

# LLP Panel Reference Files Management System

A git-based system for tracking changes to Liverpool Lymphoid Panel (LLP) reference files, with primary focus on Sequence Variant Baseline (SVB) management for targeted NGS panels using PCR-based amplification.

## Repository Structure

```
llp-reference-files/
  └── panel_v1/
    ├── cnv_baseline/
    │   └── current_cnv_baseline.vcf
    ├── false_positives/
    │   └── current_false_positives.vcf
    ├── hotspot/
    │   └── current_hotspot.vcf
    ├── design/
    │   ├── amplicons.bed
    │   ├── targets.bed
    │   └── primer_coordinates.bed
    ├── tvc_parameters/
    │   └── current_tvc_params.json
    └── svb/
        ├── current_svб.vcf      # Active SVB file
        ├── current_svб.bed      # Alternative BED format
        └── archived/
            ├── v1.0_svб.vcf
            ├── v1.1_svб.vcf
            └── v1.2_svб.vcf
  └── panel_v2/
    ├── cnv_baseline/
    ├── false_positives/
    ├── hotspot/
    ├── design/
    ├── tvc_parameters/
    └── svb/
  └── documentation/
    ├── CHANGELOG.md          # Auto-generated change log
    └── svb_tracking/
        └── false_positives_log.md  # SVB-specific discovery log
        └── validation_reports/
```

```
| |   └── 2025-01-svb-validation.md  
| |   └── 2025-02-svb-validation.md  
| ├── panel_releases/  
| |   ├── v1_release_notes.md  
| |   └── v2_release_notes.md  
| └── amplicon_issues/  
| |   ├── pcr_artifacts.md  
| |   └── primer_dimer_log.md  
└── scripts/  
    ├── generate_changelog.sh  
    ├── validate_svb.sh  
    ├── compare_panel_versions.sh  
    └── deploy_panel_config.sh  
── README.md  
└── .gitignore
```

## SVB-Focused Commit Message Format

### Template for SVB Changes

[SVB-TYPE]: Brief description - Panel vX

Sample ID: [sample\_id\_list]

Position: [genomic\_position\_or\_variant]

Amplicon: [amplicon\_name\_or\_id]

Rationale: [why\_this\_change\_was\_made]

PCR Issue: [specific\_amplification\_problem]

Validated by: [reviewer\_initials]

Date discovered: [YYYY-MM-DD]

Additional context:

- Frequency observed: X/Y samples
- Panel version: [panel\_version]
- Amplicon design: [primer\_pair\_info]
- Caller artifacts: [specific\_technical\_issue]
- Related files: [other\_config\_files\_affected]

### SVB Commit Types

- **SVB-ADD:** New SVB filter entry added
- **SVB-REMOVE:** SVB filter entry removed
- **SVB-MODIFY:** Existing SVB filter modified
- **SVB-VALIDATE:** SVB validation study results
- **SVB-MIGRATE:** SVB changes during panel version migration

## Example SVB Commit Messages

### Adding PCR Artifact Filter

bash

```
git commit -m "SVB-ADD: Filter chr14:105863407 IGH primer artifact - Panel v1"
```

Sample ID: LLP045, LLP067, LLP089

Position: chr14:105863407 A>G

Amplicon: IGH\_VDJ\_region\_amp12

Rationale: Recurrent false positive from primer-template mismatch

PCR Issue: Forward primer 3' mismatch creates systematic artifact

Validated by: GT

Date discovered: 2025-01-15

Additional context:

- Frequency: 3/50 samples this month
- Panel version: LLP v1.4
- Amplicon design: 165bp amplicon, GC-rich region
- Caller artifacts: Ion Torrent strand bias + low complexity
- Related files: May need hotspot update"

### Removing Resolved Filter

bash

```
git commit -m "SVB-REMOVE: chr13:32911035 BRCA2 filter - Panel v1"
```

Sample ID: Validation cohort (n=100)

Position: chr13:32911035 G>A

Amplicon: BRCA2\_exon11\_amp03

Rationale: Primer redesign eliminates false positive

PCR Issue: Resolved by primer pair optimization

Validated by: GT/AS

Date discovered: 2025-01-20

Additional context:

- Frequency: 0/100 samples with new primer design
- Panel version: LLP v1.5
- Amplicon design: New primer pair 20bp upstream
- Caller artifacts: Eliminated by improved amplicon balance
- Related files: Updated design/amplicons.bed"

## Panel Version Migration

bash

```
git commit -m "SVB-MIGRATE: Transfer validated filters v1->v2 - Panel v2"
```

Sample ID: Historical validation set (n=500)

Position: Multiple (see detailed log)

Amplicon: Cross-panel amplicon comparison

Rationale: Migrate proven filters to new panel version

PCR Issue: Evaluate amplicon design changes impact

Validated by: GT/AS/MB

Date discovered: 2025-02-01

Additional context:

- Frequency: Maintained filter set from v1
- Panel version: LLP v2.0
- Amplicon design: 85% amplicon overlap v1->v2
- Caller artifacts: Consistent calling parameters
- Related files: Updated all reference files for v2"

## SVB-Specific Documentation Templates

### SVB False Positives Discovery Log

markdown

## # SVB False Positive Discovery Log - LLP Panel

### ## 2025-01-15: IGH Primer Artifact

- **Position**: chr14:105863407 A>G
- **Amplicon**: IGH\_VDJ\_region\_amp12
- **Samples**: LLP045, LLP067, LLP089
- **Root cause**: Forward primer 3' mismatch with reference sequence
- **PCR issue**: Systematic primer-template mismatch
- **Frequency**: 3/50 samples (6%)
- **Action**: Added to SVB v1.4
- **Git commit**: abc123f
- **Validation**: Confirmed absent in germline DNA, present in all PCR replicates

### ## 2025-01-10: TP53 Amplicon Imbalance

- **Position**: chr17:7577120 C>T
- **Amplicon**: TP53\_exon5\_amp07
- **Samples**: LLP032, LLP041
- **Root cause**: Amplicon competition with neighboring region
- **PCR issue**: Preferential amplification of mutant allele
- **Frequency**: 2/50 samples (4%)
- **Action**: Added to SVB v1.4
- **Git commit**: def456a
- **Validation**: Absent in independent amplicon design

### ## 2025-01-05: MYC Translocation Breakpoint

- **Position**: chr8:128748315-128748320
- **Amplicon**: MYC\_translocation\_amp15
- **Samples**: LLP028
- **Root cause**: Amplicon spans common translocation breakpoint
- **PCR issue**: Chimeric amplification products
- **Frequency**: 1/50 samples (2%)
- **Action**: Added to SVB v1.4
- **Git commit**: ghi789b
- **Validation**: Confirmed germline variant, amplicon redesign needed

## SVB Validation Report Template

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## # SVB Validation Report - LLP Panel v1 - January 2025

### ## Summary

- **Review period**: 2025-01-01 to 2025-01-31
- **Panel version**: LLP v1.4
- **Samples processed**: 150
- **New SVB filters added**: 3
- **SVB filters removed**: 1
- **False positive rate**: 2.3% (before SVB filtering)
- **False positive rate**: 0.1% (after SVB filtering)

### ## Amplicon Performance Analysis

Amplicon	Total Variants	False Positives	SVB Filtered	Success Rate
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Amplicon	Total Variants	False Positives	SVB Filtered	Success Rate
IGH_VDJ_region_amp12	25	3	3	100%
TP53_exon5_amp07	18	2	2	100%
MYC_translocation_amp15	12	1	1	100%
BRCA2_exon11_amp03	8	0	0	N/A

### ## New SVB Filters Added

Position	Amplicon	Samples	Frequency	PCR Issue
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Position	Amplicon	Samples	Frequency	PCR Issue
chr14:105863407	IGH_VDJ_region_amp12	LLP045,067,089	3/150	Primer mismatch
chr17:7577120	TP53_exon5_amp07	LLP032,041	2/150	Amplicon competition
chr8:128748315	MYC_translocation_amp15	LLP028	1/150	Breakpoint spanning

### ## SVB Filters Removed

Position	Amplicon	Rationale
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Position	Amplicon	Rationale
chr13:32911035	BRCA2_exon11_amp03	Primer redesign resolved

### ## PCR-Specific Validation

- **Primer validation**: 3/3 new filters confirmed primer-related
- **Amplicon balance**: Improved after primer optimization
- **Cross-contamination**: No evidence in negative controls
- **Replicate consistency**: 100% reproducibility of filtered positions

### ## Recommendations

1. **Amplicon redesign**: Consider MYC\_translocation\_amp15 primer repositioning
2. **Primer screening**: Implement in-silico primer validation pipeline
3. **Quality metrics**: Add amplicon balance monitoring to TVC parameters
4. **Panel evolution**: Flag amplicons for v2 redesign consideration

# Panel Configuration Integration

## SVB Integration with Other Config Files

bash

```
# Check SVB consistency with other config files
./scripts/validate_panel_config.sh panel_v1

# Example validation checks:
# - SVB positions covered by design/targets.bed
# - Hotspot variants not contradicted by SVB
# - TVC parameters compatible with SVB filtering
# - CNV baseline regions don't overlap SVB filters
```

## Cross-Reference File Dependencies

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```
# SVB Dependency Map
- **design/amplicons.bed**: SVB positions must be within amplicon boundaries
- **hotspot/current_hotspot.vcf**: Hotspot variants should not be SVB filtered
- **tvc_parameters/current_tvc_params.json**: Filtering thresholds must be compatible
- **false_positives/current_false_positives.vcf**: May overlap with SVB (different filtering stages)
```

## Panel Version Management

### Version Migration Workflow

bash

```
# 1. Create new panel version directory  
mkdir -p llp-reference-files/panel_v2/svb  
  
# 2. Copy current SVB as baseline  
cp panel_v1/svb/current_svb.vcf panel_v2/svb/current_svb.vcf  
  
# 3. Evaluate amplicon changes impact on SVB  
./scripts/compare_panel_versions.sh panel_v1 panel_v2  
  
# 4. Update SVB for new amplicon design  
# (manual process based on amplicon comparison)  
  
# 5. Validate SVB against new panel design  
./scripts/validate_svb.sh panel_v2  
  
# 6. Commit migration  
git add panel_v2/svb/  
git commit -m "SVB-MIGRATE: Initialize v2 SVB from validated v1 filters"
```

## Release Management

```
bash  
  
# Tag panel releases  
git tag -a llp-v1.4 -m "LLP Panel v1.4 - SVB updates for primer artifacts"  
git tag -a llp-v2.0 -m "LLP Panel v2.0 - Major amplicon redesign + migrated SVB"  
  
# Generate release notes  
./scripts/generate_release_notes.sh llp-v1.4
```

## Automation Scripts

### SVB Validation Script

```
bash
```

```

#!/bin/bash
# validate_svb.sh

PANEL_VERSION=$1
SVB_FILE="lfp-reference-files/${PANEL_VERSION}/svb/current_svb.vcf"
DESIGN_FILE="lfp-reference-files/${PANEL_VERSION}/design/targets.bed"

echo "Validating SVB for ${PANEL_VERSION}..."

# Check SVB format
bcftools view -h $SVB_FILE > /dev/null 2>&1
if [[ $? -eq 0 ]]; then
    echo "✓ SVB VCF format valid"
    SVB_COUNT=$(bcftools view -H $SVB_FILE | wc -l)
    echo " SVB filters: $SVB_COUNT"
else
    echo "✗ SVB VCF format invalid"
    exit 1
fi

# Check SVB positions are within panel design
echo "Checking SVB positions against panel design..."
bcftools view -H $SVB_FILE | while read line; do
    CHR=$(echo $line | cut -f1)
    POS=$(echo $line | cut -f2)

    # Check if position is covered by design
    if ! bedtools intersect -a <(echo -e "${CHR}\t${POS}\t$((POS+1))") -b $DESIGN_FILE | grep -q .; then
        echo "⚠ Warning: SVB position ${CHR}:${POS} not covered by panel design"
    fi
done

echo "SVB validation complete for ${PANEL_VERSION}"

```

## Panel Configuration Deployment

bash

```

#!/bin/bash
# deploy_panel_config.sh

PANEL_VERSION=$1
DEPLOYMENT_PATH="/opt/ion/config/lip_panel"

echo "Deploying ${PANEL_VERSION} configuration..."

# Copy all current config files
cp lip-reference-files/${PANEL_VERSION}/svb/current_sv.vcf ${DEPLOYMENT_PATH}/
cp lip-reference-files/${PANEL_VERSION}/tvc_parameters/current_tvc_params.json ${DEPLOYMENT_PATH}/
cp lip-reference-files/${PANEL_VERSION}/hotspot/current_hotspot.vcf ${DEPLOYMENT_PATH}/
cp lip-reference-files/${PANEL_VERSION}/design/targets.bed ${DEPLOYMENT_PATH}/

# Validate deployment
./scripts/validate_panel_config.sh ${DEPLOYMENT_PATH}

echo "✓ ${PANEL_VERSION} deployed successfully"

```

## SVB-Specific Best Practices

### Commit Guidelines

1. **Amplicon context** - Always include amplicon information
2. **PCR issue documentation** - Specify the underlying amplification problem
3. **Cross-file impact** - Note if other config files need updates
4. **Panel version tracking** - Clearly indicate which panel version
5. **Validation depth** - Document how the filter was validated

### Quality Control Workflow

1. **Pre-commit validation** - Ensure SVB format and design compatibility
2. **Amplicon analysis** - Check for systematic amplicon-specific issues
3. **Cross-reference validation** - Verify compatibility with other config files
4. **Deployment testing** - Test on known problem samples before production
5. **Performance monitoring** - Track SVB effectiveness over time

This system provides comprehensive SVB management within the broader panel configuration framework, with particular attention to the PCR-based amplification context and multi-file dependencies inherent in targeted NGS panel management.