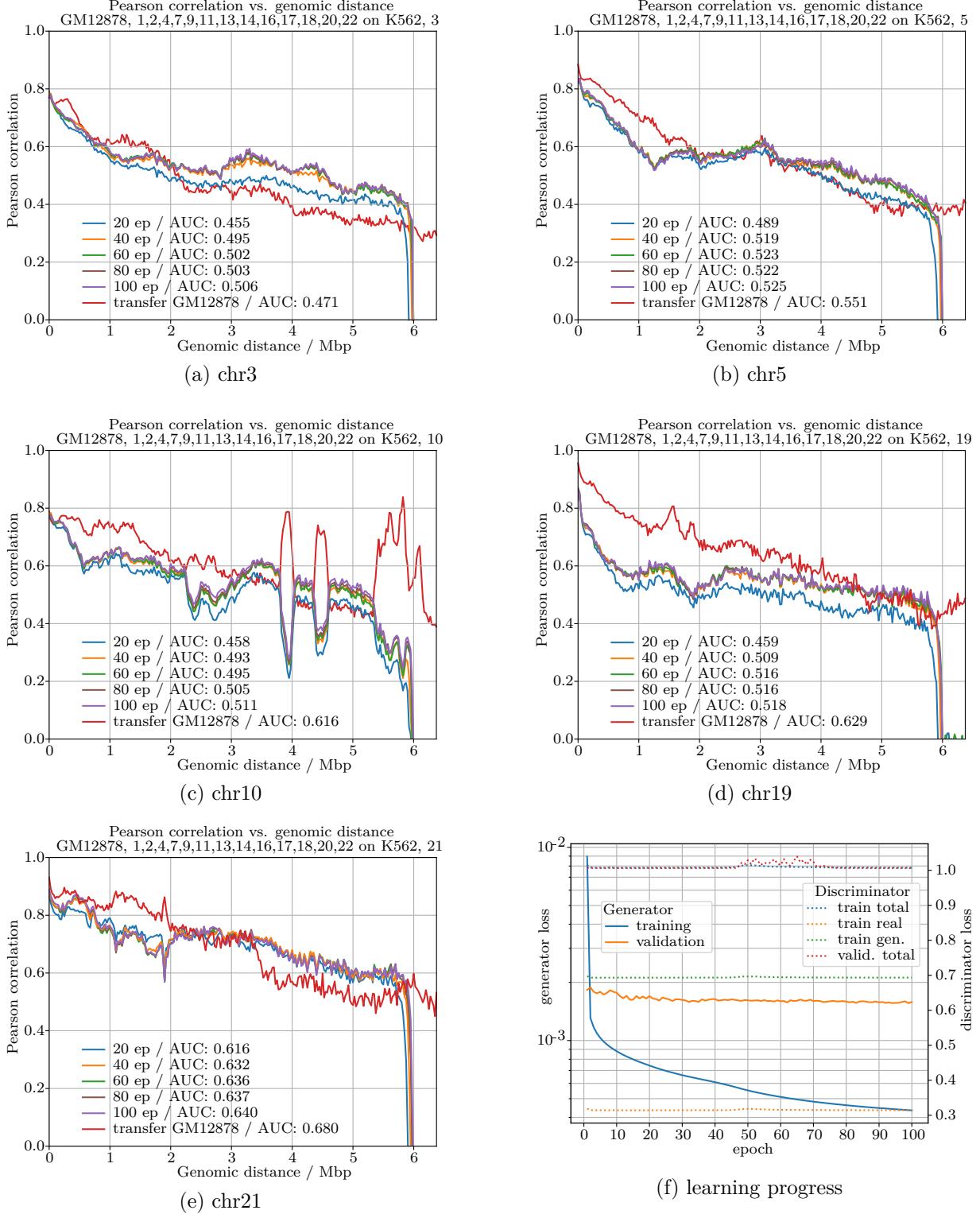
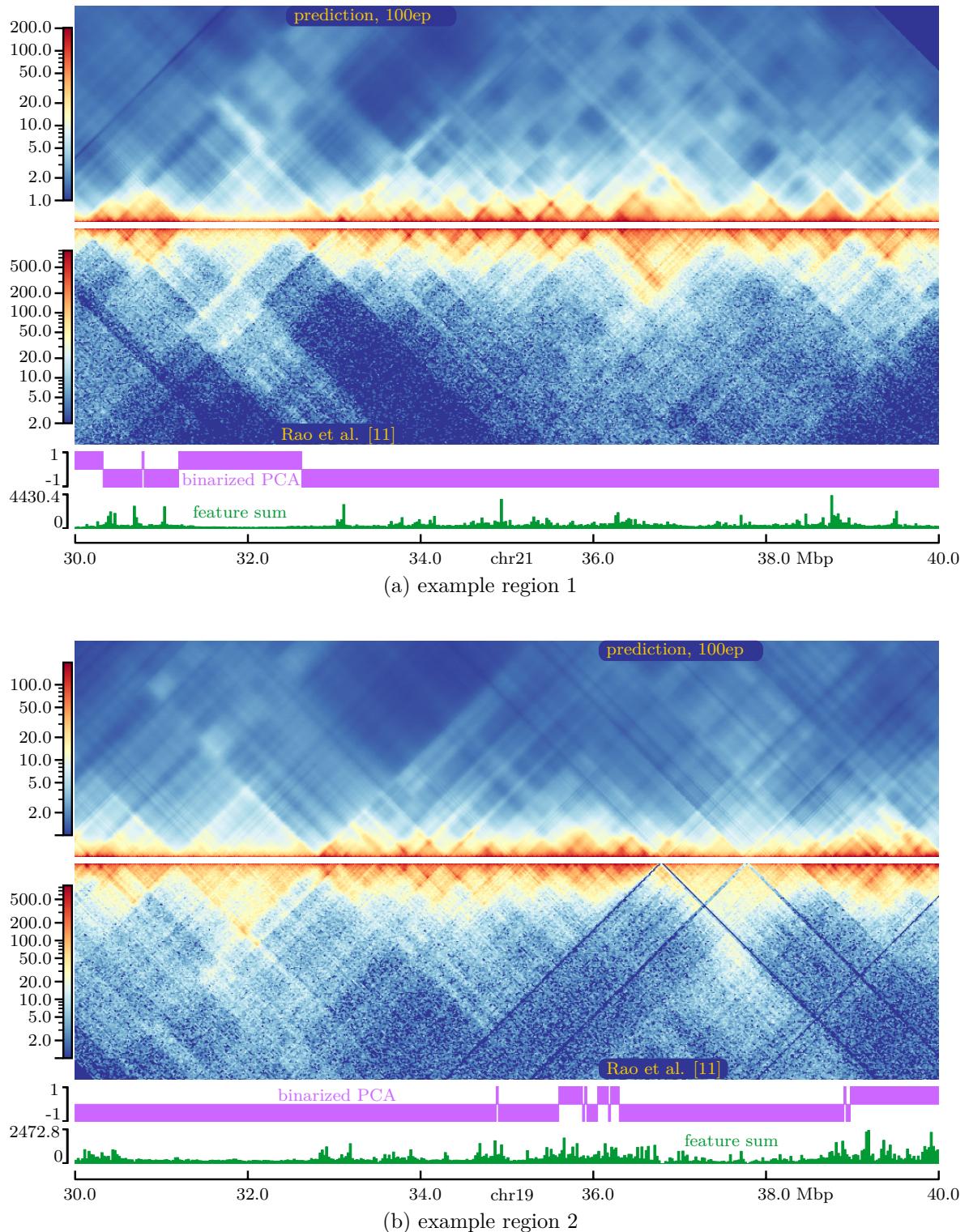


Figure 50: results / metrics cGAN, CNN embedding, $w = 256$, test chromosomes

Figure 51: example predictions, cGAN, CNN embedding, $w = 256$, 100 epochs

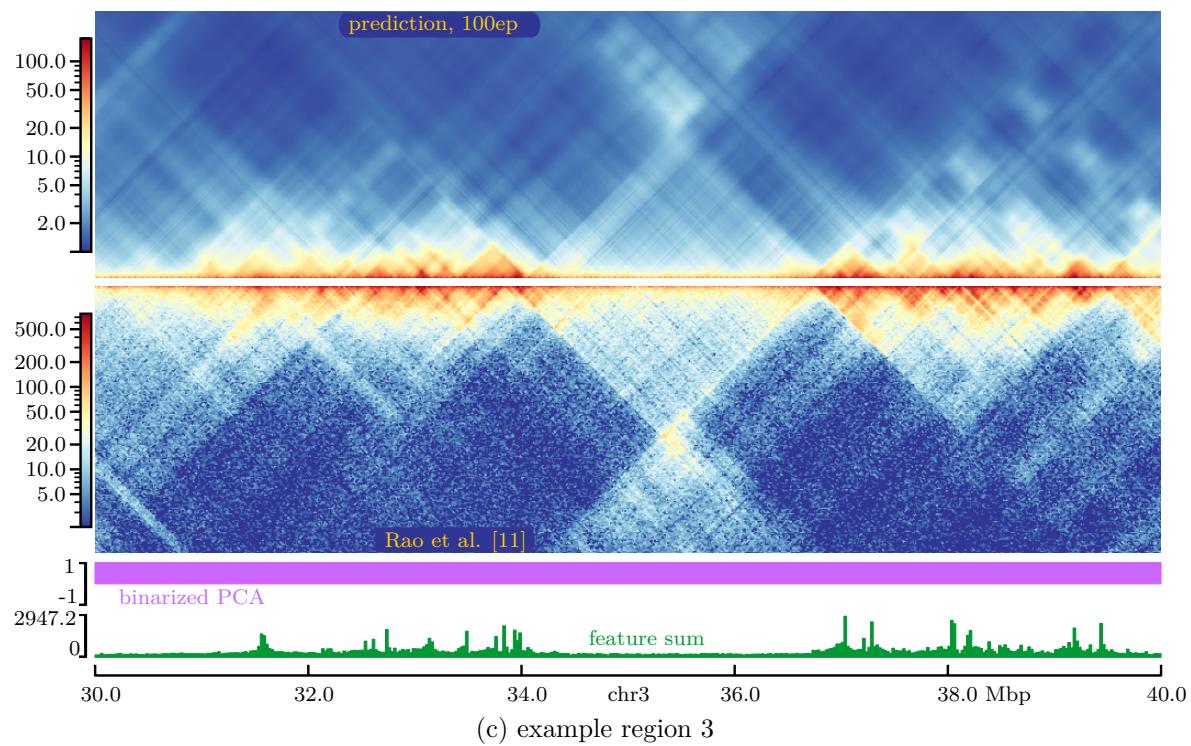


Figure 51: example predictions, cGAN, CNN embedding, $w = 256$, 100 epochs

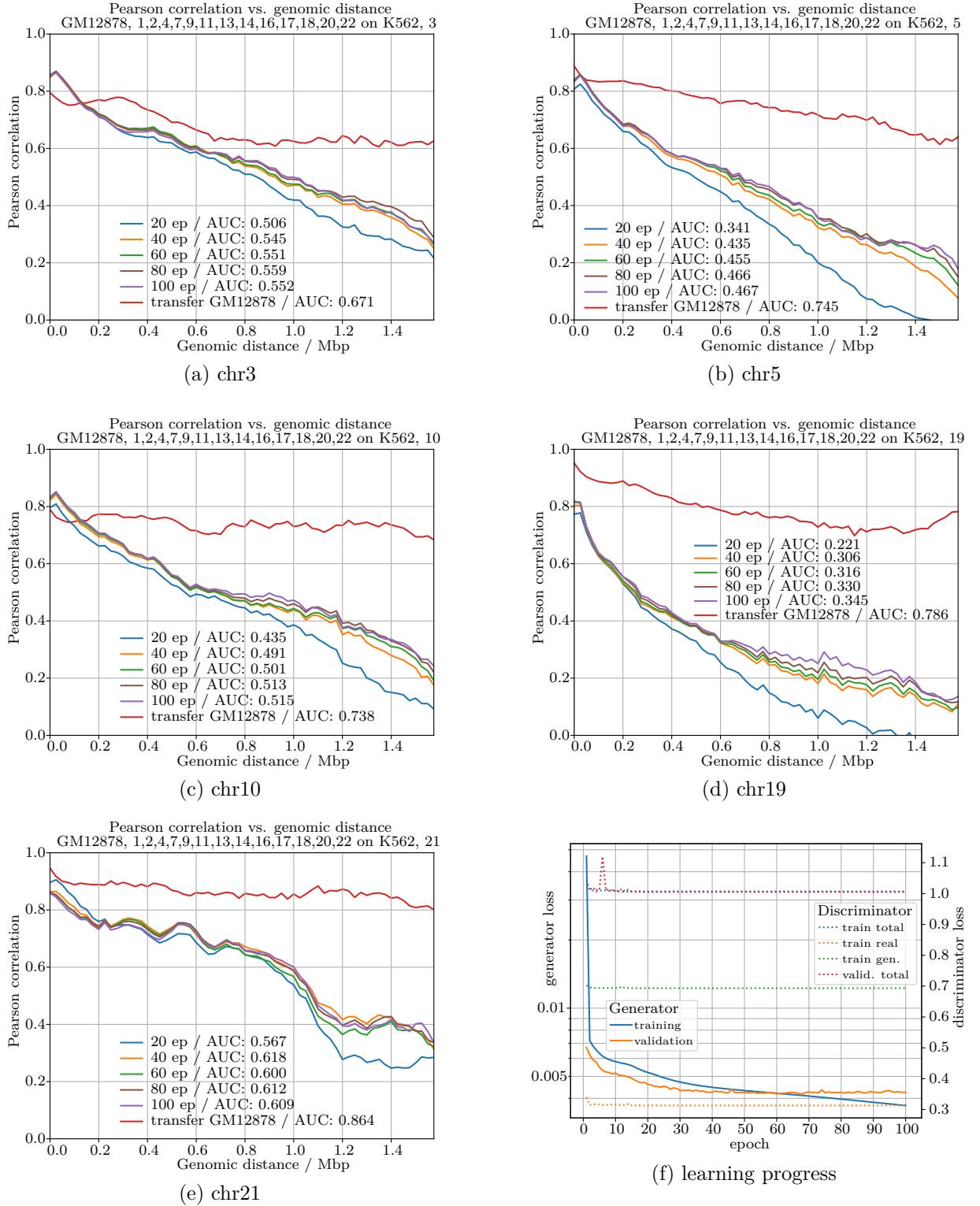


Figure 52: results / metrics cGAN, mixed embedding, no pre-training, $w = 64$, test chromosomes

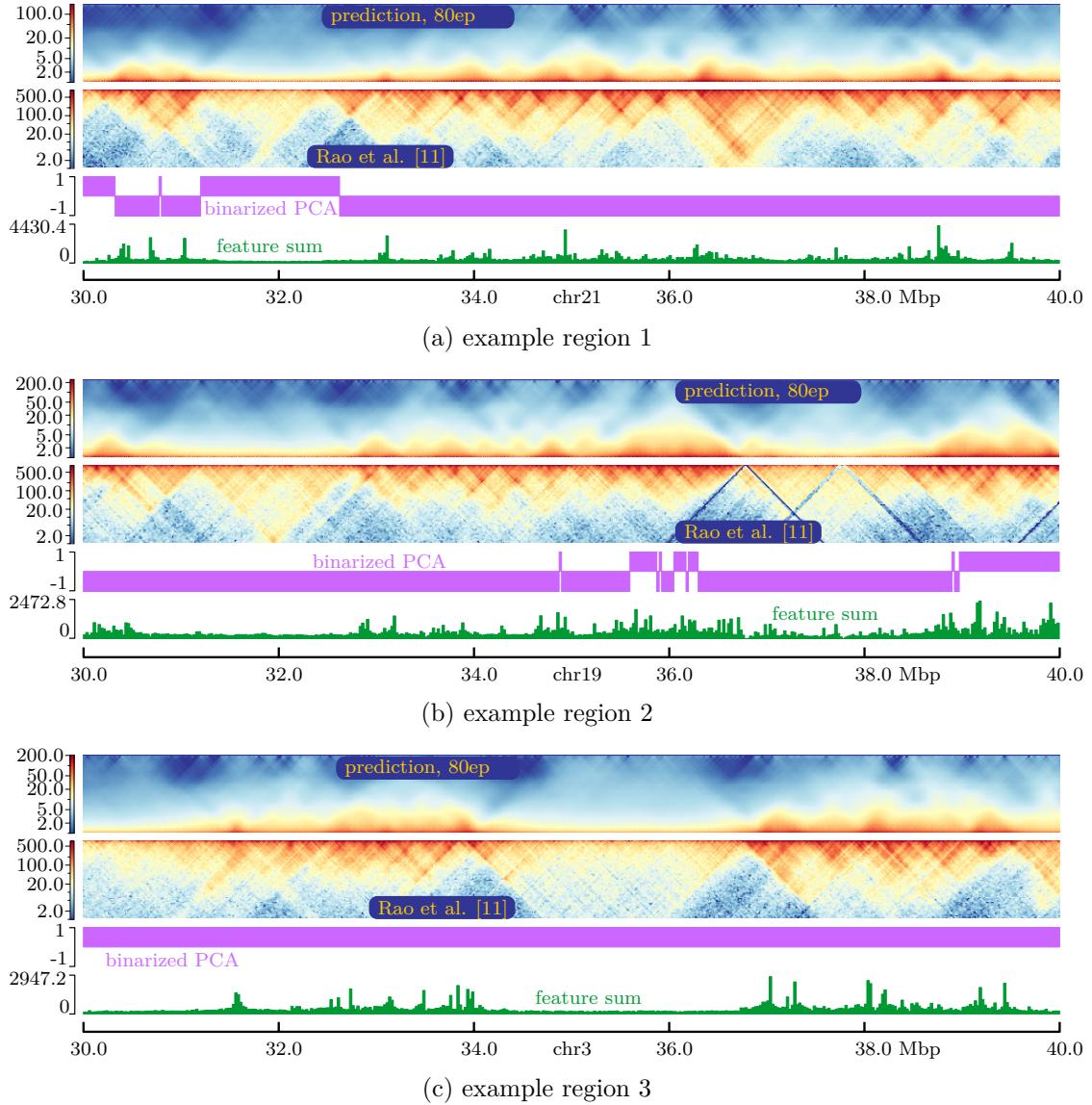


Figure 53: example predictions cGAN, mixed embedding, no pre-training, $w = 64$, 80 epochs

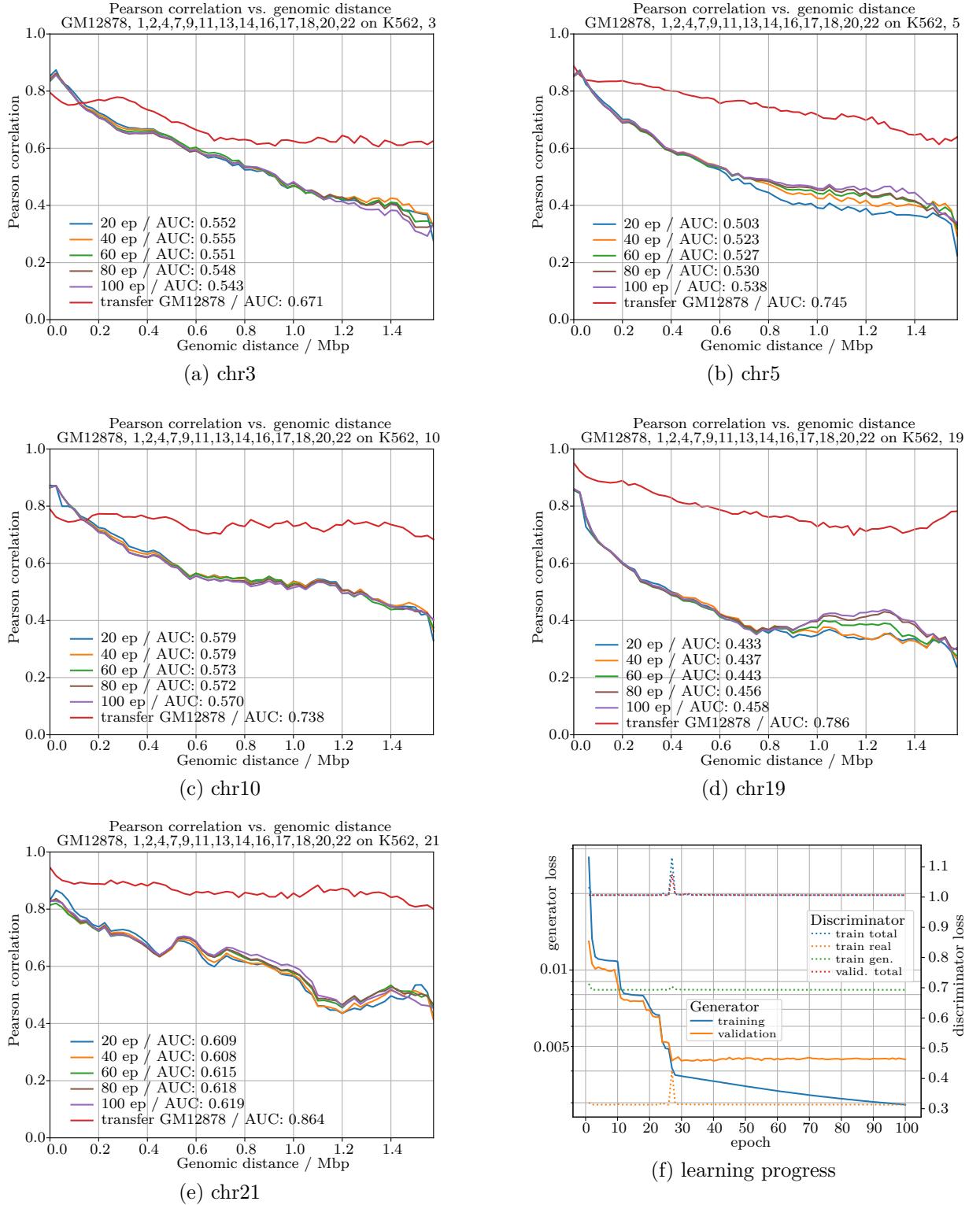


Figure 54: results / metrics cGAN, mixed embedding, DNN pre-trained, $w = 64$, test chromosomes

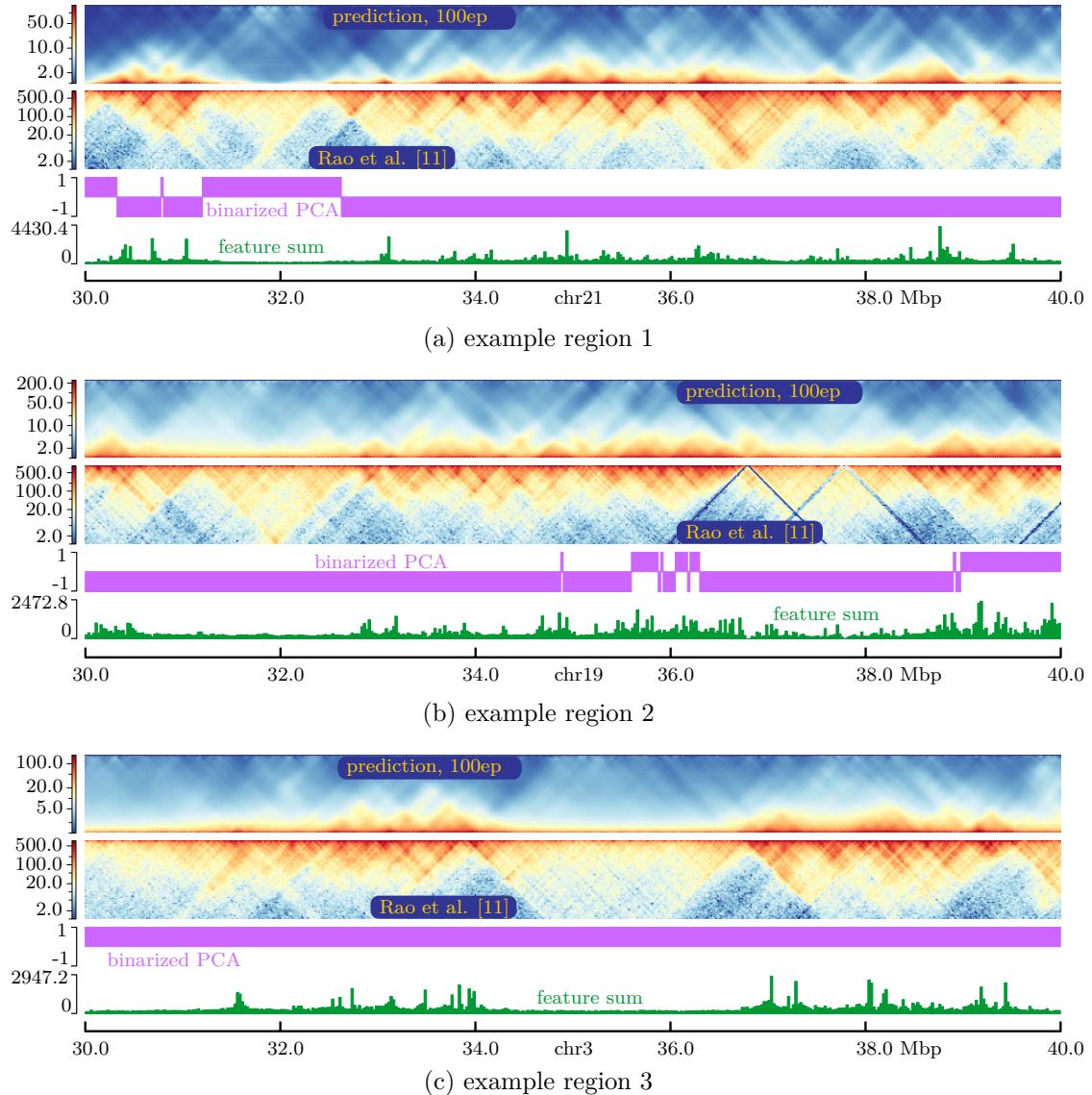


Figure 55: example predictions cGAN, mixed embedding, DNN pre-trained, $w = 64, 100$ epochs

5.3 Comparison with other approaches

When comparing the random-forest-based method by Zhang et al., HiC-Reg [28], to the cGAN model trained on the typical training chromosome set, cf. 4.1.3, the cGAN approach seemed superior to all others for distances up to about 200 kbp, while both the multicell- and window-approach by Zhang et al. outperformed DNN and Hi-cGAN for distances between 200 kbp and 1 Mbp, fig. 56. This was also reflected in the matrix plots, fig. 57. While the cGAN often predicted smaller structures up to about 400 kbp very well and offered distinct boundaries even among nested structures, the approach by Zhang et al. showed better performance for interactions in the upper half of the windowsize, see e.g. chromosome 17, 30...34.5 Mbp. Note that the HiC-Reg WINDOW data stems from a random forest trained only on chromatin features and the Hi-C matrix from GM12878, while the HiC-Reg MULTICELL data has been obtained by training on chromatin feature data from GM12878, K562, HMEC, HUVEC and NHEK and a GM12878 matrix. Both HiC-Reg methods have used only data from chromosome 14 or 17 at binsize 5 kbp, while in this setting, Hi-cGAN has been trained on feature- and matrix data from GM12878, chromosomes 1, 2, 4, 7, 9, 11, 13, 14, 16, 17, 18, 20, and 22 at binsizes of 25 kbp.

To get a better comparison with the HiC-Reg WINDOW approach, in a second setting, Hi-cGAN was trained on data from chromosome 14 or 17 only. Surprisingly, despite the low amount of training samples, the training process converged with good Pearson correlations for the (training-)chromosomes and visually good matrices, fig. 56e/56f and 57c/57f. In general, Hi-cGAN was still better than HiC-Reg MULTICELL and WINDOW at smaller distances and worse at larger ones, but structures sized approximately 500 to 1000 kbp now appeared more clearly and the “intersecting point” on the Pearson correlation graphs moved further to the right. Interestingly, interacting pairs at distances beyond approximately 1.3 kbp were all predicted zero, likely due to the small number of samples in this setting, maybe in combination with insufficient training. The effect was more pronounced for chromosome 17, which indeed has less training samples.

Additionally, we tried training our own implementation of HiC-Reg from a previous study project [13] on the same training data, binsizes and windowsizes as the cGAN model above to allow for a direct comparison. Unfortunately, we could not confirm the good results of HiC-reg, fig. 58 and 59, and it is currently unknown whether this was just due to our implementation or due to a general problem with the HiC-Reg approach in this setting. However, we have generally not been able to reproduce the results of HiC-Reg with our implementation so far.

5 Results

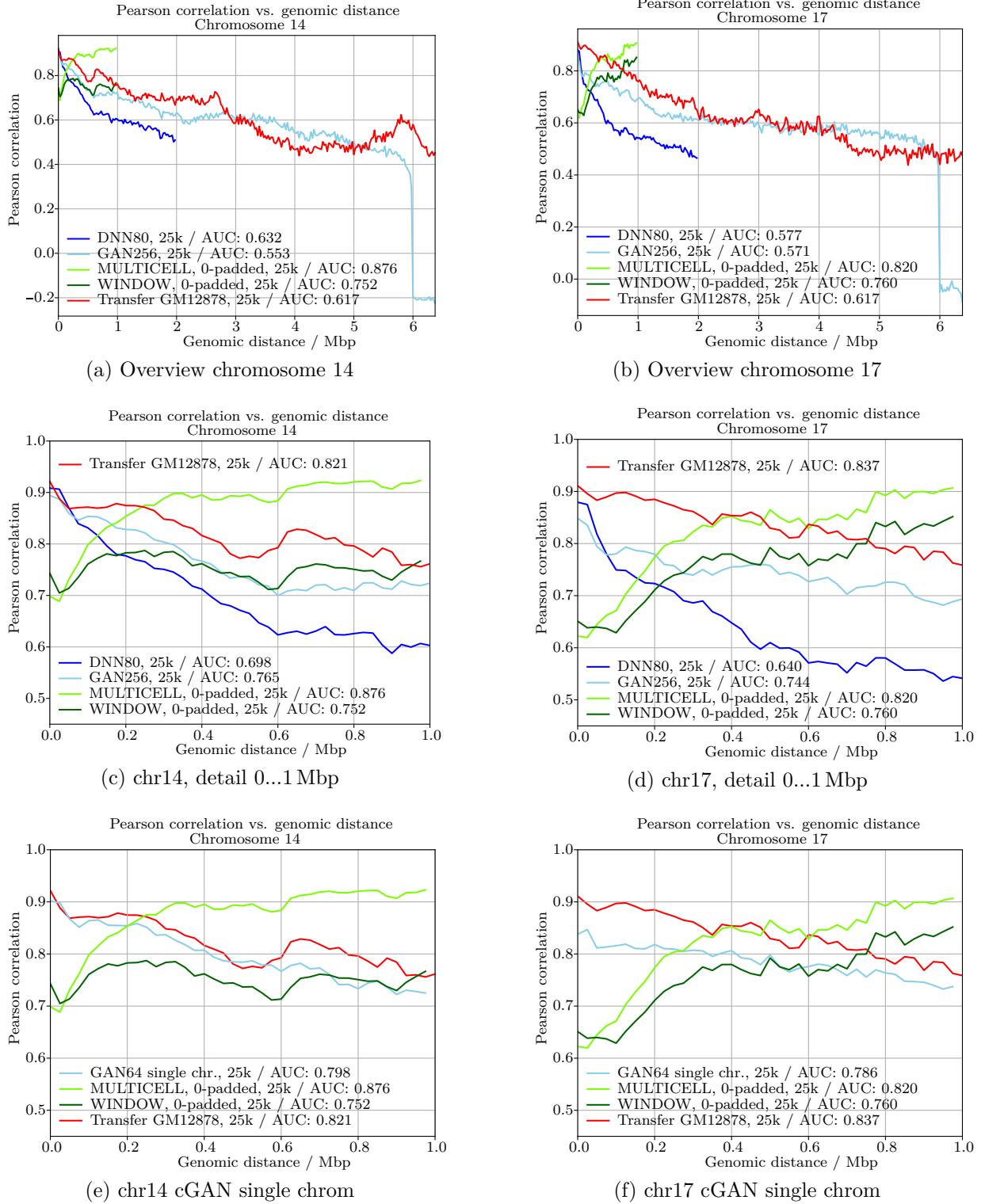


Figure 56: Pearson correlation comparison Hi-cGAN / DNN and HiC-Reg [28]

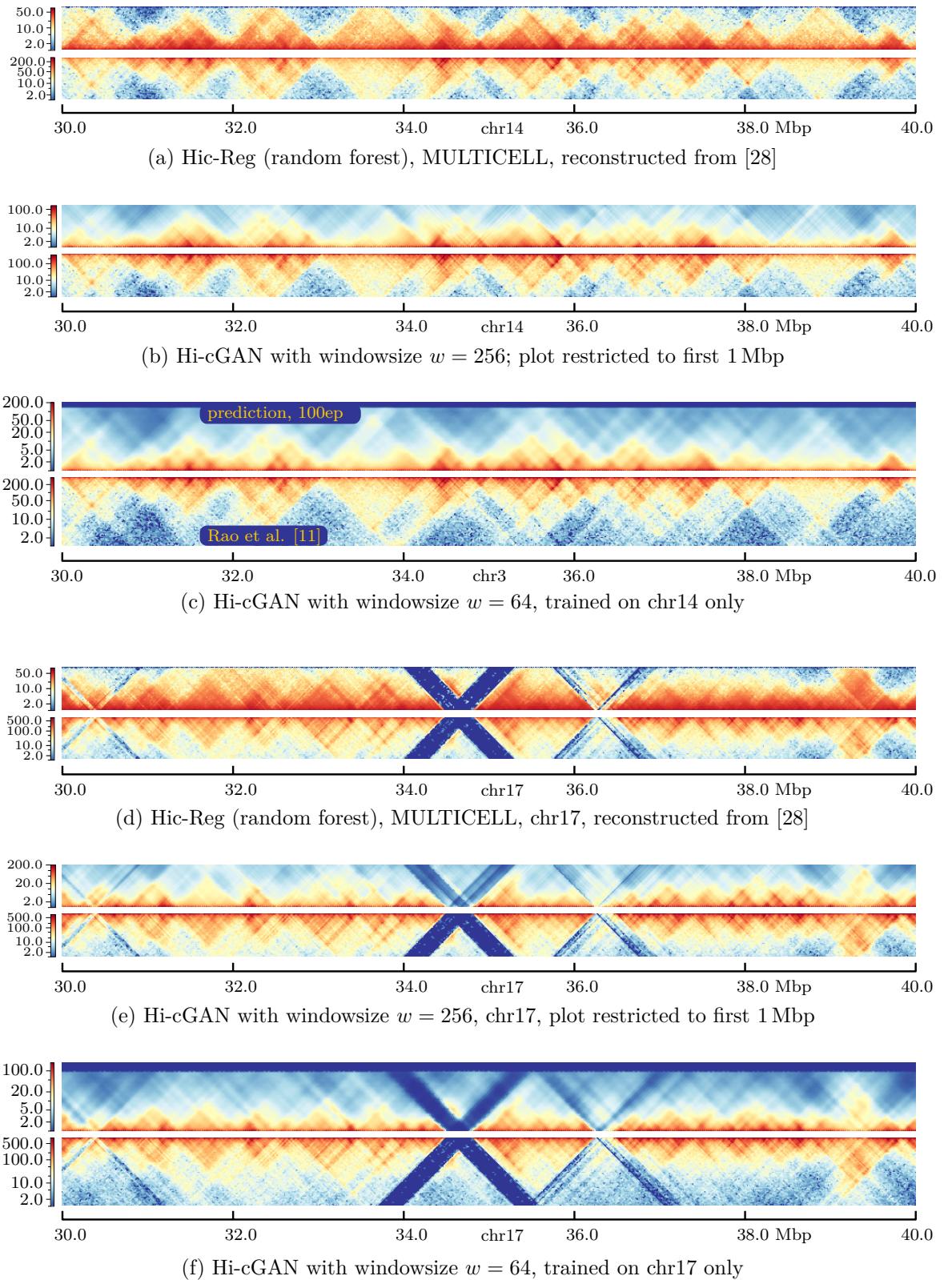


Figure 57: Comparison HiC-Reg [28] and Hi-cGAN (CNN embedding)

5 Results

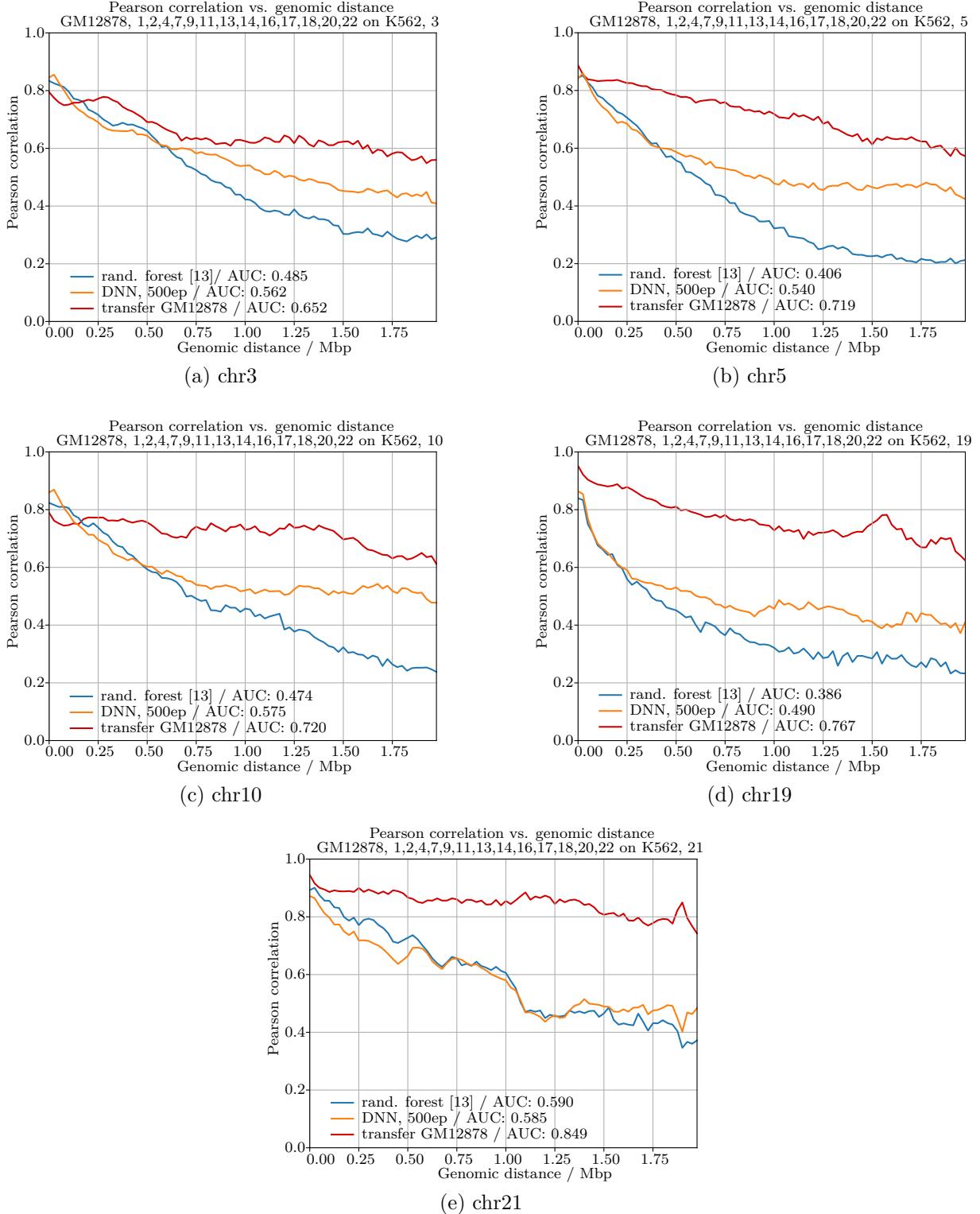


Figure 58: results / metrics, random forest from study project [13], windowsize 80, 25 kbp, test chromosomes

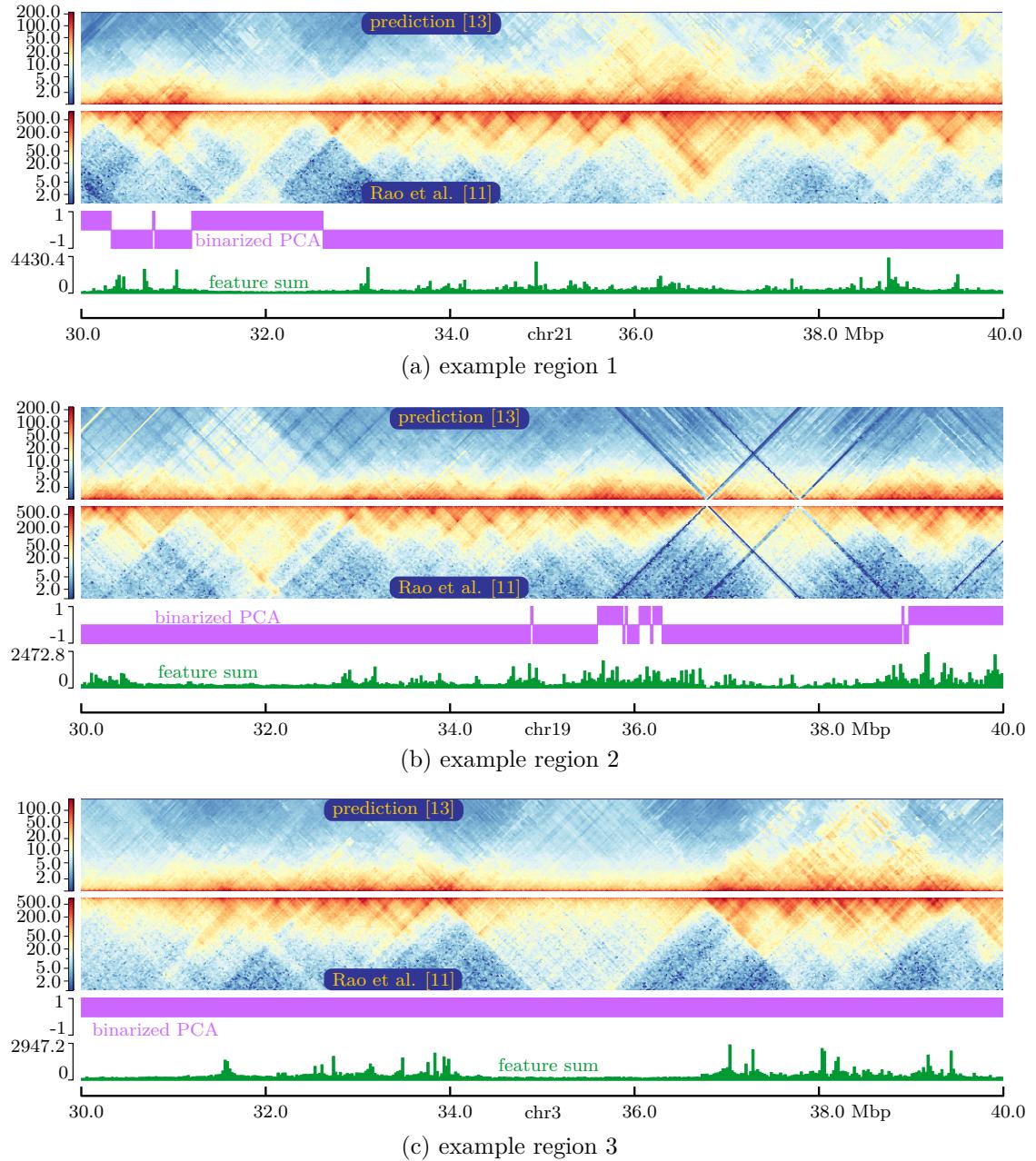


Figure 59: example predictions random forest [13], 25 kbp, $w = 80$

6 Discussion and Outlook

Approach by Farre et al also working for human data. Changes might bring benefits in certain situations, but are not a big improvement.

cGAN seems promising, but the discriminator remains to improve. Improvements still not good enough to beat transfer GM12878-K562 in many cases.

Conclusion w.r.t. comparison to other approaches

Learning from mixed up samples, true matrix but non-matching conditional input could improve things, commonly done in text-to-image synthesis networks.

7 Appendix

7.1 Chromatin feature download links

The basic download paths for all chromatin feature files in .bam format are

<https://hgdownload.cse.ucsc.edu/goldenPath/hg19/encodeDCC/wgEncodeBroadHistone/>

<https://hgdownload.cse.ucsc.edu/goldenPath/hg19/encodeDCC/wgEncodeOpenChromDnase/>

<https://hgdownload.cse.ucsc.edu/goldenPath/hg19/encodeDCC/wgEncodeSydhTfbs/>

for CTCF/Histones, DNase, RAD21/SMC3, respectively. The actual files for replicates 1 and 2 are then easily be found by searching for “*CELL_FEATURE*”, where *CELL* is the Cell line (e.g. GM12878) and *FEATURE* is the chromatin feature of interest, e.g. CTCF, H3K27ac and so on. For convenience, the pdf version of this document contains clickable links below.

GM12878	GM12878	K562	K562
replicate 1	replicate 2	replicate 1	replicate 2
CTCF	CTCF	CTCF	CTCF
H3k27ac	H3k27ac	H3k27ac	H3k27ac
H3k27me3	H3k27me3	H3k27me3	H3k27me3
H3k36me3	H3k36me3	H3k36me3	H3k36me3
H3k4me1	H3k4me1	H3k4me1	H3k4me1
H3k4me2	H3k4me2	H3k4me2	H3k4me2
H3k4me3	H3k4me3	H3k4me3	H3k4me3
H3k79me2	H3k79me2	H3k79me2	H3k79me2
H3k9ac	H3k9ac	H3k9ac	H3k9ac
H3k9me3	H3k9me3	H3k9me3	H3k9me3
H4k20me1	H4k20me1	H4k20me1	H4k20me1
DNase	DNase	DNase	DNase
Rad21	Rad21	Rad21	Rad21
SMC3	SMC3	SMC3	SMC3

7.2 Listings

```
1 #bash-style code
2 #convert from hic to cooler, single resolution
3 #MATRIXHIC is a matrix in .hic format
4 hic2cool convert -r 5000 $MATRIXHIC matrix_5k.cool
5 #coarsen the matrix from 5k to 25k, for example
6 cooler coarsen -k 5 matrix_5k.cool -o matrix_25k.cool
7 #versions used for thesis
8 #hic2cool 0.8.3, cooler 0.8.10
```

Listing 1: hic to cooler

```
1 #bash-style code
2 #indexing a bam file
3 samtools index ${BAMFILE} ${BAMFILE%bam}.bai
4 #creating a bigwig file from the bam file above
5 OUTFILE="${BAMFILE%bam}bigwig"
6 hg19SIZE="2685511504"
7 COMMAND="--numberOfProcessors 10 --bam ${BAMFILE}"
8 COMMAND="${COMMAND} --outFileName ${OUTFILE}"
9 COMMAND="${COMMAND} --outFileFormat bigwig"
10 COMMAND="${COMMAND} --binSize 5000 --normalizeUsing RPGC"
11 COMMAND="${COMMAND} --effectiveGenomeSize ${hg19SIZE}"
12 COMMAND="${COMMAND} --scaleFactor 1.0 --extendReads 200"
13 COMMAND="${COMMAND} --minMappingQuality 30"
14 bamCoverage ${COMMAND}
15 #computing mean from replicate 1 and 2 bigwig files
16 REPLICATE1="${FOLDER1}${PROTEIN}.bigwig"
17 REPLICATE2="${FOLDER2}${PROTEIN}.bigwig"
18 OUTFILE="${OUTFOLDER}${PROTEIN}.bigwig"
19 COMMAND="-b1 ${REPLICATE1} -b2 ${REPLICATE2}"
20 COMMAND="${COMMAND} -o ${OUTFILE} -of bigwig"
21 COMMAND="${COMMAND} --operation mean -bs 5000"
22 COMMAND="${COMMAND} -p 10 -v"
23 bigwigCompare ${COMMAND}
24 #versions used for thesis
25 #samtools 1.9, bamCoverage 3.5.0, bigwigCompare 3.5.0
```

Listing 2: bam to bigwig

7.3 Hardware

machine	#	make/model	CPU			GPU			RAM	
			freq.	L1	L2	L3	make/model	memory	in GB	
1	8	AMD EPYC 7742 64-Core	2.25	64	512	16384	NVIDIA Tesla T4	TU104GL	15109	20
2	40	Intel Xeon E5-2630 v4	2.20	32	4096	16384	NVIDIA Tesla T4	TU104GL	15109	116
3	20	AMD EPYC 7351P 16-Core	2.40	1300	10000	320000	–	–	–	116

Table 5: key figures of hardware used throughout the thesis

Note: CPU frequency in GHz; L1,L2,L3 cache in KB; GPU memory in MiB; RAM (main memory) in GB

7.4 Further figures

7.4.1 Combined loss function

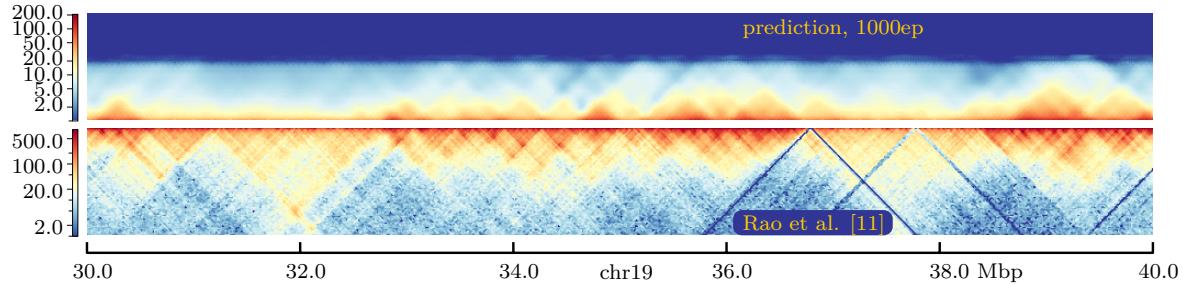


Figure 60: Combined loss with parametrization $\lambda_{MSE} = 10^{-5}$, $\lambda_{VGG} = 0.0$, $\lambda_{TV} = 1.0$

7.4.2 Results of pre-training the DNN-embedding

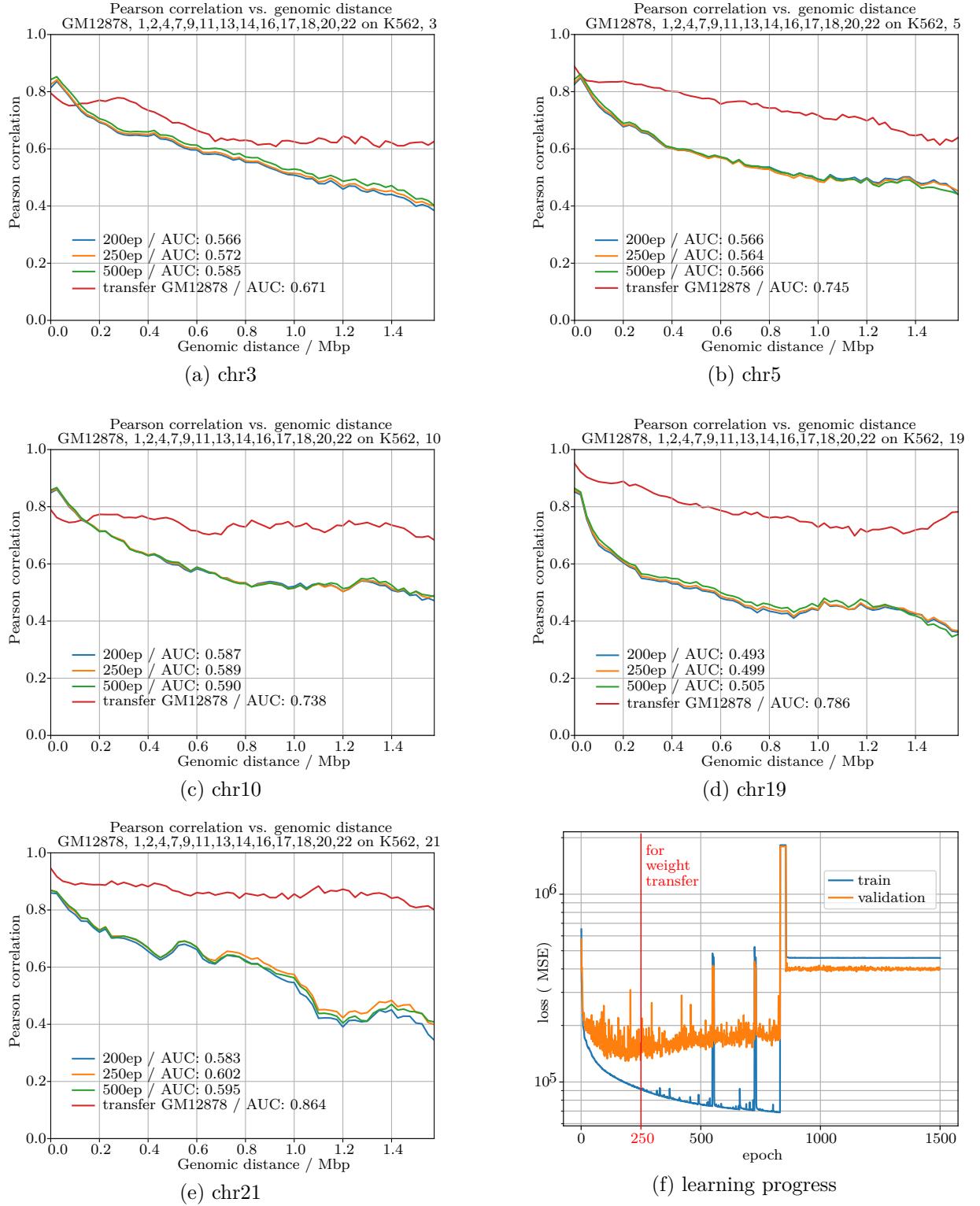


Figure 61: results / metrics DNN, windowsize 64, test chromosomes

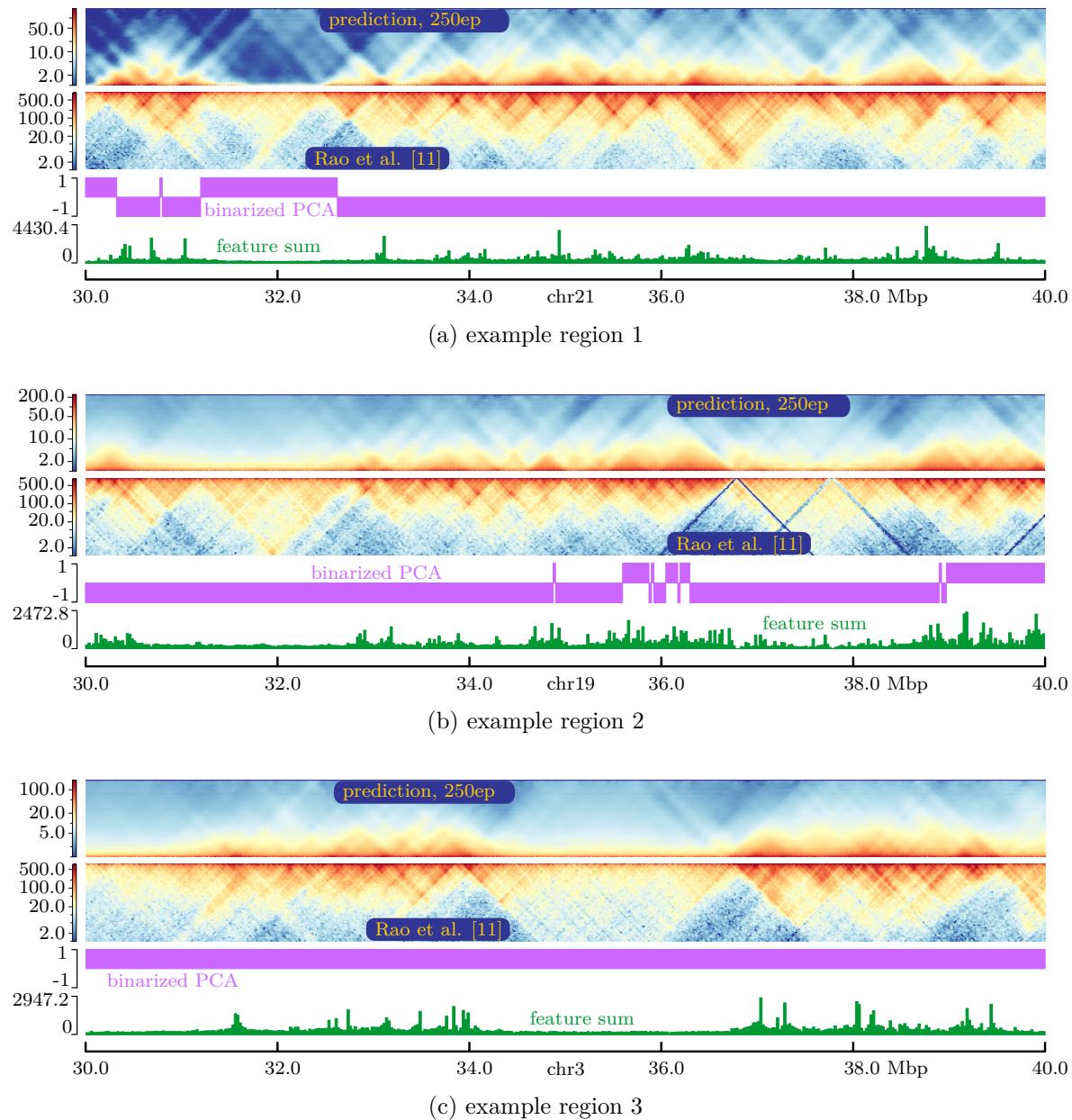
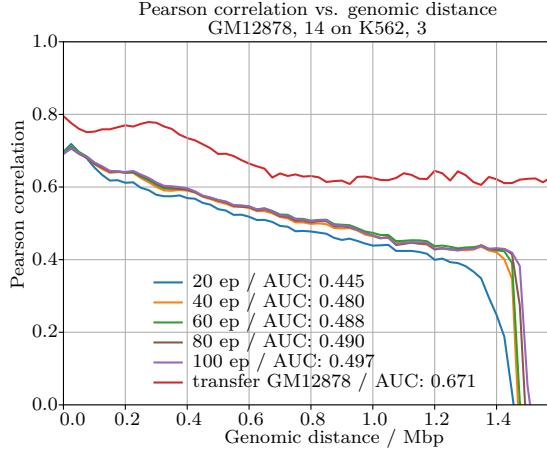
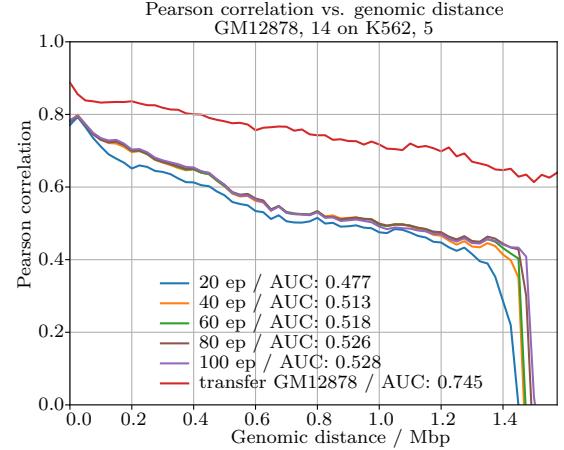


Figure 62: example predictions, DNN, $w = 64$, 250 epochs

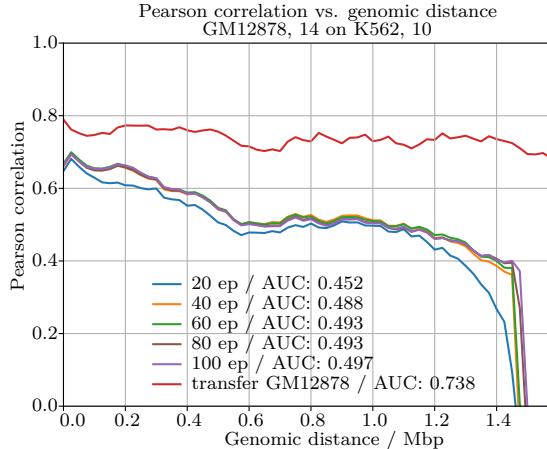
7.4.3 cGAN trained on single chromosomes predicting usual test chromosomes



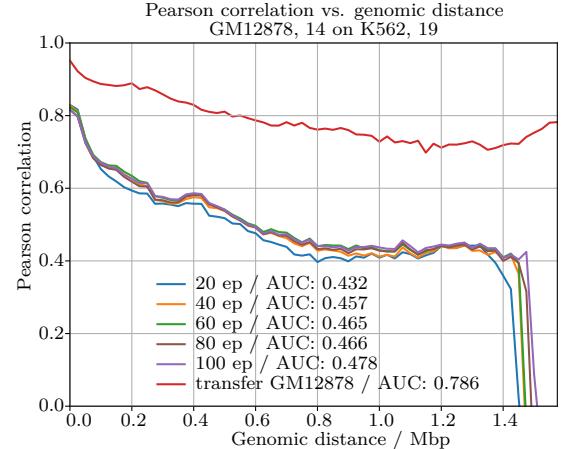
(a) chr3



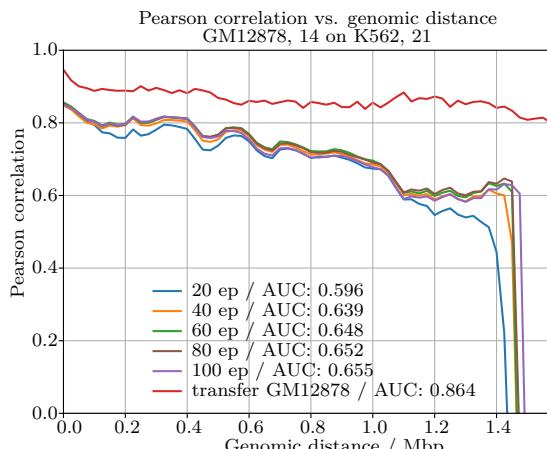
(b) chr5



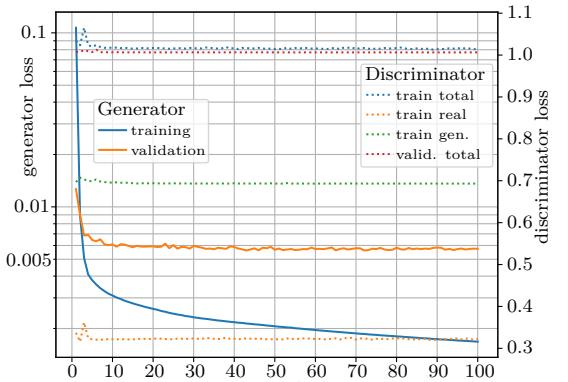
(c) chr10



(d) chr19



(e) chr21



(f) learning progress

 Figure 63: results / metrics cGAN, $w = 64$, trained on chr14 only, typical test chromosomes

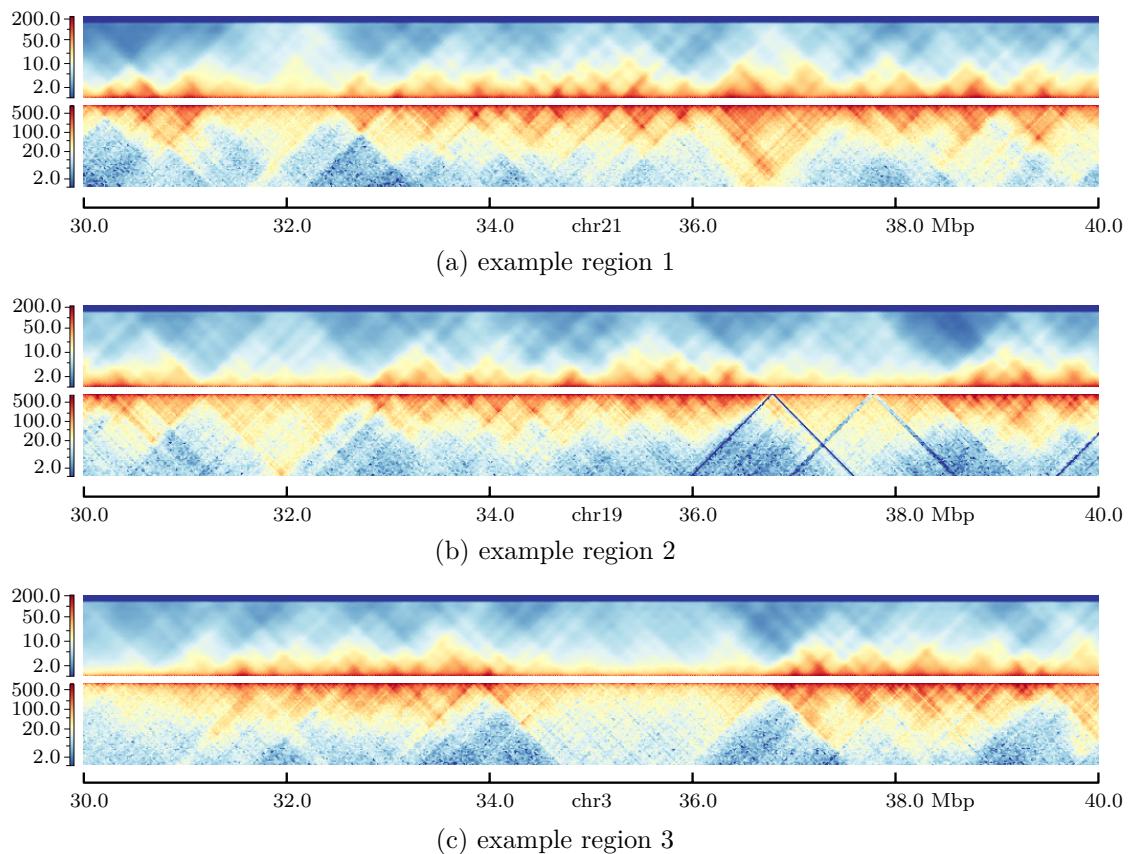
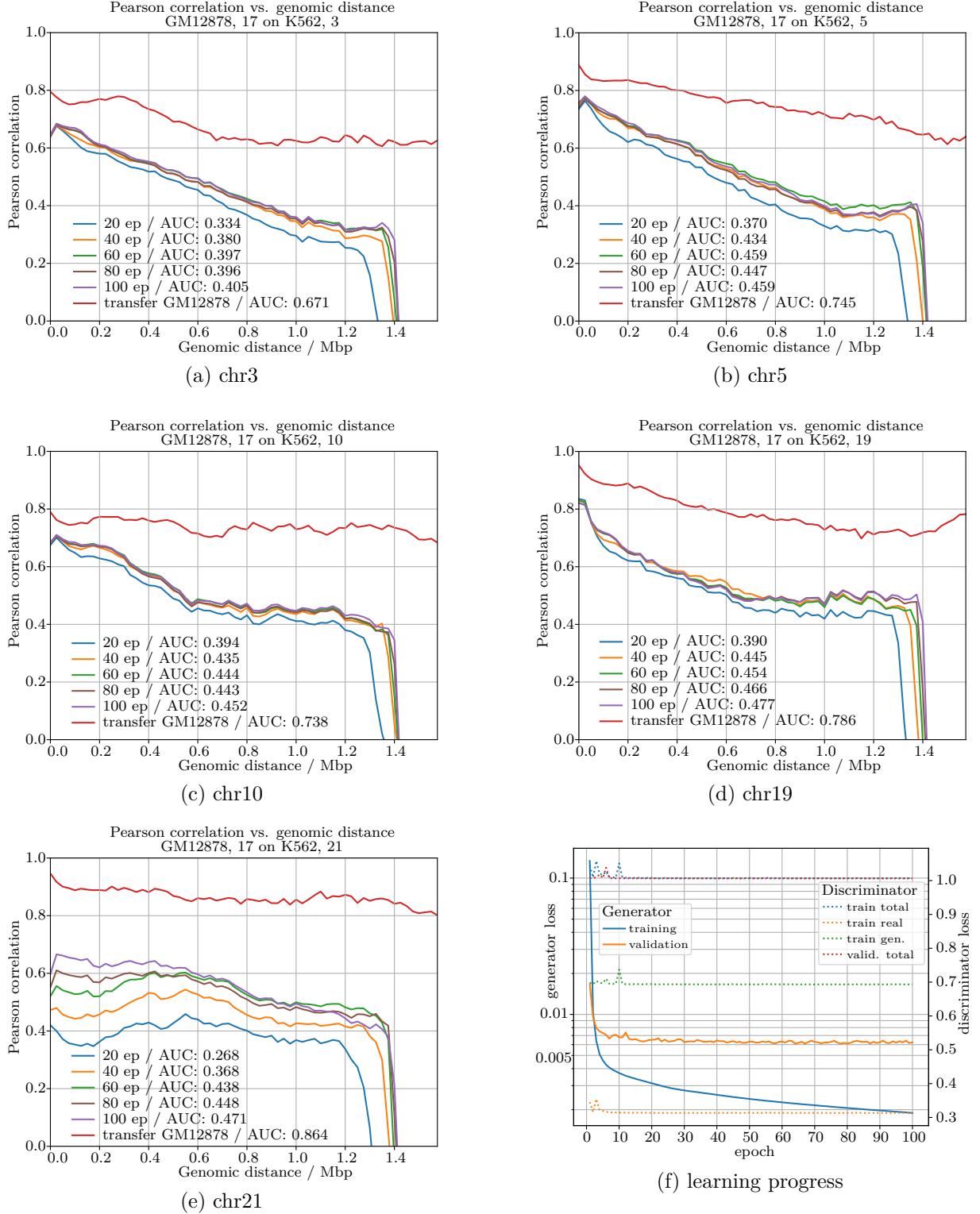


Figure 64: example predictions cGAN, trained on chr14 only, $w = 64$, 100 epochs


 Figure 65: results / metrics cGAN, $w = 64$, trained on chr17 only, typical test chromosomes

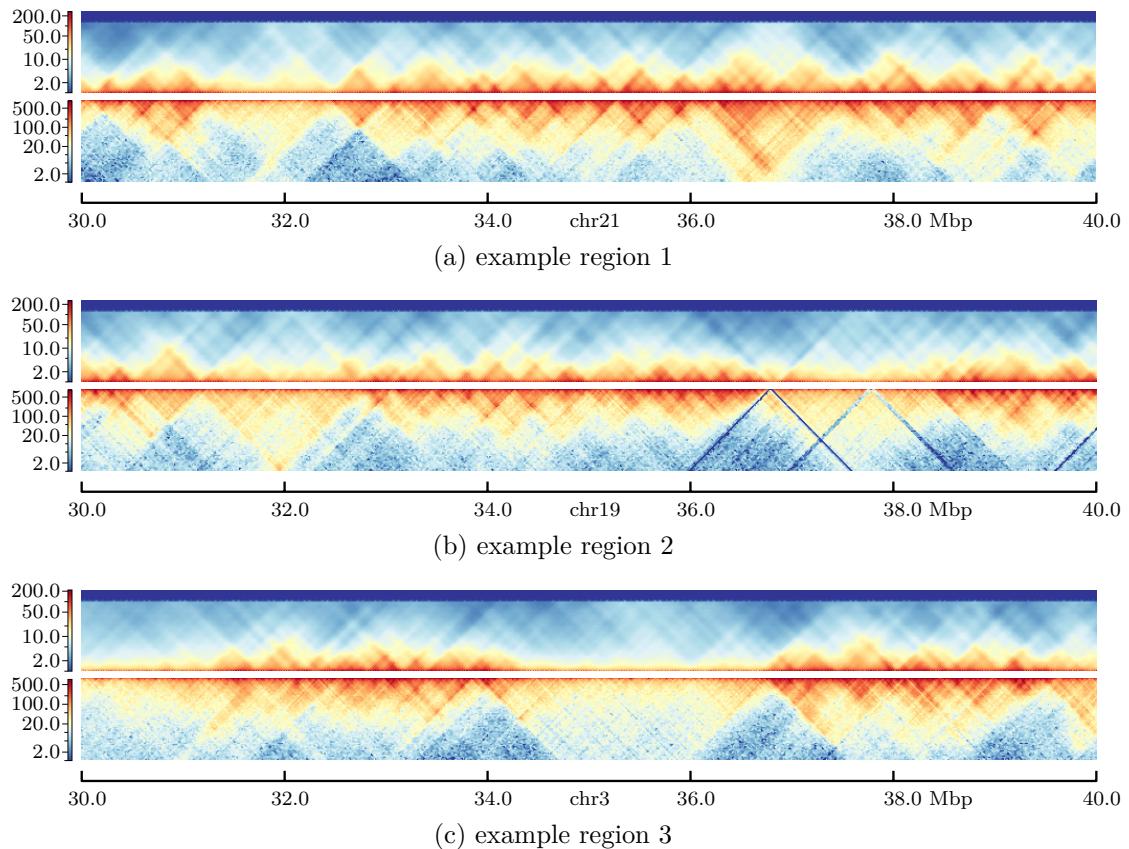


Figure 66: example predictions cGAN, trained on chr17 only, $w = 64$, 100 epochs

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Acronyms

ChIA-PET chromatin interaction analysis by paired-end tag sequencing.

ChIP-seq chromatin immunoprecipitation followed by sequencing.

CNN convolutional neural network.

DamID DNA adenine methyltransferase identification.

DNN dense neural network.

GAN generative adversarial network.

LSTM long-short-term memory.

TAD topologically associating domain.