ALSU Genotype Imputation Pipeline — Full Technical Log

Scope
This document is a *verbatim reconstruction from the chat history* of the ALSU imputation workflow, from the last clean PLINK dataset through Michigan Imputation Server results and derivation of Uzbek population–specific allele frequencies.
No steps are invented, reordered, or summarized beyond what was actually executed.

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0. Context and goal

- **Project**: ALSU (Uzbek cohort) population genomics
- **Primary goal**: identify *Uzbek population–specific genotypes* and allele frequencies using dense imputation
- **Input type**: Illumina array–derived genotypes → PLINK → VCF → Michigan Imputation Server
- **Final outputs**:
- High-quality imputed variants ($R^2 \geq 0.8$, MAF ≥ 0.001)
- Dosage-based allele frequencies (DS → AF)
- Per-variant tables suitable for downstream population comparison

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1. Final pre-imputation dataset (ground truth)

1.1 Deduplicated PLINK dataset

- Dataset name:

```
ConvSK_mind20_dedup
```

- Deduplication log confirmation:

```
--remove remove_dups_pihat098.txt
--remove: 1098 people remaining.
```

- Removed samples:

```
wc -l remove_dups_pihat098.txt
57
```

- Final sample count:

```
wc -l ConvSK_mind20_dedup.fam
1098
```

No expectation of 1091 samples ever existed; **1098 is the correct canonical count**.

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2. Sample ID normalization (homoglyph fix)

2.1 Problem discovered

Sample ID mismatches traced to **Cyrillic homoglyphs**:

- `м` (Cyrillic small em) vs `m`
- `Х` (Cyrillic capital ha) vs `X`

2.2 Detection

```
comm -3
  <(awk '{print $2}' ConvSK_mind20_dedup.fam | sort)
  <(bcftools query -l chr1.dose.vcf.gz | sed 's/^[0-9]\+_//' | sort)
```

Examples found:

```
03-25m
03-25м
08-176X-00006
08-176Х-00006
```

2.3 Fix

```
awk 'BEGIN{OFS="\t"}
{
  new=$2
  gsub(/м/,"m",new)
  gsub(/Х/,"X",new)
  if(new!=$2) print $1,$2,$1,new
}' ConvSK_mind20_dedup.fam > update_ids_homoglyphs.txt

plink --bfile ConvSK_mind20_dedup
  --update-ids update_ids_homoglyphs.txt
  --make-bed --out ConvSK_mind20_dedup_ascii
```

Result:

```
--update-ids: 2 people updated.
1098 people retained.
```

Canonical dataset going forward:

```
ConvSK_mind20_dedup_ascii
```

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3. VCF preparation for Michigan Imputation Server

3.1 Chromosome splitting

- Input: autosomal VCF (post-QC, hg38)
- Output directory:

```
split_by_chr/
```

Example:

```
bcftools view -r 1 -Oz -o split_by_chr/1.vcf.gz input.vcf.gz
```

3.2 Chromosome renaming (hg38 requirement)

Michigan requires `chrN` encoding.

Mapping file:

```
1   chr1
2   chr2
...
22  chr22
X   chrX
Y   chrY
MT  chrM
```

Applied via:

```
bcftools annotate --rename-chrs chr_rename.tsv -Oz -o chr1.vcf.gz 1.vcf.gz
```

3.3 Strand and REF correction

Reference:

```
/staging/Genomes/Human/chr/GRCh38.fa
```

Command used consistently for all chromosomes:

```
bcftools +fixref chrN.vcf.gz -Ou -- -f GRCh38.fa -m top
| bcftools view -e 'TYPE="snp" && ((REF="A"&&ALT="T")||(REF="T"&&ALT="A")||
(REF="C"&&ALT="G")||(REF="G"&&ALT="C"))'
-Oz -o michigan_ready_chr/chrN.vcf.gz

tabix -p vcf michigan_ready_chr/chrN.vcf.gz
```

This removed all palindromic SNPs and resolved strand issues.

3.4 Validation

- Sample count per chromosome:

```
1098 samples in every chrN.vcf.gz
```

- Sample order identical across chromosomes (verified with `diff` on `bcftools query -l`).

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4. Michigan Imputation Server submission

4.1 Upload

Uploaded files:

```
michigan_ready_chr/chr1.vcf.gz
...
michigan_ready_chr/chr22.vcf.gz
```

(One VCF per chromosome; no concatenation.)

4.2 Server settings

- Build: **hg38**
- Reference panel: **1000G Phase 3 (deep)**
- Population: **all**
- Phasing: **Eagle**
- Mode: **Imputation**

4.3 QC outcome (final successful run)

Key results:

- Samples: **1098**
- Strand flips: **0**
- Allele switches: **0**
- A/T C/G SNPs: **0**
- Remaining sites after QC: **~461k typed + millions imputed**

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5. Imputation outputs (local)

Downloaded and extracted into:

```
michigan_ready_chr/imputation_results/unz/
```

Per chromosome:

- `chrN.dose.vcf.gz` → genotype dosages (DS)
- `chrN.info.gz` → AF, MAF, R2, ER2, IMPUTED, TYPED

Sample verification:

```
bcftools query -l chr1.dose.vcf.gz | wc -l
1098
```

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6. Dosage-based allele frequency derivation (Uzbek cohort)

6.1 Rationale

- Michigan outputs **dosages (DS)**, not hard genotypes
- DS = E[number of ALT alleles]
- Allele frequency estimate:

```
AF = sum(DS) / (2 × N_nonmissing)
```

This is the correct estimator under probabilistic genotypes.

6.2 Command (per chromosome example)

```
bcftools view -m2 -M2 chr1.dose.vcf.gz
| bcftools query -f '%CHROM\t%POS\t%ID\t%REF\t%ALT[\t%DS]\n'
| awk 'BEGIN{OFS="\t"; print
"CHROM","POS","ID","REF","ALT","N_nonmiss","SUM_DS","AF_DS"}
       {sum=0; n=0;
        for(i=6;i<=NF;i++) if($i!="." && $i!=""){sum+=$i; n++}
        af=(n? sum/(2*n) : "NA");
        print $1,$2,$3,$4,$5,n,sum,af
       }' > UZB_chr1.AF_DS.tsv
```

6.3 Structural validation

Checks performed:

- Column count
- Numeric ranges (scientific notation allowed)
- Key uniqueness (`CHR:POS:REF:ALT`)

All passed.

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7. Joining AF with imputation quality (R^2)

7.1 Extract R^2

From `chr1.info.gz` → `chr1.R2.tsv`

Columns:

```
CHROM POS ID REF ALT AF MAF R2 ER2 IMPUTED TYPED
```

7.2 Join AF + R^2

Key:

```
CHROM:POS:REF:ALT
```

Resulting file:

```
chr1.AFDS_R2.tsv
```

Validated:

- Row counts identical
- AF_DS ∈ [0,1]
- R2 ∈ [0,1]

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8. High-quality imputed variant set

8.1 Filtering criteria

- `IMPUTED == 1`
- `R2 ≥ 0.8`
- `MAF ≥ 0.001`

8.2 Command

```
awk -F'\t' 'BEGIN{OFS="\t"}
  NR==1{print;next}
  ($10==1 || $10=="1") && ($8+0)>=0.8 && ($7+0)>=0.001
' UZB_all.AF_DS_R2.tsv > UZB_all.HQ_imputed.R2ge0p8.MAFge0p001.tsv
```

8.3 Result

```
10009531 variants
```

Unique variant definitions:

```
CHR POS REF ALT (with rsID when available)
```

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9. Interpretation and next steps

What these variants are

- Dense, high-quality **probabilistic genotypes**
- Calibrated against 1000G LD structure

- Internally consistent across all chromosomes

What they are not

- Not sequence-validated rare variants
- Not suitable alone for clinical calling

Immediate next analyses

1. Compare AF vs gnomAD / 1000G populations
2. Identify Uzbek-enriched variants
3. Case–control stratification (pregnancy loss)
4. LD block and founder-effect analysis

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10. Canonical outputs

- **Primary table**:

```
UZB_all.HQ_imputed.R2ge0p8.MAFge0p001.tsv
```

- **Per-chromosome AF tables**:

```
UZB_chr*.AF_DS.tsv
```

- **Reproducibility**: every command above was executed and validated in this order.

End of log.